Differentiating between the Adverse Effects of Nutrient-Limitation and Direct-Cold-Limitation on Tree Growth at High Altitudes

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Differentiating between the Adverse Effects of Nutrient-Limitation and Direct-Cold-Limitation on Tree Growth at High Altitudes

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Abstract

The *sink-limitation hypothesis* postulates that suppression of meristematic activity can limit growth at low temperatures (direct-cold-limitation) in situations where photosynthesis is not restricted. In accordance with this hypothesis, high concentrations of non-structural carbohydrates (NSC) have been found, in several studies, in plants at high altitudes or latitudes. However, high concentrations of NSC could also be due to nutrient deficiency. This study aimed to differentiate between nutrient- and direct-cold-limitation by comparing the growth and NSC concentrations of *Abies veitchii* saplings at two altitudes (high/low) and with two fertilization treatments (N-rich/N-poor).

N-rich saplings grew better at the low than the high site, but N-poor saplings were suppressed to similar degrees at both altitudes. Among N-rich saplings, the leaf nitrogen concentration was lower at the high site than the low site. Leaf NSC concentrations were higher in N-poor than N-rich saplings. However, NSC concentrations were higher at the high site than the low site in leaves with the same nitrogen concentrations. These results indicate that direct-cold and nutrient-limitation may occur simultaneously at high altitude, causing restricted growth and NSC accumulation. Therefore, NSC concentration should be interpreted in terms of both direct-cold and nutrient-limitation.

Introduction

Plant growth limitation at high altitudes is a well known phenomenon. Several contributing factors have been suggested, including mechanical damage or physiological stress associated with deep snow, severe frost, winter desiccation, and strong winds (c.f., Sveinbjörnsson, 2000; Holtmeier, 2003). To adapt to severe environments, plants may alter their life history strategy, for example reproducing at an earlier age (Sakai et al., 2003), or only attaining a small size in order to ensure effective resource acquisition (Stevens and Fox, 1991). Shortages of resources, such as water (Bugmann, 2001; Takahashi et al., 2003) and nutrients (Sveinbjörnsson et al., 1992; Hobbie et al., 2002), are also important factors in relation to growth limitation. Suppression of mycorrhizal infection due to low soil temperatures may cause nutrient limitation (Germino et al., 2006; Smith et al., 2009). In addition, plants may be unable to photosynthesize sufficiently in high-altitude areas due to the short growing season and associated low temperatures (Hasler, 1982; Sveinbjörnsson, 2000; Peterson and Peterson, 2001; Richardson, 2004; Li et al., 2008b). These factors would be especially critical for the growth and survival of the early life stages of tree species (Smith et al., 2009). However, most of these factors are only applicable at a local scale, and conclusive experimental evidence is still lacking to explain the phenomenon in general across the non-arid mountains of the world (Körner, 1998).

The *Sink-Limitation hypothesis* proposed by Körner (1998) is a candidate explanation of general growth limitations at high altitude/latitude globally. This hypothesis suggests that inactivity of meristematic organs (i.e., resource sinks) caused by low temperatures results in growth limitation, even though photosynthesis is not limited under the prevailing conditions. According to the hypothesis, photosynthates will be in excess, since limited sink activity means that resources are not used to add to the physical structure of the plant. As a result, the excess photosynthates are accumulated as nonstructural carbohydrates (NSC); thus, the amount of NSC is considered to be a good indicator of carbon sink limitation (Hoch and Körner, 2003). There is some evidence that plants at high elevations tend to have a higher concentration of NSC in their organs than plants growing at low elevations (Hoch et al., 2002, 2003; Li et al., 2002; Hoch and Körner, 2003; Shi et al., 2006, 2008; Bansal and Germino, 2008). However, several findings that conflict with the hypothesis have been published (e.g., Susiluoto et al., 2007; Li et al., 2008a, 2008b). Hence, the validity of the sink limitation hypothesis has not yet been established fully.

A reduction in sink demands caused by low temperatures is not the only cause of carbohydrate accumulation. Resource accumulation is usually observed when source-sink relationships are unbalanced (Bloom et al., 1985; Chapin et al., 1990). It is well known that NSC accumulation is also observed when there is a deficiency in other resources, such as nitrogen (Chapin et al., 1990; Millard et al., 2007). In addition, high-altitude regions are generally considered to experience limited nutrient availability because of the low temperatures (Page-Dumroese et al., 1990; van Miegroet et al., 1993; Morris, 1995; Michelsen et al., 1996, but see Körner, 1989; He et al., 2006). Hence, high NSC accumulation in plants in high-altitude areas may be caused by a limited nutrient supply (see e.g., Shaver and Chapin, 1980). Therefore, when NSC is used to assess sink-limitation by low temperature (direct-cold-limitation) and its negative effect on growth at high altitudes, the effect of nutrient-limitation should also be considered.

This study aimed to evaluate the effects of both nutrient-limitation and direct-cold-limitation on the growth and NSC storage of plants growing at a high-altitude site. To differentiate between the effects of nutrient-limitation and direct-cold-limitation, *Abies veitchii* Lindley saplings were grown at two different altitudes and with two levels of fertilization (resulting in a 2×2 factorial experimental design). The saplings' growth and the concentration of NSC within them were compared between the treatments.

Materials and Methods

MATERIAL

The saplings of *Abies veitchii* Lindley used in this study were all less than 10 years old at the start of the experiment. Saplings were collected from the slopes of Mt. Asahi, Nagano prefecture (35°52′N, 138°39′E, 2300 m a.s.l.) on 10 June 2004. They were placed in a nursery field until shoot elongation had ceased. On 7 July, 60 saplings were selected at random and transplanted into 2 L pots containing a mixture of vermiculite and river sand (1:1 in volume). The potted saplings were watered daily until moved to the study sites.

CULTIVATION

On 2 September 2004 the pots were divided into four groups to use for testing the effects of altitude and nutrient availability on growth and carbohydrate storage. Each group included 15 pots. Two groups of pots were placed at a "high-site," 2243 m a.s.l., on the upper slope of Mt. Ontake, about 200 m below treeline in this area, and the other two were placed at a "low-site" at 1591 m a.s.l. on the lower slope of the mountain (Table 1). Once in place, each pot was fertilized once with a commercial nutrient solution containing 6%, 10% and 5% of N, PO_4 and K, respectively (HYPONeX, HYPONeX JAPAN corp., Japan) diluted to 0.1% (v/v). Thereafter, one group of pots at each site was fertilized with 400 mL of HYPONeX solution (containing 24 mg nitrogen) once every two weeks until 6 November (N-rich treatment). The other group at each altitude received only water when the first group was fertilized (N-poor treatment). The pots were kept outside over the winter.

From 19 May 2005 (the start of the growing season in the following year), every pot was watered once a week with nutrient free water, except that each of the N-rich pots was supplied with 400 mL of 0.2% HYPONeX solution (containing 48 mg N) instead of water once every two weeks and each of the N-poor pots received 400 mL of 0.1% HYPONeX solution (containing 24 mg N) instead of water once every four weeks. The watering and fertilization regimes continued throughout the 2005 growing season. Through the watering, in conjunction with the natural precipitation (in total, 1620 mm during the growing season) and the intermediate moisture retention of the growing medium meant that the plants received sufficient water throughout the growing period.

GROWTH MEASUREMENT

To determine the radial growth rate of the *A. veitchii* saplings subjected to each treatment, the diameter at the stem base of each individual was measured every week during the 2005 growing season. The stem of every sapling was marked 1 cm above the soil surface, and two diameters, at right angles to each other, were measured using calipers. An average of the two diameters was used in the analysis. For each sapling, attributes associated with radial stem growth were determined by estimating the parameters in the growth function:

$$D = \frac{D_{initial} - D_{final}}{1 + \exp(\frac{x - x_{50}}{m})} + D_{final} \tag{1}$$

where $D_{initial}$ (the diameter at the beginning of growth), D_{final} (the diameter at the end of growth), x_{50} (the midpoint for steady-state phase; when x becomes x_{50}, D reaches 50% of the total increment, and it is also the inflection point of the growth function), and m (slope factor) are estimated parameters, while D and x are variables (stem diameter and measurement days from 19 May 2005, respectively). The growth rate of each sapling was calculated as the slope of the tangent of the growth function at x_{50}, at which the slope becomes maximum:
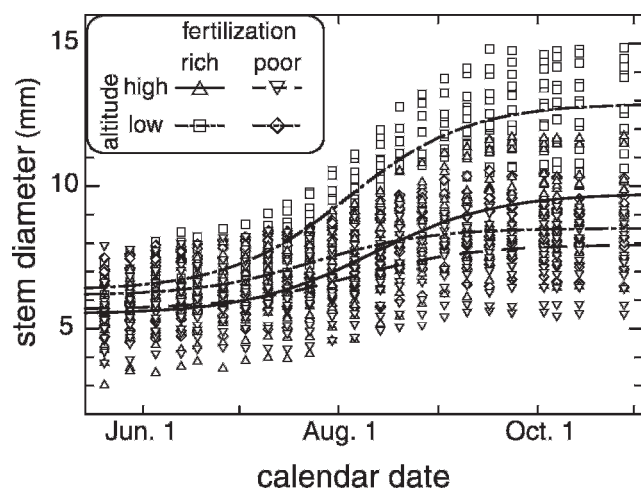
$$growth\ rate = \frac{D_{final} - D_{initial}}{4m} \tag{2}$$

LEAF HARVESTING

To assess the amount of carbohydrate storage and the nitrogen content in leaves, these were harvested sequentially. In order to account for the effect of age on the content of carbohydrates and nitrogen in leaves, two age classes (current year leaves and one-year-old leaves) were collected. From 22 July to 13 October 2005, two or three one-year-old leaves from different branches were harvested every two weeks. Two or three current year leaves were collected at the same time, except on 22 July when the current year leaves were insufficiently mature. Leaves were collected in the morning and stored immediately in a cool box; leaf area was measured in the laboratory using image processing software (ImageJ, National Institutes of Health, U.S.A.) after scanning with an image scanner (CanoScan FB 636U, Canon, Japan), then the samples were placed in a deep freezer (−75 °C) whilst awaiting lyophilization. The lyophilized samples were weighed, crushed into small fragments, and subsamples were used for the quantification of carbohydrates and nitrogen in the leaves.

CARBOHYDRATE AND NITROGEN ANALYSIS

The carbohydrates in the leaves of *A. veitchii* were measured according to the method of Kabeya and Sakai (2005). The water-soluble fraction was extracted three times with 80% ethanol. Ethanol solutions from each separate extraction were combined, then the ethanol was evaporated off and the precipitate was dissolved in pure water. All soluble sugars in the resulting solution were quantified according to the phenol-sulfuric acid method. To extract starch, the precipitate from the ethanol extraction was boiled with 0.2N KOH for 30 minutes. After neutralization with

TABLE 1

Study site description.

Site	Elevation (m a.s.l.)	Air temperature (°C) during study period (mean ± SD)	Pot soil temperature (°C) during study period (mean ± SD)
high	2243	12.5 ± 3.5	13.3 ± 3.8
low	1591	17.1 ± 3.4	18.3 ± 3.3

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FIGURE 1. Stem diameter change over time in *Abies veitchii* saplings cultivated at two altitudes (high/low) × two fertilization levels (rich/poor). Each point represents an individual stem diameter measurement. Growth function curves, the parameters of which were estimated from the average of the individuals in each treatment (see Table 2), are also shown.

1N CH_3COOH, the starch in the solutions was hydrolyzed into glucose using amyloglucosidase (from *Rizophs* mould, Sigma) for 30 minutes at 55 °C. The glucose was selectively quantified using a glucose-peroxidase testing kit (glucose C-II test, Wako corp., Japan). For both measurements, a glucose solution was used as the standard. The NSC concentration was calculated as the sum of the concentrations of soluble sugars and of starch (glucose equivalent).

The concentration of leaf nitrogen was determined by the Indophenol-Blue method, after digestion of the subsamples with a sulfuric acid–hydrogen peroxide solution (Matsunaga and Shiozaki, 1989). The weighed subsamples were placed in the mixture of sulfuric acid and hydrogen peroxide, and maintained at a temperature of 120 °C for 1.5 hours. After cooling, the volume was adjusted to a standard volume by adding pure water, and subsamples of the solution were used for the Indophenol-Blue method. NH_4 solution was used as the standard.

STATISTICAL ANALYSES

To estimate the parameters of the growth function, nonlinear regression (using the *nlm* function) was performed in the *R* software package (version 2.1.1).

The effects of altitude and fertilization on the growth parameters of the saplings ($D_{initial}$, D_{final}, *growth rate*, and *growing interval*) were tested by a two way ANOVA after normality and

homoscedasticity had been confirmed. The Tukey-Kramer HSD test was used for *post-hoc* testing. In the analysis of the leaf nitrogen content, two main effects (altitude and fertilization) and one random effect (individual effect) were considered to account for the repeated measurements. In these cases, a mixed model was applied after normality had been confirmed. In the analyses of NSC (soluble sugar + starch) concentrations, two fixed effects (altitude and leaf nitrogen concentration) and one random effect (sampling date) were included the model. When the interaction between the fixed effects was not significant, an ANCOVA model including a random effect (sampling date) was applied. In all mixed model analyses, an unstructured covariance structure was adopted. All the statistical analyses were conducted using SAS/STAT 9.2 (SAS Institute).

Results

STEM RADIAL GROWTH

At the time that observations commenced, the basal diameter ($D_{initial}$) was larger for the low-site saplings than the high-site ones (Fig. 1). The dates that the diameter size reached 5% and 95% of the maximum radial increment ($D_{final} - D_{initial}$) were regarded as the beginning and end of the growing period, respectively. The dates of the start of the growing period for the high-rich, high-poor, low-rich, and low-poor treatment combinations were 6/14 ± 4, 6/8 ± 5, 6/8 ± 2, and 5/31 ± 6, respectively (mean dates ± SE in days). The dates when growth ceased in each treatment were 10/6 ± 2, 9/29 ± 3, 9/27 ± 2, and 9/13 ± 2, respectively. The growing interval was not affected significantly by location, fertilization, or their interaction (Table 2). There was a significant difference in the radial growth rate in the N-rich treatment: the growth rate was higher for the low-rich saplings than for the high-rich ones (Table 2). As a result, the diameter at the end of the growing period (D_{final}) was significantly larger for the low-site saplings than high-site ones subjected to the N-rich treatment (Table 2).

CARBOHYDRATE CONCENTRATION IN LEAVES

The leaves of *A. veitchii* contained more soluble sugars than starch in both the current year and the one-year-old leaves (Fig. 2). The concentrations of the soluble sugars and the starch in the leaves varied among the sampling occasions. At the time of each harvest, the concentration of soluble sugars in the leaf was higher in the N-poor saplings than in the N-rich ones in leaves of both ages (Fig. 2). The effect of fertilization on the leaf carbohydrate concentration was clear with respect to starch. Regardless of altitude and leaf age, the saplings subjected to the N-poor treatment contained more starch in their leaves than those subjected to the N-rich treatment (Fig. 2).

TABLE 2

Estimated parameters of the radial growth function for stems of *A veitchii* saplings initial size ($D_{initial}$), final size (D_{final}), midpoint for steady-state phase (x_{50}), and slope factor (m) and growth parameters calculated from them (growth rate and growing interval). Means and SEs of each parameter are shown. Different lower-case letters indicate significant differences between parameters ($p < 0.05$; Tukey-Kramer HSD-test).

Altitude	Fertilization	n	$D_{initial}$ (mm) mean	SE	D_{final} (mm) mean	SE	x_{50} (day) mean	SE	m mean	SE	Growth rate (mm*day^{-1}) mean	SE	Growing interval (days) mean	SE
high	rich	14	5.5	0.3 a	9.8	0.4 b	83.4	1.3	19.5	1.0	5.6E–2	2.8E–3 b	114.6	5.6 a
high	poor	15	5.7	0.3 a	7.8	0.3 c	76.9	1.6	19.1	1.2	2.8E–2	1.7E–3 c	112.4	7.1 a
low	rich	13	6.3	0.2 a	12.9	0.4 a	76.1	1.2	18.8	0.7	8.8E–2	3.3E–3 a	110.8	4.2 a
low	poor	14	6.2	0.3 a	8.5	0.3 bc	65.1	3.6	17.7	1.0	3.4E–2	2.0E–3 c	104.5	6.0 a
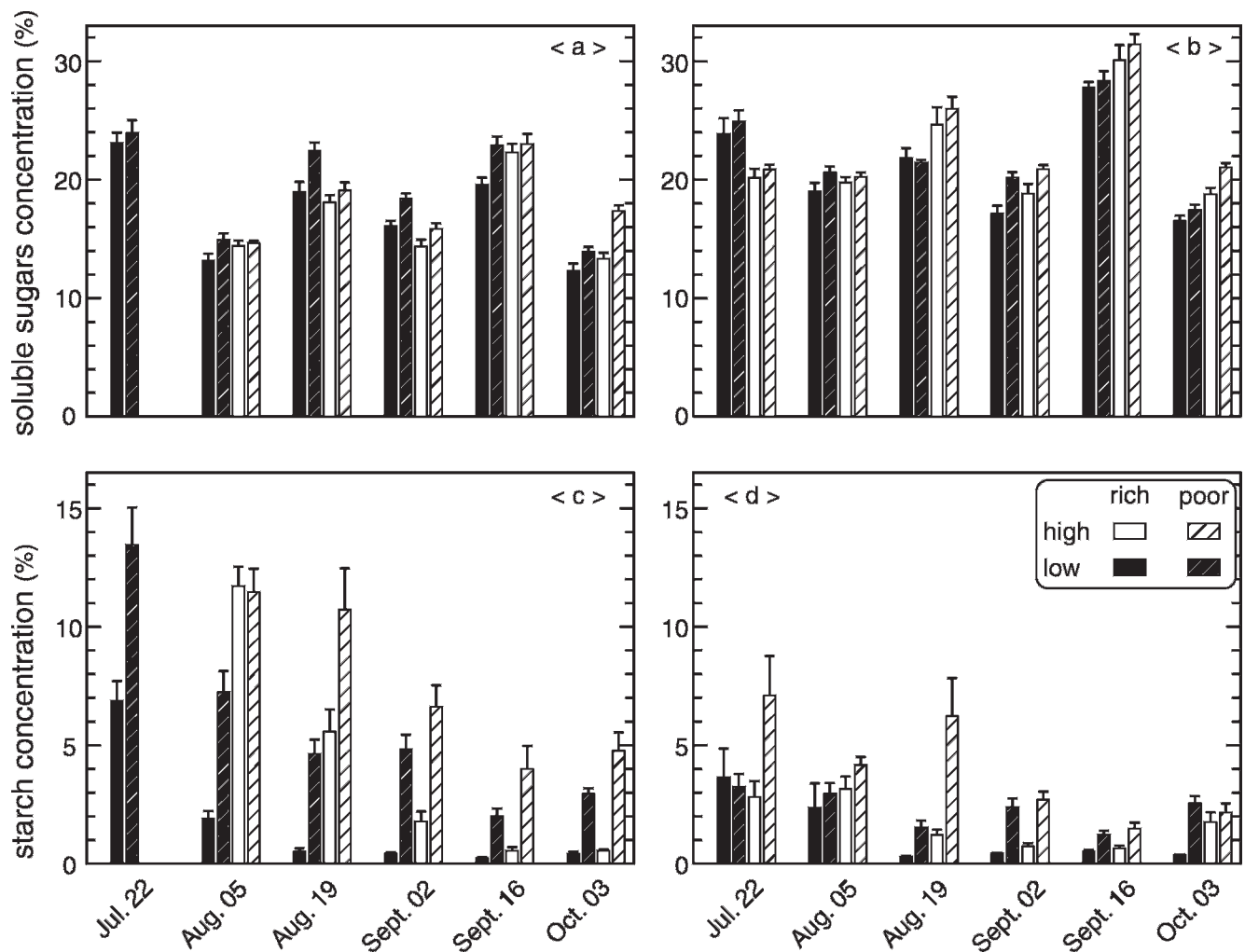
FIGURE 2. The concentration of soluble sugars and starch in current year leaves (a, c) and one-year-old leaves (b, d) of *Abies veitchii* saplings cultivated at two altitudes (high/low) × two fertilization levels (rich/poor). Means and SEs are shown.

NITROGEN CONCENTRATION IN LEAVES

Nitrogen concentration was higher in the N-rich saplings than in the N-poor saplings at both sites (Fig. 3). In the current year leaves, the effect of altitude on nitrogen concentration was not significant (Table 3). However, the N concentration based on structural leaf mass (total leaf mass – NSC pool in the leaf) of the current year leaves was higher in the low-site saplings than the high-site ones. Leaf nitrogen concentration was higher in the low-site plants than the high-site plants in one-year-old leaves (Table 3). A significant difference in nitrogen concentration with respect to structural leaf mass was only found for the N-rich treatment (Table 3, significant interaction).

THE EFFECT OF ALTITUDE AND FERTILIZATION LEVEL ON THE NSC CONCENTRATION

Across the harvests, significant altitude effects on leaf NSC concentrations were found (Table 4), and leaf nitrogen concentration correlated negatively with leaf NSC concentration in leaves of both ages (Fig. 4). In addition, a significant interaction between altitude and fertilization was found in the current year leaves. Within the range of the data collected, the leaf NSC concentration was higher in the high-site saplings than the low-site ones for leaves of both ages (Fig. 4). Least square (LS) means of the NSC concentrations in current year leaves, which were adjusted for the

effect of the leaf nitrogen concentrations, were $21.6 \pm 0.3\%$ and $18.6 \pm 0.4\%$ in the high-site and the low-site saplings, respectively (mean $\pm$ SE). In one-year-old leaves, LS means of the NSC concentrations were $24.4 \pm 0.3\%$ and $23.1 \pm 0.4\%$ in the high-site and the low-site saplings, respectively.

Discussion

Although the high-site examined in this study was situated about 200 m below the treeline altitude, radial growth of *Abies veitchii* saplings was suppressed at this site. However, the saplings stored higher levels of NSC at the high-site than at the low-site, after adjusting for the fertilization effect. Therefore, carbon resource limitation, at least, does not constrain the growth of *A. veitchii* saplings at high altitudes. Furthermore, their radial growth was suppressed at the high-site even under well-fertilized conditions, so their growth was not limited by inadequate nutrient supply. In addition, although low leaf nitrogen concentrations were observed at the high-site, implying nutrient uptake limitation, the high NSC accumulation at this site (after adjusting fertilization effects) indicates that the direct-cold-limitation may affect *A. veitchii* saplings at high altitude, even if nutrient acquisition is limited.

Low temperatures, such as those encountered at high altitudes and latitudes, have severe effects on plant nutrition in natural conditions (but see Körner, 1989), for several reasons.

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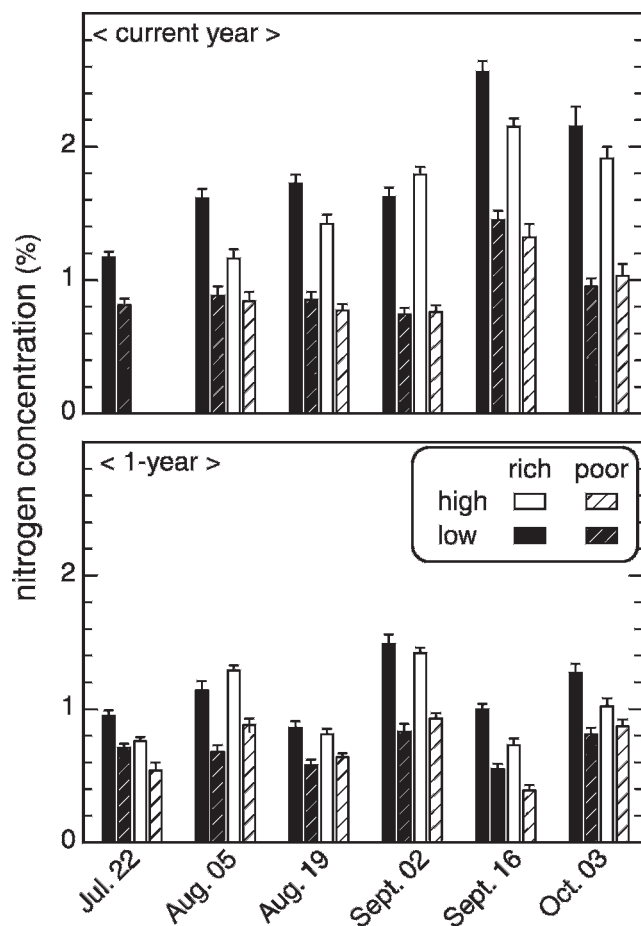
FIGURE 3. Nitrogen concentrations in current year and one-year-old leaves of *Abies veitchii* saplings cultivated at two altitudes (high/low) × two fertilization levels (rich/poor). Means and SEs are shown.

First, soil available nutrients are limited by the slow decomposition rate (Herrera, 1991; Radwan, 1992; Schulze et al., 1994; Hobbie and Chapin, 1998; Hobbie et al., 2002; Crosti et al., 2006). Nutrient absorption activity also depends on temperature (Karlsson and Nordell, 1996; Weih and Karlsson, 2001). Moreover, limited mycorrhizal symbiosis at high altitude (Germino et al., 2006) may suppress nutrient acquisition. In *A. veitchii* saplings, leaf nitrogen concentration was lower at the high-altitude site than at the lower site under the higher fertilization regime.

This could be due to suppressed nutrient absorption at the high-altitude site.

A shortage of nitrogen is one of the major causes of carbohydrate accumulation (Warren-Wilson, 1966; Bloom et al., 1985; Chapin et al., 1990; Schulze et al., 1994; Stitt and Krapp, 1999; Lerdau and Coley, 2002; Millard et al., 2007). In this study, *A. veitchii* saplings accumulated a large amount of non-structural carbohydrates in their leaves when they were grown with limited fertilization at both altitudes studied. This trend was particularly clear when starch was considered; starch is the main storage substance in many plants. This result suggests that nutrient-limitation may explain the high levels of carbohydrates in plants growing in high-altitude/latitude areas recorded in previous studies (Hoch et al., 2002, 2003; Li et al., 2002; Hoch and Körner, 2003; Bansal and Germino, 2008). In fact, Shaver and Chapin (1980) showed that fertilization caused a reduction in NSC concentration in several plant species of tundra regions, thus indicating the existence of NSC accumulation due to nutrient limitation in natural environments.

Plants growing in high-altitude areas tend to contain higher concentrations of nitrogen than those growing at low altitudes (Körner, 1989; Kudo, 1995; Weih and Karlsson, 1999; Hikosaka et al., 2002; Reich and Oleksyn, 2004, but see He et al., 2006). This could result from ecotypic acclimatization to low temperature environments (Weih and Karlsson, 1999). In the current study, saplings originating from a single location were cultivated for one and a half years at each study site. This may have been insufficient time for acclimatization to have occurred. Moreover, optimal nitrogen concentration in leaves increases with altitude. As predicted by Stevens and Fox (1991), plants may restrict their size and concentrate nitrogen in order to ensure optimal nitrogen levels. As a result, plants at high altitudes would be able to photosynthesize sufficiently, but photosynthates may not be used to their optimum efficiency, because plant size is actually determined by the amount of available nitrogen. This would mean that a plant growing at high altitude would exhibit a higher NSC concentration than a plant growing at low altitude, even though the leaves of the former contained a higher concentration of nitrogen. In addition, some fertilization studies have recorded an increase in nitrogen content in plants, particularly at high altitudes (Herrera, 1991; Sveinbjörnsson et al., 1992; van Miegroet et al., 1993). Plants may experience nutrient-limitation at high altitudes, even though their leaves have higher nitrogen concentrations than plants from lower altitudes.

The higher recorded concentration of NSC in *A. veitchii* saplings growing at high altitudes compared to those at low

TABLE 3

Type III test in the mixed model including leaf N concentration and leaf N as a proportion of structural mass in *Abies veitchii* saplings. The growing sites (Altitude), the fertilization levels (Fertilization), and their interaction are considered as the fixed effects.

Independent variables	Leaf age	Fixed effect	Numerator *df*	Denominator *df*	*F* value	*p*
N concentration	current	Altitude	1	53.3	3.1	0.087
		Fertilization	1	53.3	257.1	<0.001
		A*F	1	53.3	0.3	0.585
	1-year	Altitude	1	45.0	9.8	0.003
		Fertilization	1	45.0	102.0	<0.001
		A*F	1	45.0	1.2	0.283
N proportion of structural mass	current	Altitude	1	51.3	5.2	0.028
		Fertilization	1	51.3	119.4	<0.001
		A*F	1	51.3	0.4	0.534
	1-year	Altitude	1	43.3	3.0	0.091
		Fertilization	1	43.3	135.0	<0.001
		A*F	1	43.3	5.2	0.028

TABLE 4

Type III test in the mixed model for leaf NSC concentration in *Abies veitchii* saplings. Two growing sites (categorical data), leaf N concentration (continuous data), and their interaction were considered as the fixed effects in the model. In the one-year-old leaves, the interaction between Altitude and N concentration was excluded from the model, because it was insignificant.

Leaf age	Fixed effect	Numerator *df*	Denominator *df*	*F* value	*p*
current	Altitude	1	90.7	18.0	<0.001
	N conc.	1	104	174.7	<0.001
	A*N	1	104	6.7	0.01
1 year	Altitude	1	36.4	9.2	0.005
	N conc.	1	90.9	90.1	<0.001

altitudes, irrespective of nutrient treatment, supports data from other studies that have provided evidence for the direct-cold-limitation (e.g., Hoch et al., 2002; Li et al., 2002; Hoch et al., 2003). Therefore, direct-cold-limitation does seem to occur in *A. veitchii*. However, a high level of NSC storage does not conclusively confirm the sink-limitation hypothesis; source-sink manipulations (such as the defoliation experiment described by Li et al., 2002) will be required in order to provide verification. Therefore, further investigation of the hypothesis is necessary.
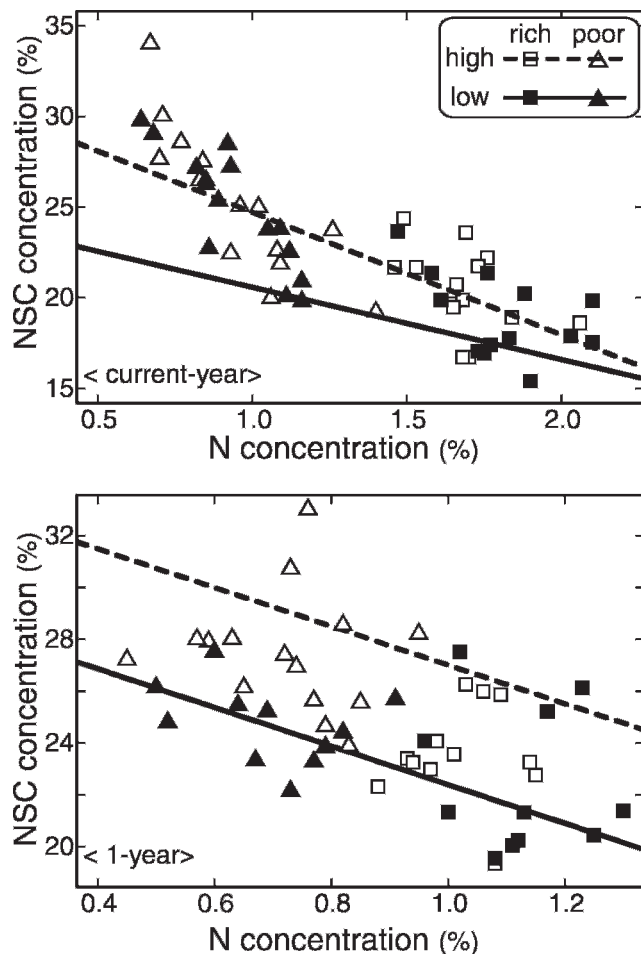

FIGURE 4. Relationships between nitrogen concentration and non-structural carbohydrate (NSC) concentration in current year leaves and one-year-old leaves of *Abies veitchii* saplings cultivated at two altitudes (high/low) × two fertilization levels (rich/poor). Each point represents the average of multiple samplings for each individual. Lines are the results of a mixed model (see Table 4).

The leaves of *A. veitchii* saplings contained a higher concentration of soluble sugars than of starch. High concentrations of soluble sugars in plant leaves are thought to provide osmotic control for cryoprotection (Repo et al., 2004; Reyes-Díaz et al., 2005; Morin et al., 2007; Bansal and Germino, 2009, 2010). Therefore, it is possible that the high concentrations of sugars in the leaves of *A. veitchii* at the high-altitude site are a result of physiological adaptation to low temperature conditions. However, the difference in starch concentration between altitudes was more pronounced than the difference in the concentration of soluble sugars. In addition, all the leaf samples were collected during the growing season when the lowest daily temperatures were well above freezing. Therefore, higher concentrations of soluble sugars in *A. veitchii* leaves at the high-altitude site are unlikely to be only associated with winter hardiness.

The high level of NSC storage in plants growing at high altitude may have a different ecological significance (Sveinbjörnsson, 2000). Resource storage is an important adaptation to fluctuating environments, because plants can use stored resources for the rapid growth of specially adapted organs when the environment changes (Chapin et al., 1990). Therefore, a high allocation of carbon resources to storage can be an adaptive strategy for plants in high-altitude areas that are subjected to greater environmental fluctuations than those in low-altitude areas (Grace et al., 2002). Artificial experiments, such as controlling the range of environmental fluctuation, would be needed to test this hypothesis.

Unlike the growth rate, the length of the growing interval did not differ between altitudes in this experiment, even though the average temperature during the growing season was 3 °C lower at the high-altitude site. The similar length of growing interval between altitudes may result from phylogenetic constraints. *Abies veitchii* has a determinant shoot growth pattern, with the shoot elongating during spring and early summer. Hence, the length of the summer may not directly affect the increase in leaf area during the current season, and, therefore, may not be related to the radial growth of the shoot.

Conclusion

The results of this study suggest that, in the high-altitude area, both meristematic inactivity and nutrient-limitation occur as a result of low temperatures during the growing season; these responses result in growth limitation and NSC accumulation in the leaves. The amount of NSC is a good indicator that growth limitation was not the result of photosynthetic inactivity, although it is difficult to differentiate between the possible reasons for NSC accumulation in plants growing at high altitude. Therefore, care should be taken when using NSC concentration to evaluate direct-cold-limitation, because nutrient-limitation may provide an alternative and equally valid interpretation.

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