

Seed-based treeline seedlings are vulnerable to freezing events in the early growing season under a warmer climate: Evidence from a reciprocal transplant experiment in the Sergyemla Mountains, southeast Tibet



Wei Shen^{a,b}, Lin Zhang^a, Xinsheng Liu^a, Tianxiang Luo^{a,*}

^a Key Laboratory of Tibetan Environment Changes and Land Surface Processes, Institute of Tibetan Plateau Research, Chinese Academy of Sciences, Beijing 100085, China

^b University of Chinese Academy of Sciences, Beijing 100049, China

ARTICLE INFO

Article history:

Received 20 May 2013

Received in revised form

29 November 2013

Accepted 13 December 2013

Keywords:

Alpine treeline

Climate change

Frost

Mortality

Seedling

Transplant

ABSTRACT

Seedling mortality is important to the formation and dynamics of alpine treeline. There is a need to understand the mechanisms governing seedling mortality at and above treelines under a warmer climate. We tested the hypothesis that under a warmer climate, seed-based treeline seedlings are especially vulnerable to freezing events in the early growing season. Using space-for-time substitution, we conducted a 5-yr reciprocal transplant experiment for >10 yr-old seedlings of seed-based fir and root-sprouting juniper between north-facing and south-facing slopes of a valley with elevations of 4200–4600 m in the Sergyemla Mountains, and additional experiments of seed germination and younger seedling transplants (with ages of 3–5 yr) at and above the fir treeline. Between both slopes, annual precipitation was similar but annual mean air-temperature above the treeline differed by 2.0 °C, being comparable to the temporal difference of 2.3 °C between the warmest and coldest years and the unchanged trend of precipitation during 1960–2008 at Nyingchi station nearby the study sites. The frequency, intensity and duration of growing-season freezing events were much higher under the warmer climate on the south-facing slope. Across years and non-forested sites above both treelines, annual mean air-temperature was well correlated with the early-season (April–June) freezing events. In pooled data across years and sites, annual mortality increased in fir seedlings but varied little in juniper seedlings with increasing freezing events in the early growing season. Similar patterns were also found in their annual growth rates. Partial correlation analysis indicated that the early-season freezing event was the major limiting factor determining annual mortality of fir seedlings, while that of juniper seedlings varied little with all the microclimate factors. Harsh environments above the treeline did not limit fir seed germination. The finding that the early-season freezing events under a warmer climate increased fir seedling mortality can explain the cause for the unique distribution pattern of fir and juniper treelines on opposite slopes of a valley in southeast Tibet, and suggests an explanation for the phenomenon that the world's highest fir-treeline position did not advance with climatic warming in past 200 years.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

It has been suggested that the likelihood of freezing events will tend to increase under future global warming (Inouye, 2000; IPCC, 2007; You et al., 2008). The number of freezing events generally increases with increasing elevation (Wieser and Tausz, 2007;

Barry, 2008; Li et al., 2013). At high elevations, extreme low temperatures can occur during the growing season because of the severe radiation cooling at night under clear skies, light winds and dry conditions (Sakai and Larcher, 1987; Taschler and Neuner, 2004; Wieser and Tausz, 2007; Larcher et al., 2010). This can be fatal to the survival of alpine plants because they have the weakest ability to resist the unpredictable freezing events in the early growing season when alpine plants are fully active (Inouye, 2008; Mayr et al., 2012; Neuner and Hacker, 2012; Rixen et al., 2012). Alpine treeline seedlings are expected to be especially vulnerable to freezing events, resulting in the difficulty of seedling establishment at and above treelines (Smith et al., 2003; Kullman, 2007; Harsch and Bader, 2011). However, continuous and detailed

* Corresponding author at: Key Laboratory of Tibetan Environment Changes and Land Surface Processes, Institute of Tibetan Plateau Research, Chinese Academy of Sciences, Building 3, 16 Lincui Road, Chaoyang District, Beijing 100101, China. Tel.: +86 10 84097060; fax: +86 10 84097079.

E-mail address: luotx@itpcas.ac.cn (T. Luo).

measurements of microclimate at alpine treeline ecotones are rare (Barry, 2008; Körner, 2012), and characteristics of growing-season freezing events and their effects on the survival and growth of seedlings at and above treelines remain poorly understood (Kullman, 2007; Barbeito et al., 2012). Such knowledge is important to understand the formation and dynamics of alpine treeline since seedling mortality is one of the three general ‘first level’ mechanisms that determine treeline position and dynamics (Harsch and Bader, 2011).

Southeast Tibet, which is characterized by a cold and moist climate, has the highest elevation treeline in the world (Miehe et al., 2007; Opgenoorth et al., 2010). The instrumental climatic data indicate a significant warming trend since the 1960s, which has been recorded in ring-width chronologies of treeline trees (Liang et al., 2009) and alpine shrubs (Liang and Eckstein, 2009; Kong et al., 2012). In the Sergyemla Mountains, the seed-regenerated fir treeline (*Abies georgei* var. *smithii*) generally dominates on the north-facing slope, while the sprout-regenerated juniper treeline (*Juniperus saltuaria*) dominates on the south-facing slope, forming a unique distribution pattern on opposite slopes of a valley where annual mean air-temperature above both treelines differs greatly and annual precipitation is similar (Zhang et al., 2010; Liu and Luo, 2011). There is evidence that little change has occurred in the seed-regenerated fir treeline position after 200 years of warming even though the fir population density has increased (Liang et al., 2011), which is consistent with the worldwide data that almost half of 130 alpine/boreal treelines did not advance in response to global warming (Harsch et al., 2009). This points to the importance of microsite availability in seedling establishment above treeline (Smith et al., 2003; Harsch and Bader, 2011; Wang et al., 2012). The unique distribution pattern of fir and juniper treelines on opposite slopes of a valley in the Sergyemla Mountains provides a natural laboratory to explore characteristics of growing-season freezing events and their effects on seedling mortality at and above both treelines, which might be useful for understanding why the world’s highest fir-treeline position has not advanced with climatic warming. There is evidence that space-for-time substitution can be used for predicting climate-change effects on biodiversity (Blois et al., 2013).

In this study, we hypothesize that under a warmer climate, seed-based treeline seedlings are especially vulnerable to freezing events in the early growing season. Using space-for-time substitution, we conducted a 5-yr reciprocal transplant experiment for >10-yr-old seedlings of fir and juniper between opposite slopes of a valley with elevations of 4200–4600 m in the Sergyemla Mountains, and additional experiments of seed germination and younger seedling transplants at and above the fir treeline. Our aims are to: (1) examine if growing-season freezing events are significantly higher under the warmer climate on the south-facing slope; (2) determine whether the annual mortality and growth rates of transplanted fir and juniper old-seedlings are generally associated with the growing-season freezing events across sites and years; (3) investigate to what extent harsh environments above the treeline limit fit seed germination and its young-seedling growth and survival.

2. Materials and methods

2.1. Study sites

This study was conducted at the treeline observation sites of the Southeast Tibet Observation and Research Station for Alpine Environments, Chinese Academy of Sciences. The study sites are located on the opposite slopes (north-facing vs. south-facing) of a U-shaped valley at the peak of the Sergyemal Mountains (29°36′ N,

94°36′ E, 4200–4600 m a.s.l.). *A. georgei* var. *smithii* and *J. saltuaria* are the dominant species of treeline forests on the north-facing and south-facing slopes, respectively. Above both treelines, the vegetation changes to an open mosaic of shrublands and grasslands. In August 2005, four automatic weather stations were installed at the sites for treeline forests of fir (4320 m, N2) and juniper (4425 m, S2) and their nearby low-shrubland (4390 m, N3) and grassland (4441 m, S3). Air (1 m and 3 m aboveground) and soil (–5 cm and –20 cm) temperatures, radiation (global radiation, net radiation, photosynthetically active radiation, long wave radiation, red light radiation), wind speed, relative humidity, rainfall, snow depth and soil volumetric moisture were recorded hourly by HL20 data loggers (Jauntering Inc., Taiwan). According to the treeline meteorological observations during 2006–2012, the difference of annual mean air-temperature between the warmest and coldest years was 0.7–1.2 °C for south-facing sites (S2 and S3) and 0.8–1.0 °C for north-facing sites (N2 and N3). Both forested sites (S2 and N2) had similar annual precipitation (850–940 mm) and growing-season mean air-temperature (6.5–6.6 °C). Annual mean air-temperature differed by 0.5 °C between S2 and N2, and by 2.0 °C between S3 and N3 with a warmer climate on the south-facing slope. This is comparable to the temporal difference of 2.3 °C between the warmest and coldest years and the unchanged trend of precipitation during 1960–2008 at Nyingchi station (3000 m, ca. 10 km away from our study sites; Liang et al., 2009; Kong et al., 2012). The maximum snow depth was 80–100 cm on the north-facing slope and 20–30 cm on the south-facing slope. The spring soil warming dates (with –20 cm soil temperature >0 °C) were 20–30 days earlier on the south-facing slope than on the north-facing slope. The –5 cm soil temperature amplitude in the growing season was much lower at N2 (<1 °C) than at the sites of N3, S2 and S3 (2–5 °C). The daily mean soil moisture during the growing season was typically >20% across slopes and sites. Detailed information on the sensor types and site conditions is found in Liu and Luo (2011).

2.2. Reciprocal transplant experiment of fir and juniper old-seedlings

Our previous survey indicated that the seed-based fir seedlings were limited to a stable soil temperature environment (the –5 cm temperature amplitude <1 °C) in the north-facing forests, compared to the relatively widespread root-sprouting juniper seedlings (Liu and Luo, 2011). This suggests an explanation for the unique distribution pattern of both species treelines on opposite slopes of a valley in the Sergyemla Mountains. Knowledge of how the survival and growth of fir and juniper seedlings differ between north-facing and south-facing treeline ecotones is helpful for understanding the mortality of fir seedlings under a warmer climate. We performed a reciprocal transplant experiment of both species old-seedlings between the two slopes. In early June of 2008, fir and juniper old-seedlings (20–50 cm in height) growing well at forest edges in the two lower slopes (4180–4200 m) were selected, and then carefully dug out with soil to a depth of 20 cm. In total, 60 old-seedlings per species were sampled and randomly divided into four groups. The grouped seedlings of each species were transplanted to the four study sites (N2, N3, S2, S3) at and above both treelines. The four study sites represented two typical habitats across both slopes (forested: N2 and S2; non-forested: N3 and S2). At each site, the seedlings were planted around the weather station, at least 1 m apart. Before transplanting, the height and/or age of each seedling were measured. The ages of transplanted fir old-seedlings ranged from 10 to 15 years, which was determined by counting the internodes along the main stem. However, it was difficult to determine the ages of juniper seedlings because the internodes were not obvious. During 2008–2012, the mortality of transplanted seedlings was recorded and their new top-shoot growth rates (cm yr^{–1}) were

measured at the end of the growing season once a year. Annual mortality (%) across sites and years was calculated as the ratio of dead seedlings to total transplanted seedlings. The survival rate (%) was calculated as the ratio of living seedlings to total transplanted seedlings.

2.3. Additional experiments of seed germination and young-seedling transplants at and above the fir treeline

Knowledge about the dispersal and germination of seeds and the survival of younger seedlings is also important to understand the limitations to seedling establishment and treeline dynamics (Harsch and Bader, 2011). In the study area, our transect investigation of fir seedling distribution above the treeline indicated that young seedlings (<10 cm in height with ages of 3–5 yr) growing under alpine shrubs were found up to 4360 m in elevation (40 m higher above the fir treeline), suggesting that seed dispersal may not be the bottleneck of fir seedling establishment above the treeline. To further explore the effects of microclimate factors on fir seed germination and its young-seedling growth and survival, we additionally conducted a seed germination experiment and a young-seedling transplant experiment at N2 and N3 on the north-facing slope. Annual mean air and soil temperatures were about 0.5 °C higher at N3 (non-forested) than at N2 (forested).

In early June of 2011, fir young-seedlings (<10 cm in height, 3–5 yr) growing well at forest edges in the lower north-facing slope (4200 m) were selected, and then carefully dug out with soil to a depth of 10 cm. In total, 140 young-seedlings were sampled and then transplanted to N2 (4320 m, forested) and N3 (4390 m, non-forested). At each site, seven 1-m² plots were established around the weather station, at least 5 m apart. Ten young-seedlings were planted in a plot. During 2011–2013, the mortality of transplanted seedlings was recorded and their new top-shoot growth rates (mm yr⁻¹) were measured at the end of the growing season once a year. The total mortality (%) of a plot after 3-yr transplantation was calculated as the ratio of total dead seedlings to total transplanted seedlings.

In the seed year of 2012, mature seeds of fir in late October were randomly collected from five parent trees at 4320 m (treeline, height 12–15 m, DBH 40–50 cm) and 3800 m (distribution center, height 10–14 m, DBH 35–50 cm), respectively. About twenty cones per tree were collected with the help of ladder and lopper. Seeds were obtained from the natural air-drying cones. After measurements of seed size (including length, width and thickness) and 1000-seed mass, the seeds from the two elevations were sowed at N2 and N3 in early November of 2012. At each site, five 1-m² plots per seed source were established around the weather station, at least 5 m apart. There were fifty seeds in a plot, and each seed was marked by a toothpick to avoid mixing up with natural dispersal seeds. During June–August of 2013, the germinated seeds of each plot were marked and recorded once a week. By the end of August, the germination rate of a plot was calculated as the ratio of total germinated seeds to total sowed seeds.

2.4. Microclimate data

Belowground root growth will start later than the aboveground shoot growth because soil warming in spring lagged weeks behind the increased air temperature due to seasonal snow cover and soil thawing (Liu and Luo, 2011). Körner and Paulsen (2004) defined the fully active growing season as the period at which the daily soil temperatures at –10 cm exceeded 3.2 °C in spring and dropped below 3.2 °C for the first time in autumn. The cambium activity of *A. georgei* var. *smithii* trees generally started when a daily mean temperature of 3–5 °C or daily minimum temperature of 1–2 °C was reached (Li and Liang, unpublished data). In this study, we used

the threshold of 3.2 °C to define the beginning and ending of the growing season based on daily mean soil temperature at –5 cm, assuming that the seedlings (rooting depth < 10 cm) are fully active when the –5 cm soil temperature is above this threshold (Liu and Luo, 2011).

The hourly recorded data of air temperature (1 m aboveground) obtained from the four automatic weather stations (2006–2012) were used for calculating the frequency, intensity and duration of growing-season freezing events across the four study sites. The frequency of growing-season freezing event was defined as the number of days with daily minimum air temperature (T_{\min}) < 0 °C during the growing season, and its intensity was defined as the seasonal absolute air temperature minimum (AT_{\min}). The duration of a freezing event was defined as daily time (hours) with the air temperature (T) < 0 °C during the growing season. To characterize the seasonal changes in freezing events, we further calculated the monthly days with T_{\min} < 0 °C over the growing season.

To understand the causes of freezing events, the hourly recorded data from the two automatic weather stations above both treelines were used for analyzing the relationships between daily minimum air temperature and other meteorological factors (wind speed, net radiation, vapor pressure deficit and soil moisture) during the growing season within a site. Vapor pressure deficit, an indicator of the dry condition of air, was calculated from air temperature and relative humidity.

2.5. Statistical analysis

Two-way analysis of variance (ANOVA) in the general linear model (GLM) was used for assessing the differences of the growing-season freezing events and the annual mortality and growth rates of transplanted old-seedlings among slopes (south-facing vs. north-facing) and habitats (forested vs. non-forested), regarding the 5-yr measurements as repetitions. We aim to examine the long-term effects of slope, habitat and the interaction on the freezing events and the transplanted old-seedling mortality and growth rates over the five years.

Partial correlation analysis of multiple linear regression was used for assessing the relative influences of wind speed, net radiation, vapor pressure deficit and soil moisture on daily minimum air temperature, and examining the relative importance of freezing events and soil conditions (soil temperature and moisture) to annual mortality and growth rates of fir and juniper old-seedlings. Our previous study found that soil organic matter and total nitrogen at the depth of 0–20 cm varied little with slopes and habitats in the study area (Kong et al., 2012).

The linear regression ($y = a + bx$) was used for examining the relationships between annual mean air-temperature and the characteristics of growing-season freezing events, and between microclimate factors (freezing events, soil temperature and moisture) and the mortality and growth rates of transplanted seedlings in pooled data across sites and years. Differences of fir seed germination rates and its young-seedling mortality and growth rates at and above the fir treeline were tested by independent samples *t*-test.

All statistical analysis were performed using the SPSS 19 for Windows (SPSS Inc., Chicago, USA), and all significant differences were at $P < 0.05$.

3. Results

3.1. Characteristics of the growing-season freezing events across slopes and habitats

During 2006–2012, the frequency, intensity and duration of growing-season freezing events were generally greater under the

Table 1
Characteristics of the growing-season freezing events at and above treelines across north-facing and south-facing slopes during 2006–2012. N.A., the data were not available because of power failure.

Freezing event Characteristics	Year	Forested sites		Non-forested sites	
		N-slope	S-slope	N-slope	S-slope
Frequency (days with $T_{\min} < 0^{\circ}\text{C}$, d)	2006	0	0	0	59
	2007	N.A.	N.A.	N.A.	49
	2008	4	6	14	N.A.
	2009	12	31	49	65
	2010	1	18	31	63
	2011	9	12	25	57
	2012	0	8	16	44
	Average	4.3 ± 2.1	12.5 ± 4.4	22.5 ± 6.8	56.2 ± 3.3
Intensity (seasonal AT_{\min} , $^{\circ}\text{C}$)	2006	0	0	0	-7.9
	2007	N.A.	N.A.	N.A.	-7.0
	2008	-1.9	-2.7	-8.0	N.A.
	2009	-2.4	-5.4	-8.1	-8.0
	2010	-1.5	-4.3	-7.9	-9.2
	2011	-2.4	-2.4	-8.8	-8.2
	2012	0	-4.23	-7.1	-8.2
	Average	-1.4 ± 0.4	-3.2 ± 0.8	-6.7 ± 1.4	-8.1 ± 0.3
Duration (daily time with $T < 0^{\circ}\text{C}$, h)	2006	0	0	0	10.0
	2007	N.A.	N.A.	N.A.	9.91
	2008	7.5	8.2	6.2	N.A.
	2009	5.8	8.6	10.2	11.9
	2010	5.0	5.1	7.7	11.5
	2011	5.6	7.2	10.4	9.9
	2012	0.0	6.6	7.6	8.9
	Average	4.0 ± 1.3	5.9 ± 1.3	7.0 ± 1.5	10.4 ± 0.5

warmer climate on the south-facing slope (Table 1). For the average frequency, the days with $T_{\min} < 0^{\circ}\text{C}$ differed by 8–9 d between both treeline forests and by 33–34 d between both non-forested sites above the treeline. For the average intensity, the seasonal AT_{\min} was 1.8 $^{\circ}\text{C}$ lower in the south-facing juniper forest than in the north-facing fir forest, and was 1.4 $^{\circ}\text{C}$ lower at the south-facing non-forested site than at the north-facing non-forested site. The average duration of a freezing event was 2–4 h longer at the south-facing sites than at the north-facing sites.

Seasonal patterns of freezing days differed greatly with slopes and habitats (Table 2). In the north-facing fir forest, the freezing days with $T_{\min} < 0^{\circ}\text{C}$ were only found in the late growing season, compared to the frequent occurrences in the early (April–June) and late (September–October) growing seasons across the south-facing juniper forest and the two non-forested sites above the treeline (Table 2). Two-way analysis of variance indicated that slope (south-facing vs. north-facing) and habitat (forested vs. non-forested) showed significant effects on the three variables of freezing events in the early growing season, while only habitat significantly influenced those in the late growing season (Table 3). Slope (39%), habitat (32%) and the interaction (17%) explained most of the variations in the occurrence early-season freezing days.

Across years and non-forested sites above both treelines, annual mean air-temperature was well correlated with early-season freezing events ($R^2 = 0.63\text{--}0.92$, $P < 0.01$), compared to low or insignificant correlations across both treeline forests (Fig. 1a–c). Freezing events in the late growing season did not show a correlation with annual mean air-temperature across both slopes (Fig. 1d–f). Partial correlation analysis indicated that seasonal variations in daily minimum air temperature within a non-forested site above the treeline were mainly determined by net radiation and air humidity (i.e. vapor pressure deficit, except for that in early season at the N-slope site) (Table 4). Additional effects of soil moisture at -5 cm differed with seasons, in which daily minimum air temperature decreased with decreasing soil moisture in the late growing season but increased or varied little in the early growing season (Table 4). Wind speed had minor or unclear effects on daily minimum air temperature.

3.2. Variations in mortality and growth of fir and juniper old-seedlings associated with freezing events

In the fifth year after transplanting, the survival rate of transplanted fir old-seedlings across forested and non-forested habitats decreased to 23–46% on the south-facing slope and to 73% on the north-facing slope (Fig. 2a and b), while the survival rate of transplanted juniper old-seedlings decreased to 54–75% with unclear change trends with slopes and habitats (Fig. 2c and d). Two-way analysis of variance indicated that only slope significantly explained 23% of the variation in annual mortality of fir old-seedlings, while annual mortality of juniper old-seedlings and annual growth rates of both species varied little with slopes and habitats (Table 5).

In pooled data across years and sites, annual mortality increased in fir old-seedlings ($P < 0.05$, Fig. 3a–c) but varied little in juniper old-seedlings (Fig. 3d–f) with increasing freezing events in the early growing season, both showed no correlation with the freezing events in the late growing season (data not shown). Similar patterns were also found in the variations of annual growth rates (Fig. 4). Partial correlation analysis indicated that the early-season freezing event was the major limiting factor determining annual mortality of fir old-seedlings, while the annual growth rate was mainly limited by soil moisture at -20 cm (Table 6). In contrast, annual mortality and growth rates of juniper old-seedlings varied little with all the microclimate factors (Table 6).

3.3. Variations in fir seed germination and its young-seedling growth and mortality at and above the fir treeline

Seed size and 1000-seed mass were generally higher for low-elevation seeds (8.6 mm × 2.9 mm × 1.7 mm and 9.6 g, seed source at 3800 m) than for high-elevation seeds (6.6 mm × 2.2 mm × 1.6 mm and 7.1 g, seed source at 4320 m), but both seed source had similar germination rates in the fir treeline forest (6.4% vs. 7.6%, $P > 0.05$) (Table 7). Under the alpine low-shrubs above the treeline, low-elevation seeds (30.0%) had a much higher germination rate than high-elevation seeds (4.0%).

Table 2

Monthly days with daily minimum air temperature $<0^{\circ}\text{C}$ over the growing season at and above treelines across north-facing and south-facing slopes during 2006–2012. N.A., the data were not available because of power failure.

		April	May	June	July	August	September	October
<i>Forested sites</i>								
N-slope	2006			0	0	0	0	0
	2007			N.A.	N.A.	N.A.	N.A.	N.A.
	2008			0	0	0	0	4
	2009			0	0	0	4	8
	2010			0	0	0	0	1
	2011			0	0	0	0	9
	2012			0	0	0	0	0
S-slope	2006		0	0	0	0	0	0
	2007		N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
	2008		0	0	0	0	0	6
	2009		15	2	0	0	3	11
	2010	3	9	0	0	0	0	6
	2011		4	0	0	0	0	8
	2012		2	0	0	0	0	6
<i>Non forested sites</i>								
N-slope	2006		0	0	0	0	0	0
	2007		N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
	2008		0	3	2	0	0	9
	2009		16	2	0	1	4	25
	2010		4	1	0	0	2	24
	2011		3	0	0	1	0	21
	2012		0	0	0	0	4	12
S-slope	2006	14	17	0	0	0	2	26
	2007	19	11	0	0	0	3	16
	2008	N.A.	N.A.	0	0	0	0	25
	2009	20	17	2	0	0	4	22
	2010	24	15	1	0	0	0	23
	2011	14	18	0	0	0	0	25
	2012	7	18	0	0	0	0	19

Table 3

Summary of GLM-ANOVA for the effects of slope, habitat and the interaction on characteristics of freezing events in early and late growing seasons of 2006–2012.

Variables	df	Days with $T_{\min} < 0^{\circ}\text{C}$			Seasonal AT_{\min}			Daily time with $T < 0^{\circ}\text{C}$		
		SS	F	%SS	SS	F	%SS	SS	F	%SS
Early growing season (April–June)^a										
Slope	1	1802.667	68.33***	39.49	68.919	32.20***	38.79	135.727	25.72***	38.29
Habitat	1	1441.500	54.64***	31.57	59.252	27.69***	33.35	107.637	20.40***	30.37
Slope \times habitat	1	793.500	30.08***	17.39	6.668	3.12	3.75	5.524	1.05	1.56
Error	20	527.667		11.56	42.805		24.09	105.530		29.78
Late growing season (September–October)^b										
Slope	1	112.667	2.49	4.88	8.473	2.09	3.24	44.827	4.09	12.48
Habitat	1	1261.500	27.88***	54.67	171.628	42.30***	65.69	95.122	8.68***	26.48
Slope \times habitat	1	28.167	0.62	1.22	0.010	0.00	0.00	0.077	0.01	0.02
Error	20	905.000		39.22	81.141		31.06	219.219		61.02

df, degree of freedom; SS, sum of squares; %SS, % of variation explained by the main factors and the interactions.

^a In early growing season, overall model for days with $T_{\min} < 0^{\circ}\text{C}$, $R^2 = 0.88$, $F = 51.01$, $P < 0.001$; for seasonal AT_{\min} , $R^2 = 0.76$, $F = 21.00$, $P < 0.001$; for daily time with $T < 0^{\circ}\text{C}$, $R^2 = 0.72$, $F = 15.72$, $P < 0.001$.

^b In late growing season, overall model for days with $T_{\min} < 0^{\circ}\text{C}$, $R^2 = 0.61$, $F = 10.33$, $P < 0.001$; for seasonal AT_{\min} , $R^2 = 0.69$, $F = 14.80$, $P < 0.001$; for daily time with $T < 0^{\circ}\text{C}$, $R^2 = 0.39$, $F = 4.26$, $P < 0.05$.

*** $P < 0.001$.

Table 4

Partial correlation coefficients of multiple linear regressions for relationships of daily minimum air temperature to wind speed, net radiation, vapor pressure deficit, and soil moisture (-5 cm) within a non-forested site above treeline. The hourly recorded data in the growing seasons of 2006–2012 were obtained from two automatic weather stations, each placed above one of the treelines (sample size in early growing season (April–June): N-slope, $n = 174$; S-slope, $n = 245$; in late growing season (September–October): N-slope, $n = 219$; S-slope, $n = 234$).

Independent variables	Early growing season		Late growing season	
	N-slope	S-slope	N-slope	S-slope
Wind speed	0.11 ^{NS}	0.10 ^{NS}	0.13*	-0.15*
Net radiation	0.33***	0.47***	0.14*	0.33***
Vapor pressure deficit	-0.02 ^{NS}	-0.28***	-0.25***	-0.23***
Soil moisture (-5 cm)	-0.20***	0.11 ^{NS}	0.13*	0.38***

NS, no significance.

* $P < 0.05$.

*** $P < 0.001$.

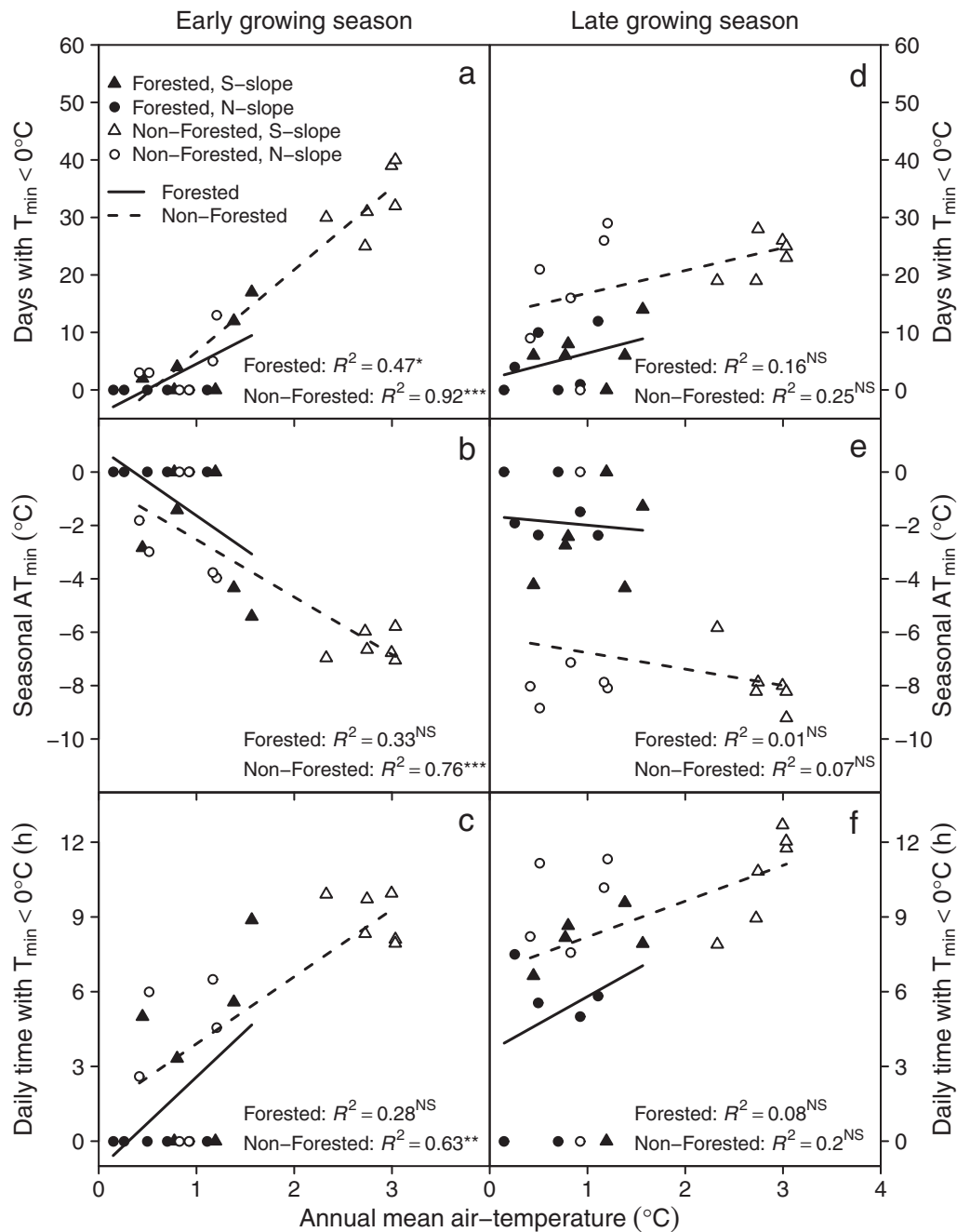


Fig. 1. Relationships between annual mean air-temperature (3 m aboveground) and the characteristics of freezing events in the early (a–c) and late (d–f) growing seasons across years, slopes and habitats.

However, the germination rate of high-elevation seeds did not significantly differ between sites at and above the treeline (Table 7).

In the third year after transplanting, the total mortality of fir young-seedlings was significantly higher in alpine shrubland (85.7%) than in treeline forest (41.4%), and the opposite trend was found in annual growth rate (Table 7).

4. Discussion

4.1. Increased early-season freezing events under warmer climate at treeline ecotones

Compared to lowland area, the radiative cooling at calm, clear and dry nights is more significant at high elevations because the air is more clean and less dense (Barry, 2008). Then freezing events

may commonly occur in the early and late growing season across alpine ecosystems (Mayr et al., 2012; Neuner and Hacker, 2012). Above treeline, the spatial pattern of microclimate forms a complex mosaic according to topographical differences in radiation, wind exposure, depth of snow cover, and plant stature and coverage (Barry, 2008). In this study, there was a great difference in snow pack depth between north-facing (80–100 cm) and south-facing (20–30 cm) slopes. In high mountains, a south-facing slope generally receives more radiation and then has higher net radiation and drier conditions of air and soil than a north-facing slope (Barry, 2008; Liu and Luo, 2011). On those clear nights (lack of downwelling cloud radiation), the surface air with low humidity absorbs less long-wave radiation from the ground, resulting in lower daily minimum temperature and higher number of freezing events in the growing season on the south-facing slope than on the

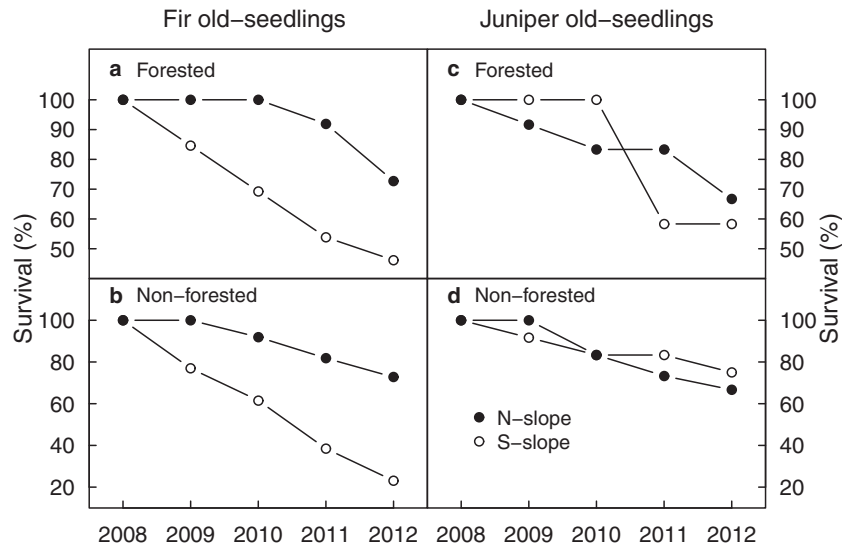


Fig. 2. Accumulated survival rates of transplanted old-seedlings for fir (a and b) and juniper (c and d) across forested and non-forested habitats at and above treelines on the north-facing and south-facing slopes during 2008–2012.

Table 5

Summary of GLM-ANOVA for the effects of slope, habitat and the interaction on the annual mortality and growth rates of transplanted fir and juniper old-seedlings during 2008–2012.

Variables	df	Annual mortality			Annual growth rate		
		SS	F	%SS	SS	F	%SS
Fir old-seedlings^a							
Slope	1	290.503	5.12*	23.21	1.265	1.98	9.78
Habitat	1	26.627	0.47	2.13	0.734	1.15	5.68
Slope × habitat	1	26.627	0.47	2.13	0.696	1.09	5.38
Error	16	908.015		72.54	10.243		79.18
Juniper old-seedlings^b							
Slope	1	3.472	0.027	0.17	5.440	2.46	13.29
Habitat	1	31.250	0.247	1.51	0.005	0.00	0.01
Slope × habitat	1	3.472	0.027	0.17	0.054	0.02	0.13
Error	16	2027.778		98.15	35.419		86.56

df, degree of freedom; SS, sum of squares; %SS, % of variation explained by the main factors and the interactions.

^a For fir old-seedlings, overall model for annual mortality, $R^2 = 0.28$, $F = 2.02$, $P = 0.152$; for annual growth rate, $R^2 = 0.21$, $F = 1.40$, $P = 0.278$.

^b For juniper old-seedlings, overall model for annual mortality, $R^2 = 0.02$, $F = 0.10$, $P = 0.959$; for annual growth rate, $R^2 = 0.13$, $F = 0.83$, $P = 0.498$.

* $P < 0.05$.

Table 6

Partial correlation coefficients of multiple linear regressions for relationships of annual mortality and growth rates of transplanted fir and juniper old-seedlings to microclimate factors of early growing-season freezing events, soil temperature and moisture during 2006–2012 (sample size: $n = 20$).

Independent variables	Annual mortality		Annual growth rate	
	Fir	Juniper	Fir	Juniper
Days with $T_{\min} < 0^\circ\text{C}$	0.59**	0.04	0.41	0.19
Soil temperature (–5 cm)	0.04	0.30	0.29	–0.02
Soil moisture (–5 cm)	0.13	0.24	0.15	–0.11
Seasonal AT_{\min}	–0.42	0.25	0.39	–0.15
Soil temperature (–5 cm)	–0.10	0.34	0.38	–0.06
Soil moisture (–5 cm)	0.06	0.06	0.11	–0.11
Daily time with $T < 0^\circ\text{C}$	0.54*	–0.17	–0.22	0.12
Soil temperature (–5 cm)	–0.23	0.33	0.35	–0.07
Soil moisture (–5 cm)	0.13	0.12	0.21	–0.13
Days with $T_{\min} < 0^\circ\text{C}$	0.62**	–0.01	–0.07	–0.13
Soil temperature (–20 cm)	–0.17	0.17	–0.05	0.07
Soil moisture (–20 cm)	0.26	0.14	0.39	–0.43
Seasonal AT_{\min}	–0.42	0.41	0.10	–0.01
Soil temperature (–20 cm)	–0.22	0.41	0.01	0.01
Soil moisture (–20 cm)	–0.10	0.06	0.48*	0.43
Daily time with $T < 0^\circ\text{C}$	0.52*	–0.25	0.05	0.06
Soil temperature (–20 cm)	–0.29	0.30	–0.10	–0.02
Soil moisture (–20 cm)	–0.19	0.16	0.52*	–0.44

* $P < 0.05$.

** $P < 0.001$.

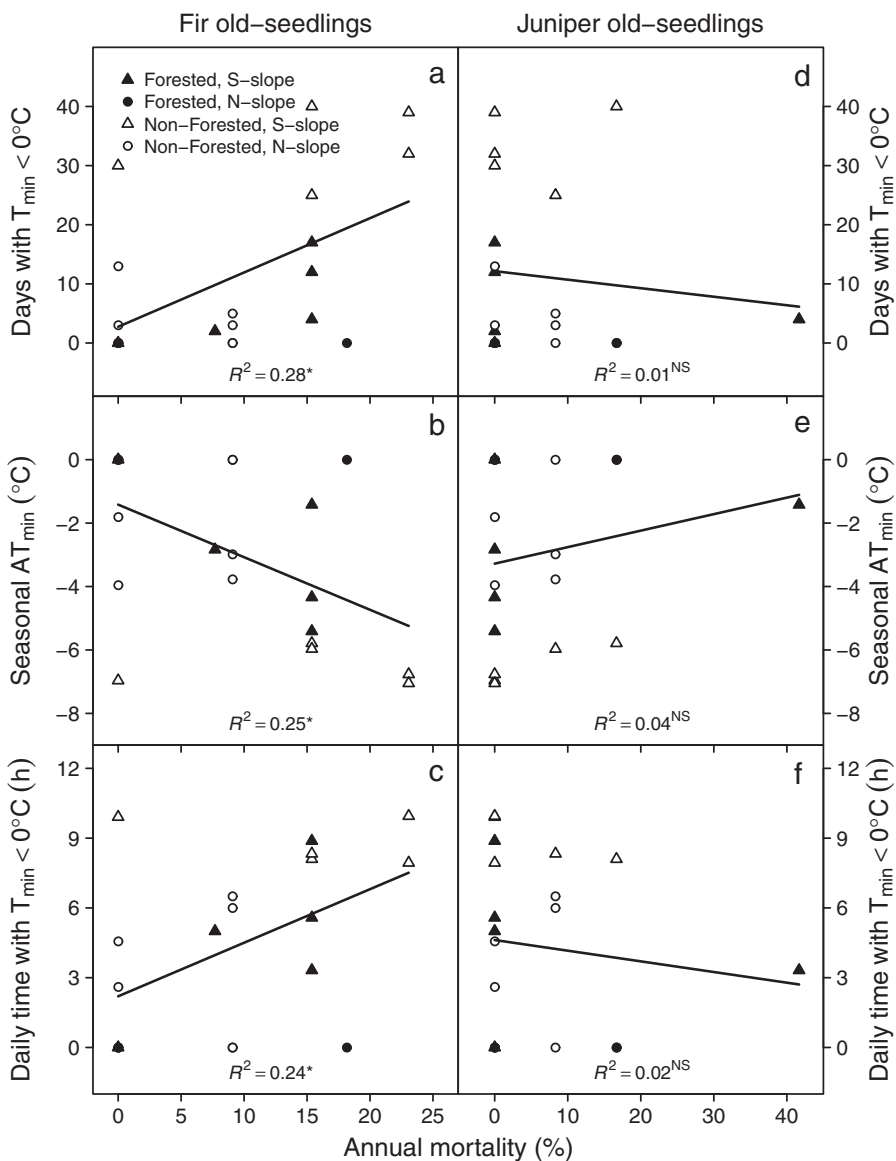


Fig. 3. Relationships between annual mortality and the number of freezing events in the early growing season for transplanted fir (a–c) and juniper (d–f) old-seedlings across sites and years.

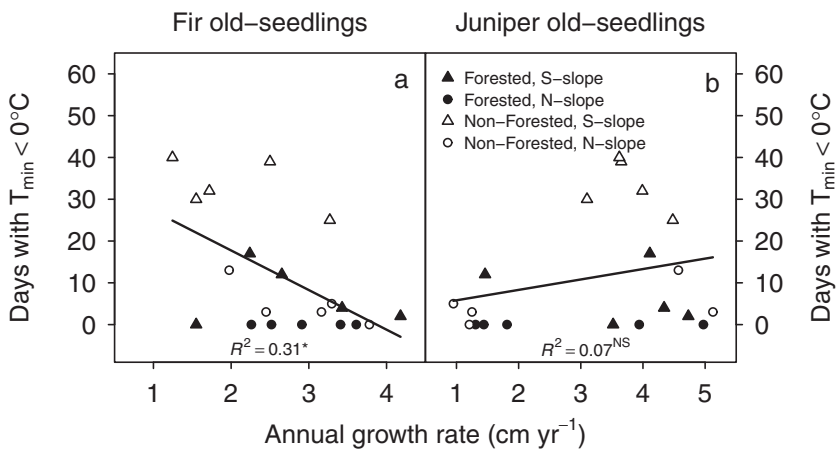


Fig. 4. Relationships between annual growth rate and the number of freezing days in the early growing season for transplanted fir (a) and juniper (b) old-seedlings across sites and years.

Table 7

Differences in germination rate of fir seeds and the mortality and growth rate of fir young-seedlings at and above the fir treeline (mean \pm SE). Different letters of low case within a row indicate the significant difference between habitats at $P < 0.05$. Different letters of upper case within a column indicate the significant difference between two seed source at $P < 0.05$.

Measure variables	Treeline forest	Alpine shrubland
Germination experiment of fir seeds (2012.11–2013.8)		
Seeds from 3800 m (%)	6.40 \pm 4.12 ^{aA}	30.00 \pm 7.13 ^{bA}
Seeds from 4320 m (%)	7.60 \pm 4.66 ^{aA}	4.00 \pm 2.19 ^{aB}
Transplant experiment of fir young-seedlings (2011.6–2013.8)		
3-yr total mortality (%)	41.43 \pm 9.37 ^a	85.71 \pm 6.85 ^b
Annual growth rate (mm yr ⁻¹)	9.31 \pm 0.70 ^a	5.47 \pm 0.59 ^b

north-facing slope (Tables 1–3). Temperature extremes were less under a closed forest canopy than in an open non-forested habitat (Table 1) because of reduced convective heat exchange with the air above the canopy. Within a non-forested site above treeline, seasonal variations in daily minimum air temperature were mainly determined by net radiation and the dry condition of air (Table 4). Because of snow melting and soil thawing in the early growing season, daily minimum air temperature decreased with increasing soil moisture, compared to the positive correlation in the late growing season (Table 4). Then both temporal and spatial variations in freezing events would be explained by the energy balance principles.

The variation of extreme temperature can be more important to alpine plant survival and distribution than that of mean temperature (Katz and Brown, 1992; Karl and Easterling, 1999; Inouye, 2008). It has been predicted that the probability of frost events may increase under future global warming (Inouye, 2000; IPCC, 2007). On the Tibetan Plateau, the frequency of extreme temperatures has increased over the last 45 years (1961–2005) associated with increased summer and winter temperatures (You et al., 2008). However, the direct evidence for increased growing-season freezing events with climate warming at and above alpine treelines is still rare. At the treeline in the Swiss Alps, climate data of 1975–2010 indicate a warming trend of summer temperature, which is associated with earlier snow melt and constant trend in the number of freezing events during the growing season (Rixen et al., 2012). The warming-induced increase of growing-season freezing events has been observed in a short-term warming experiment in the central Chilean Andes (Sierra-Almeida and Cavieres, 2010).

Predictions relying on space-for-time substitution can be well consistent with the time-for-time predictions when the temporal variation in climate is similar to the spatial variation (Blois et al., 2013). In the Sergyemal Mountains, annual precipitation was similar but annual mean air-temperature above the treeline differed by 2.0 °C between south-facing and north-facing slopes. This is comparable to the temporal difference of 2.3 °C between the warmest and coldest years and the unchanged trend of precipitation during 1960–2008 at Nyingchi station nearby the study sites. In this study, we found that there were more severe growing-season freezing events under the warmer climate on the south-facing slope. The number of freezing events in the early growing season increased with increasing annual mean air-temperature across non-forested sites and years (Fig. 1). Our data suggest that the number of early-season freezing events at alpine treeline ecotones may increase under a warmer climate, which can affect the establishment of tree seedlings above the treeline.

4.2. The reason why the fir treeline position did not advance with climatic warming

In the past 100 years, 47% of worldwide treelines did not advance in response to global warming (Harsch et al., 2009). To explain the unconformity between treeline dynamics and global

warming, Harsch and Bader (2011) suggested three general ‘first level’ mechanisms (growth limitation, seedling mortality and dieback) that determine treeline forms and dynamics. In theory, the treeline forms controlled by dieback and seedling mortality are relatively unresponsive to climatic warming (Harsch and Bader, 2011). Numerous studies have suggested that the lack of suitable habitats can impede seedling establishment above alpine treelines (Cuevas, 2001; Smith et al., 2003; Juntunen and Neuvonen, 2007; Battlori et al., 2009; Holtmeier and Broll, 2011; Dufour-Tremblay et al., 2012; Harsch et al., 2012). However, there are few field data documenting the limiting environmental factors that lead to seedling mortality directly.

Our transplant experiments of old- and young-seedlings indicated that the survival of seed-based fir seedlings transplanted to the warmer climate on the south-facing slope and at non-forested sites above both treelines were strongly limited by the freezing events in the early growing season (Figs. 2 and 3, Tables 6 and 7). This can explain why the seed-regenerated fir saplings and trees are excluded from the south-facing juniper treeline and non-forested sites above the treeline (Liu and Luo, 2011). Körner (2012) suggested that freezing events occurring in the transition period from dormancy to active growth are much more critical to the seedling survival at and above alpine treelines, in which most plants would freeze below -1.8 °C. In this study, the average seasonal absolute air temperature minimum (AT_{\min}) ranged from -8.1 ± 0.3 °C to -3.2 ± 0.8 °C on the south-facing slope and at non-forested sites above both treelines, and was -1.4 ± 0.4 °C at the fir treeline (Table 1). Therefore, freezing temperature in the early growing season may lead to physical damage of seedling leaves and new shoots, and consequently decrease the carbon gain and height growth and further increase their sensitivity to other environmental factors (Cavieres et al., 2000; Awada et al., 2003; Johnson et al., 2004; Coop and Givnish, 2008; Rixen et al., 2012). On the other hand, high light intensity at non-forested sites above the treeline may be adverse to the fir seedling survival due to low-temperature photoinhibition (Bader et al., 2007; Germino and Smith, 1999; Slot et al., 2005).

In contrast, the survival of transplanted juniper old-seedlings varied little with slopes and habitats, suggesting that the difference of microclimate conditions may not be the main factor that causes the juniper seedling mortality. Since the juniper seedlings generally regenerate from the basal trunk and root bud (Zhang et al., 2010), the transplanted seedling mortality might be due to lack of nutrition and water supply from their parent trees. This suggests that juniper seedlings can live on their parent trees, and the dynamics of juniper treeline mainly depends on the horizontally growing roots under the ground.

The amount and quality of seeds generally decrease with increasing altitude, which may be another control to treeline dynamics (Holtmeier, 2009). For *A. georgei* var. *smithii*, the field data of seed germination experiment with seed source of low and high elevations indicated that harsh environments above the treeline did not limit fir seed germination though the quality of seed source at treeline was lower (Table 7). There is evidence that the population density of fir treeline stands has increased under the recent 60-year warming (Liang et al., 2011), suggesting that seed availability and germination may not be the bottleneck of treeline change in this study area. Also, seed dispersal may not limit the fir treeline position because we observed that the young seedlings (<10 cm in height with ages of 3–5 yr) growing under alpine shrubs were found up to 4360 m in elevation (40 m higher above the fir treeline).

5. Conclusions

Our transplant experiments provided evidence that the warmer climate on the south-facing slope and at non-forested sites above

treeline generally had more severe growing-season freezing events, and the seed-based fir seedlings were especially vulnerable to the increased freezing events in the early growing season. This can explain the cause for the unique distribution pattern of fir and juniper treelines on opposite slopes of a valley in the Sergyemla Mountains (Liu and Luo, 2011), and suggests an explanation for the phenomenon that the fir treeline position did not advance with climate warming in past 200 years (Liang et al., 2011). Also, such knowledge might be important to understand why almost half of 130 alpine/boreal treelines did not advance in response to global warming (Harsch et al., 2009).

Acknowledgements

We thank XX Li and EY Liang for communication of their unpublished data, and the Southeast Tibet Station for Alpine Environment Observation and Research, Chinese Academy of Sciences for their help in the filed work. This work was funded by the National Key Projects for Basic Research of China (2010CB951301), the Strategic Priority Research Program (B) of the Chinese Academy of Sciences (XDB03030402), and the National Natural Science Foundation of China (40901038, 40671069).

References

- Awada, T., Radoglou, K., Fotelli, M.N., Constantinidou, H.I.A., 2003. Ecophysiology of seedlings of three Mediterranean pine species in contrasting light regimes. *Tree Physiology* 23, 33–41.
- Bader, M.Y., van Geloof, I., Rietkerk, M., 2007. High solar radiation hinders tree regeneration above the alpine treeline in northern Ecuador. *Plant Ecology* 191, 33–45.
- Barbeito, I., Dawes, M.A., Rixe, C., Senn, J., Bebi, P., 2012. Factors driving mortality and growth at treeline: a 30-year experiment of 92,000 conifers. *Ecology* 93, 389–401.
- Barry, R.G., 2008. *Mountain Weather and Climate*. Cambridge University Press, Cambridge.
- Batllori, E., Camarero, J.J., Ninot, J.M., Gutierrez, E., 2009. Seedling recruitment, survival and facilitation in alpine *Pinus uncinata* tree line ecotones: implications and potential responses to climate warming. *Global Ecology and Biogeography* 18, 460–472.
- Blois, J.L., Williams, J.W., Fitzpatrick, M.C., Jackson, S.T., Ferrier, S., 2013. Space can substitute for time in predicting climate-change effects on biodiversity. *Proceedings of the National Academy of Sciences* 110, 9374–9379.
- Cavieres, L.A., Rada, F., Azócar, A., García-Núñez, C., Cabrera, H.M., 2000. Gas exchange and low temperature resistance in two tropical high mountain tree species from the Venezuelan Andes. *Acta Oecologica* 21, 203–211.
- Coop, J.D., Givnish, T.J., 2008. Constraints on tree seedling establishment in montane grasslands of the Valles Caldera, New Mexico. *Ecology* 89, 1101–1111.
- Cuevas, J.G., 2001. Tree recruitment at the *Nothofagus pumilio* alpine timberline in Tierra del Fuego, Chile. *Journal of Ecology* 88, 840–855.
- Dufour-Tremblay, G., De Vriendt, L., Lévesque, E., Boudreau, S., 2012. The importance of ecological constraints on the control of multi-species treeline dynamics in eastern Nunavik, Québec. *American Journal of Botany* 99, 1638–1646.
- Germine, M.J., Smith, W.K., 1999. Sky exposure, crown architecture, and low-temperature photoinhibition in conifer seedlings at alpine treeline. *Plant Cell and Environment* 22, 407–415.
- Harsch, M.A., Bader, M.Y., 2011. Treeline form – a potential key to understanding treeline dynamics. *Global Ecology and Biogeography* 20, 582–596.
- Harsch, M.A., Hulme, P.E., McGlone, M.S., Duncan, R.P., 2009. Are treelines advancing? A global meta-analysis of treeline response to climate warming. *Ecology Letters* 12, 1040–1049.
- Harsch, M.A., Buxton, R., Duncan, R.P., Hulme, P.E., Wardle, P., Wilmshurst, J., 2012. Causes of tree line stability: stem growth, recruitment and mortality rates over 15 years at New Zealand *Nothofagus* tree lines. *Journal of Biogeography* 39, 2061–2071.
- Holtmeier, F.K., 2009. *Mountain Timberlines: Ecology, Patchiness, and Dynamics*. Springer, New York.
- Holtmeier, F.K., Broll, G., 2011. Response of Scots pine (*Pinus sylvestris*) to warming climate at its altitudinal limit in northernmost subarctic Finland. *Arctic* 64, 269–280.
- IPCC, 2007. *The Physical Science Basis Working Group I Contribution to the Fourth Assessment Report of the IPCC Intergovernmental Panel on Climate Change*. Cambridge University, Cambridge, UK.
- Inouye, D.W., 2000. The ecological and evolutionary significance of frost in the context of climate change. *Ecology Letters* 3, 457–463.
- Inouye, D.W., 2008. Effects of climate change on phenology, frost damage, and floral abundance of montane wildflowers. *Ecology* 89, 353–362.
- Johnson, D.M., Germine, M.J., Smith, W.K., 2004. Abiotic factors limiting photosynthesis in *Abies lasiocarpa* and *Picea engelmannii* seedlings below and above the alpine timberline. *Tree Physiology* 24, 377–386.
- Juntunen, V., Neuvonen, S., 2007. Natural regeneration of Scots pine and Norway spruce close to the timberline in northern Finland. *Silva Fennica* 40, 443–458.
- Katz, R.W., Brown, B.G., 1992. Extreme events in a changing climate: variability is more important than averages. *Climatic Change* 21, 289–302.
- Karl, T.R., Easterling, D.R., 1999. Climate extremes: selected review and future research directions. *Climatic Change* 42, 309–325.
- Kong, G.Q., Luo, T.X., Liu, X.S., Zhang, L., Liang, E.Y., 2012. Annual ring widths are good predictors of changes in net primary productivity of alpine *Rhododendron* shrubs in the Sergyemla Mountains, southeast Tibet. *Plant Ecology* 213, 1843–1855.
- Körner, C., Paulsen, J., 2004. A world-wide study of high altitude treeline temperatures. *Journal of Biogeography* 31, 713–732.
- Körner, C., 2012. *Alpine Treelines: Functional Ecology of the Global High Elevation Tree Limits*. Springer, Basel.
- Kullman, L., 2007. Treeline population monitoring of *Pinus sylvestris* in the Swedish Scandes, 1973–2005: implications for tree line theory and climate change ecology. *Journal of Ecology* 95, 41–52.
- Larcher, W., Kainmüller, C., Wagner, J., 2010. Survival types of high mountain plants under extreme temperatures. *Flora* 205, 3–18.
- Li, R.C., Luo, T.X., Tang, Y.H., Du, M.Y., Zhang, X.Z., 2013. The altitudinal distribution center of a widespread cushion species is related to an optimum combination of temperature and precipitation in the central Tibetan Plateau. *Journal of Arid Environments* 88, 70–77.
- Liang, E.Y., Shao, X.M., Xu, Y., 2009. Tree-ring evidence of recent abnormal warming on the southeast Tibetan Plateau. *Theoretical and Applied Climatology* 98, 9–18.
- Liang, E.Y., Eckstein, D., 2009. Dendrochronological potential of the alpine shrub *Rhododendron nivale* on the south-eastern Tibetan Plateau. *Annals of Botany* 104, 665–670.
- Liang, E.Y., Wang, Y.F., Eckstein, D., Luo, T.X., 2011. Little change in the fir treeline position on the southeastern Tibetan Plateau after 200 years of warming. *New Phytologist* 190, 760–769.
- Liu, X.S., Luo, T.X., 2011. Spatiotemporal variability of soil temperature and moisture across two contrasting timberline ecotones in the Sergyemla Mountains, southeast Tibet. *Arctic, Antarctic, and Alpine Research* 43, 229–238.
- Mayr, S., Schmid, P., Beikircher, B., 2012. Plant water relations in alpine winter. In: Lütz, C. (Ed.), *Plants in Alpine Regions: Cell Physiology of Adaptation and Survival Strategies*. Innsbruck, Springer Wien New York, pp. 153–162.
- Miehe, G., Miehe, S., Vogel, J., Co, S., Duo, L., 2007. Highest treeline in the northern hemisphere found in southern Tibet. *Mountain Research and Development* 27, 169–173.
- Neuner, G., Hacker, J., 2012. Ice formation and propagation in alpine plants. In: Lütz, C. (Ed.), *Plants in Alpine Regions: Cell Physiology of Adaptation and Survival Strategies*. Springer Wien New York, Innsbruck, pp. 163–174.
- Opgenoorth, L., Vendramin, G.G., Mao, K.S., Miehe, G., Miehe, S., Liepelt, S., Liu, J.Q., Ziegenhagen, B., 2010. Tree endurance on the Tibetan Plateau marks the world's highest known tree line of the Last Glacial Maximum. *New Phytologist* 185, 332–342.
- Rixen, C., Dawes, M.A., Wipf, S., Hagedorn, F., 2012. Evidence of enhanced freezing damage in treeline plants during six years of CO₂ enrichment and soil warming. *Oikos* 121, 1532–1543.
- Sakai, A., Larcher, W., 1987. *Frost Survival of Plants: Responses and Adaptation to Freezing Stress*. Springer-Verlag, Berlin.
- Sierra-Almeida, A., Cavieres, L.A., 2010. Summer freezing resistance decreased in high-elevation plants exposed to experimental warming in the central Chilean Andes. *Oecologia* 163, 267–276.
- Slot, M., Wirth, C., Schumacher, J., Mohren, G.M.J., Shibistova, O., Lloyd, J., Ensminger, I., 2005. Regeneration patterns in boreal Scots pine glades linked to cold-induced photoinhibition. *Tree Physiology* 25, 1139–1150.
- Smith, W.K., Germine, M.J., Hancock, T.E., Johnson, D.M., 2003. Another perspective on altitudinal limits of alpine timberlines. *Tree Physiology* 23, 1101–1112.
- Taschler, D., Neuner, G., 2004. Summer frost resistance and freezing patterns measured in situ in leaves of major alpine plant growth forms in relation to their upper distribution boundary. *Plant, Cell and Environment* 27, 737–746.
- Wang, Y.F., Camarero, J.J., Luo, T.X., Liang, E.Y., 2012. Spatial patterns of Smith fir alpine treelines on the south-eastern Tibetan Plateau support that contingent local conditions drive recent treeline patterns. *Plant Ecology and Diversity* 5, 311–321.
- Wieser, G., Tausz, M., 2007. *Trees at Their Upper Limit: Treeline Limitation at the Alpine Timberline*. Springer, Dordrecht, The Netherlands.
- You, Q.L., Kang, S.C., Pepin, N., Yan, Y.P., 2008. Relationship between trends in temperature extremes and elevation in the eastern and central Tibetan Plateau, 1961–2005. *Geophysical Research Letters* 35, L04704, <http://dx.doi.org/10.1029/2007GL032669>.
- Zhang, L., Luo, T.X., Liu, X.S., Kong, G.Q., 2010. Altitudinal variations in seedling and sapling density and age structure of timberline tree species in the Sergyemla Mountains, southeast Tibet. *Acta Ecologica Sinica* 30, 76–80.