Performance of germinating tree seedlings below and above treeline in the Swiss Alps

Journal Article

Author(s):
Zurbriggen, Natalie; Hättenschwiler, Stephan; Frei, Eva S.; Hagedorn, Frank; Bebi, Peter

Performance of germinating tree seedlings below and above treeline in the Swiss Alps

Natalie Zurbriggen · Stephan Hättenschwiler ·
Eva S. Frei · Frank Hagedorn · Peter Bebi

Abstract The germination and early survival of tree seedlings is a critical process for the understanding of treeline dynamics with ongoing climate change. Here we analyzed the performance of 0–4 year-old seedlings of seven tree species at three sites above and below the current altitudinal treeline in the Swiss Central Alps near Davos. Starting from sown seeds, we monitored the seedling performance as proportions of living seedlings, seedling shoot height growth, and biomass allocation over 4 years to examine changes along an elevational gradient. We evaluated the relative importance of the environmental factors soil temperature, light conditions, water use efficiency, and nitrogen availability on seedling performance.

During the 4 years, the proportions of living seedlings differed only slightly along the elevational gradient even in species currently occurring at lower elevations. Microsite-specific soil temperature and light availability had only little effect on the proportion of living seedlings and seedling biomass across the elevational gradient. Conversely, seedling biomass and biomass allocation correlated well with the foliar stable nitrogen isotope abundance ($\delta^{15}N$) that was used as an indicator for nitrogen availability. Collectively, our results suggested that the early establishment of seedlings of a variety of tree species in the treeline ecotone was not limited by current climatic conditions even beyond the species' actual upper distribution limit. Nitrogen dynamics appeared to be an important environmental co-driver for biomass production and allocation in very young tree seedlings.

N. Zurbriggen (✉)
WSL Swiss Federal Institute for Forest,
Snow and Landscape Research, Zürcherstrasse 111,
8903 Birmensdorf, Switzerland
e-mail: natalie.zurbriggen@wsl.ch

N. Zurbriggen
Forest Ecology, Department of Environmental Sciences,
Swiss Federal Institute of Technology ETH,
Universitätstrasse 22, 8092 Zürich, Switzerland

S. Hättenschwiler
Centre of Functional Ecology and Evolution,
CEFE-CNRS, 1919 route de Mende,
34293 Montpellier Cedex 5, France

E. S. Frei
Agrofutura, Agronomy, Economy & Ecology,
Ackerstrasse, Postfach, 5070 Frick, Switzerland

F. Hagedorn
WSL Swiss Federal Institute for Forest, Snow and
Landscape Research, Zürcherstrasse 111, 8903
Birmensdorf, Switzerland

P. Bebi
WSL Institute for Snow and Avalanche Research SLF,
Flüelastrasse 11, 7260 Davos Dorf, Switzerland

Introduction

Many alpine treeline ecotones have shown upward elevational shifts as a result of climate warming (see reviews by Holtmeier and Broll 2005; Harsch et al. 2009). Further treeline advances are anticipated because, in the absence of large-scale natural disturbances or human land use, low temperature appears to be the primary cause of high altitude treeline formation globally (Körner and Paulsen 2004). In the Swiss Alps, where temperature has increased more rapidly than the global average (Beniston et al. 1997), the treeline might advance at higher rates than expected based on the global average. It has been suggested that many treelines may currently be below their potential maximum elevation even in the absence of anthropogenic impacts, and their reported advances may lag behind current climate warming rates (Holtmeier 1994; Harsch et al. 2009). During these lag phases, factors other than temperature may be important for treeline dynamics at regional and local scales (Holtmeier and Broll 2005; Danby and Hik 2007).

The establishment of seedlings (≤10-year-old trees, sensu Smith et al. 2003) and their performance during early years are key processes determining shifts in treeline positions (Germino et al. 2002; Smith et al. 2009), and it is essential to analyze seedling establishment, survival, and growth in combination with various environmental factors to be able to understand the underlying mechanisms of treeline dynamics. Seedlings, moreover, respond differently to environmental drivers compared to upright trees in later stages of development because they are less coupled to atmospheric conditions than upright trees (Wilson et al. 1987) and often have different sensitivities to environmental factors (Hofgaard et al. 2009; Bansal and Germino 2010). Numerous studies have investigated how seedlings of treeline species respond to changes in a variety of environmental factors, such as soil temperature (e.g., Domisch et al. 2002; Peng and Dang 2003; Juntunen and Neuvonen 2006), exposure to light (e.g., Johnson and Smith 2005), nitrogen

availability (e.g., Sveinbjörnsson et al. 1992; Kabeya 2010), drought (e.g., Hättenschwiler and Körner 1995), competition and facilitation (e.g., Moir et al. 1999; Germino et al. 2002; Batllori et al. 2009; Venn et al. 2009), and herbivory (e.g., Myster 2009). While interactions between several of these factors have been studied before (e.g., Domisch et al. 2002; Myster 2009; Munier et al. 2010), there are only few studies on the relative importance of multiple factors (e.g., Kabeya 2010; Barbeito et al. 2012). In the present study, we measured proxies for four of these important factors—soil temperature, light conditions, water use efficiency, and nitrogen availability—and evaluated their relative importance for seedling performance of seven tree species at and above the current treeline.

Analyses of stable carbon and nitrogen isotopes ($\delta^{13}C$, $\delta^{15}N$) can provide an integrated measure of water use efficiency and plant nitrogen availability (Farquhar and Richards 1984; Craine et al. 2009; Resco et al. 2011). However, foliar $\delta^{13}C$ is also influenced by temperature, atmospheric pressure, and higher carboxylation efficiency at higher elevations, and is, therefore, expected to increase with elevation (Körner et al. 1991; Körner 2003), making causal interpretations difficult. Foliar $\delta^{15}N$ has been shown to be closely linked to soil N availability (Garten and Van Miegroet 1994; Craine et al. 2009) where $\delta^{15}N$ values decrease with tighter N cycles, characterized by stronger microbial N immobilization, a smaller and more available soil N pool, and higher plant N-uptake rates via mycorrhizal associations (Hobbie et al. 2000; Hobbie and Colpaert 2003).

Our analysis of early species-specific seedling performance, over several years and along an elevational gradient, aims at a more detailed understanding of the key processes determining the position of the upper treeline and of potential future species-specific elevational shifts. Seeds of seven regionally important tree species were sown at three sites along an elevational transect, and seedlings were monitored over four growing seasons. We hypothesized that (1) measures of seedling performance—proportions of living seedlings, and seedling growth and biomass—should be higher at sites at or below the current treeline than above, and that such performance differences among sites should reflect (2) the species' current elevational ranges, and (3) local environmental factors such as nitrogen or light availability, in addition to soil temperature.

Methods

Study area and tree species

We conducted the study across an elevational gradient (1,990–2,400 m a.s.l.) on a NE-exposed slope of the Dischma Valley near Davos (Switzerland). The transect crosses the Stillberg treeline research site, where the treeline is currently at 2,100 m a.s.l. (Barbeito et al. 2012). At the meteorological station Stillberg, at 2,090 m a.s.l., the annual mean air temperature is 2.0 °C and the annual precipitation is 1,150 mm (years 1975–2005). The average snow cover lasts from mid October to the end of May, with a difference of 1–2 weeks in snowmelt date between the highest and lowest site of the transect (Walder 1983). The growing season lasts (on average) from end of May to end of September (Rixen et al. 2012). The local treeline ecotone is characterized by a Larch-Swiss stone pine forest (*Larici-Pinetum cembrae*) with dwarf shrubs on siliceous (Paragneis) bedrock (Schönenberger 1975).

Seeds of the tree species *Alnus viridis* (Chaix) DC., *Larix decidua* L., *Picea abies* (L.) Karst, *Pinus cembra* L., *Pinus mugo* ssp. *uncinata* Ramond, *Pinus sylvestris* L., and *Sorbus aucuparia* L. were used; all of which occur naturally in close vicinity of the study area, but have different elevational ranges and thus are thought to represent a range of temperature sensitivities: *P. cembra*, *L. decidua*, and *P. mugo* are treeline species, whereas *P. abies*, *P. sylvestris*, *S. aucuparia*, and *A. viridis* have lower elevational ranges (Table 1; Brändli 1998). The seeds were obtained from natural populations (one population per species) from the Swiss Central Alps in the upper elevational range of each species (Table 1), and the numbers of seeds sown per plot were adjusted to the expected species-specific viability rates based on greenhouse germination tests (A. Burkart, pers. comm.). Due to the low initial germination success of *P. cembra* and *S. aucuparia* in the first year of the experiment, pre-germinated seedlings of these two species were added in the second year.

Experimental design

The elevational transect was set up in May 2007, with one site each at 1,990 m (ca. 100 m below the current treeline), 2,220 m (ca. 100 m above the current treeline), and 2,400 m a.s.l. (ca. 300 m above the current treeline). All sites were in areas with similar microtopography in treeless alpine vegetation. The three sites were outside forested areas and without direct shading from trees, but with varying densities and heights of surrounding ground vegetation, which consisted mostly of graminoid and herbaceous species, with scattered deciduous and evergreen dwarf shrubs (e.g., Dawes et al. 2011). Potential N-fixing species (e.g., *Lotus alpinus* (DC) Ramond) occurred at similar very low densities at all sites. Each elevation level ("site") contained five replications ("groups") of seven species-specific circular plots ("plots") of ca. 15 cm diameter. Within each site, each of the five groups spanned a diameter of 2–3 m, with distances of 5–6 m between group centers. Within each group, the seven species-specific plots were located at distances of 0.5–2 m between plots. At the beginning of the experiment, above-ground vegetation was removed inside and within a 10-cm radius of each plot. A 2-cm layer of sieved soil taken from the top 5-cm layer near the intermediate elevation site was added to each plot before sowing. This treatment ensured similar starting conditions for all plots. Reinvasion of ground vegetation into plots was minor, and any reinvading plants were removed twice yearly. Plots were covered over the course of the experiment with a 2-mm metal mesh attached to a plastic ring around the seeds, in order to exclude herbivores while only slightly changing light availability.

Measurements

For each plot, photosynthetically active radiation (PAR) was measured once in July 2010, when the surrounding ground vegetation (outside the weeded 17.5 cm radius from the center of the plots) had reached its maximum height. Measurements were taken with a light sensor (LI-190SA Quantum Sensor, LI-COR Environmental, USA) held horizontally directly above the seedling canopy at c. 10 cm height, and well above any grass or shrub layers near the seedling canopies at 60 cm height. In order to avoid direct radiation that could negatively influence the measurements of relative differences in radiation, the measurements were taken during mostly cloudy conditions. The percentage PAR reaching the seedling crowns, relative to the PAR at 60 cm (radC), provides an indirect estimate for light competition in the immediate surroundings of the plots.

Table 1 Current elevational ranges of seven species in Switzerland, with ranges spanning 90 % of all observations in Switzerland (Brändli 1998); and elevation of seed sources (A. Burkart, personal communication)

Species	Current elev. range (m a.s.l.)	Elev. of seed source (m a.s.l.)
Larix decidua	600–2,100	2,160
Picea abies	500–1,800	2,000
Pinus cembra	1,700–2,200	2,100
Pinus mugo	1,300–2,100	1,860
Pinus sylvestris	400–1,500	1,750
Sorbus aucuparia	900–1,700	1,960
Alnus viridis	1,400–2,100	1,900

Soil temperatures at 5 cm depth were measured using one data logger (Thermochron iButtons, Maxim Integrated Products, USA) per site at 0.5 h intervals during July–September 2010. Due to the lack of continuous data over the whole experiment, soil temperatures were not used as predictor variable in seedling performance analysis, but used for post hoc interpretation of site differences instead.

For each plot, the seedlings were counted and measured twice per year during 2007–2010 (only once in 2008), over a total of 39 months. Yearly census dates were early and late in the vegetation period (June and October). Since it was impossible to follow the many seedlings individually, germination and survival rates could not be explicitly calculated, and the proportion of living seedlings relative to the expected number of viable seeds per plot was calculated instead for each time point at M months after sowing (%aM).

Individual shoot length was measured to the nearest mm from the soil surface to the apical meristem for three random seedlings per plot, and averaged for each plot. Relative shoot growth rate was calculated as $RGR_{sh} = [\ln(L_{2010}) - \ln(L_{2007})] \times \Delta t^{-1}$, where L_{2007} and L_{2010} were shoot lengths at the end of the growing seasons of 2007 and 2010, respectively, and Δt was 3 years (growing seasons 2008–2010). For the two species with delayed germination (*P. cembra* and *S. aucuparia*), the last two growing seasons were used (2009–2010). To assess accumulated biomass at the end of the experiment, three seedlings per plot were harvested in September 2010, separated carefully with tweezers, cleaned, and separated into roots, stems, and foliage. The seedlings were oven-dried for 2 days at 60 °C and weighed to ±0.1 mg accuracy for all

compartments separately, but pooled over the three individuals per plot. These biomass (bm) data were also used for the calculation of root to shoot ratio, $R:S = bm_{root}/(bm_{stem} + bm_{foliage})$, and the foliar mass ratio, $FMR = bm_{foliage}/bm_{total}$. Foliar C and N concentrations (%C, %N) and stable C and N isotope ratios ($\delta^{13}C$, $\delta^{15}N$) were measured from the dried foliage samples (see Online Resource 1A).

Data analysis

To analyze potential differences in nine variables (%a39, RGR_{sh}, bm, R:S, FMR, and foliar %N, N allocation, $\delta^{15}N$ and $\delta^{13}C$) among sites for each species, we used 50–50-Manova (Langsrud et al. 2007; see Online Resource 1B).

In a second analysis, we examined the relationship of seedling performance with different environmental variables, in a mixed effects regression model on the data pooled over all species (spc) and sites. The response variables used as measures for seedling performance were %a4, %a16, %a28, %a39, i.e., the proportion of living seedlings at the end of each vegetation period, and RGR_{sh}, bm, R:S, and FMR. For each response variable (y), site, spc, radC, and $\delta^{15}N$ were used as fixed predictors, and group identity (1–15) as random factor (Eq. 1). The spc:radC interaction was included because *L. decidua*, *P. sylvestris*, *P. mugo* were expected to require more light than the other species (Landolt 2010), and the spc:$\delta^{15}N$ interaction was included after an initial correlation test showed that $\delta^{15}N$ was positively correlated with %N and negatively correlated with R:S in the same three species (Table 2).

We did not include $\delta^{13}C$ as a predictor variable because it was not significantly different from the expected altitudinal lapse rate (see Results section). To better meet the assumptions of the mixed effects regression model, response variables were transformed where necessary (see Online Resource 1B).

$$y_{transf} \sim site + spc + radC + \delta^{15}N + spc:radC$$
$$+ spc:\delta^{15}N + (1|group) \qquad (1)$$

The significance of the predictor variables was assessed with likelihood ratio tests (Bolker et al. 2009), and a Bonferroni correction was used for the four time-series response variables (see Online Resource 1B). Overall model fit for each response

Table 2 Species-specific Pearson's correlation coefficients (*r*) between foliar N concentration (%N) and foliar δ^{15}N natural abundance heavy isotope, and between foliar δ^{15}N and root to shoot ratio (R:S)

Species	r (%N, δ^{15}N)	r (δ^{15}N, R:S)
Larix decidua	0.76**	−0.78***
Picea abies	−0.47(*)	0.37
Pinus cembra	0.34	−0.25
Pinus mugo	0.64*	−0.63*
Pinus sylvestris	0.78***	−0.72**
Sorbus aucuparia	−0.11	0.17

Data were pooled over sites for each species, and *p* values are given as: ***<0.001; **<0.01; *<0.05; (*)<0.1

variable was calculated as linear regression of the predicted versus the observed values (Byrnes et al. 2006). The parameter estimation methods and results can be found in the Online Resource 1B.

Results

Site characteristics

The relative light availability at the seedling crown level (radC) increased with site elevation, with means (±SD) of 79.3 % (±5.9 %) at the lowest site, 86.6 % (±1.7 %) at the intermediate site, and 95.0 % (±0.5 %) at the highest site.

Soil temperature at 5 cm depth averaged over the 0.5 h-measurements in July–September 2010 decreased with site elevation: mean (±SD) temperatures were 10.9 (±3.2) °C at the lowest site, 9.3 (±4.1) °C at the intermediate site, and 9.0 (±4.8) °C at the highest site. Site differences were more pronounced during cloudy weather (4–5 °C), while in clear weather, temperature differences were smaller (<1 °C). Overall, 0.5 h-measurements in July–September 2010 ranged from 4.4 to 22.8, 2.2 to 22.5, and 1.2 to 24.8 °C at the lowest, intermediate, and highest site, respectively. No temperatures below 0 °C were recorded. Temperatures below 2 °C, at which the surface soil may have been frozen, were only recorded at the highest site, during a total of c. 93 h in September. Soil temperatures below 5 °C, the approximate minimum temperature required for tissue formation (Körner 2003), occurred during 4, 15, and 22 % of the temperature measurement period at the lowest, intermediate, and highest site, respectively.
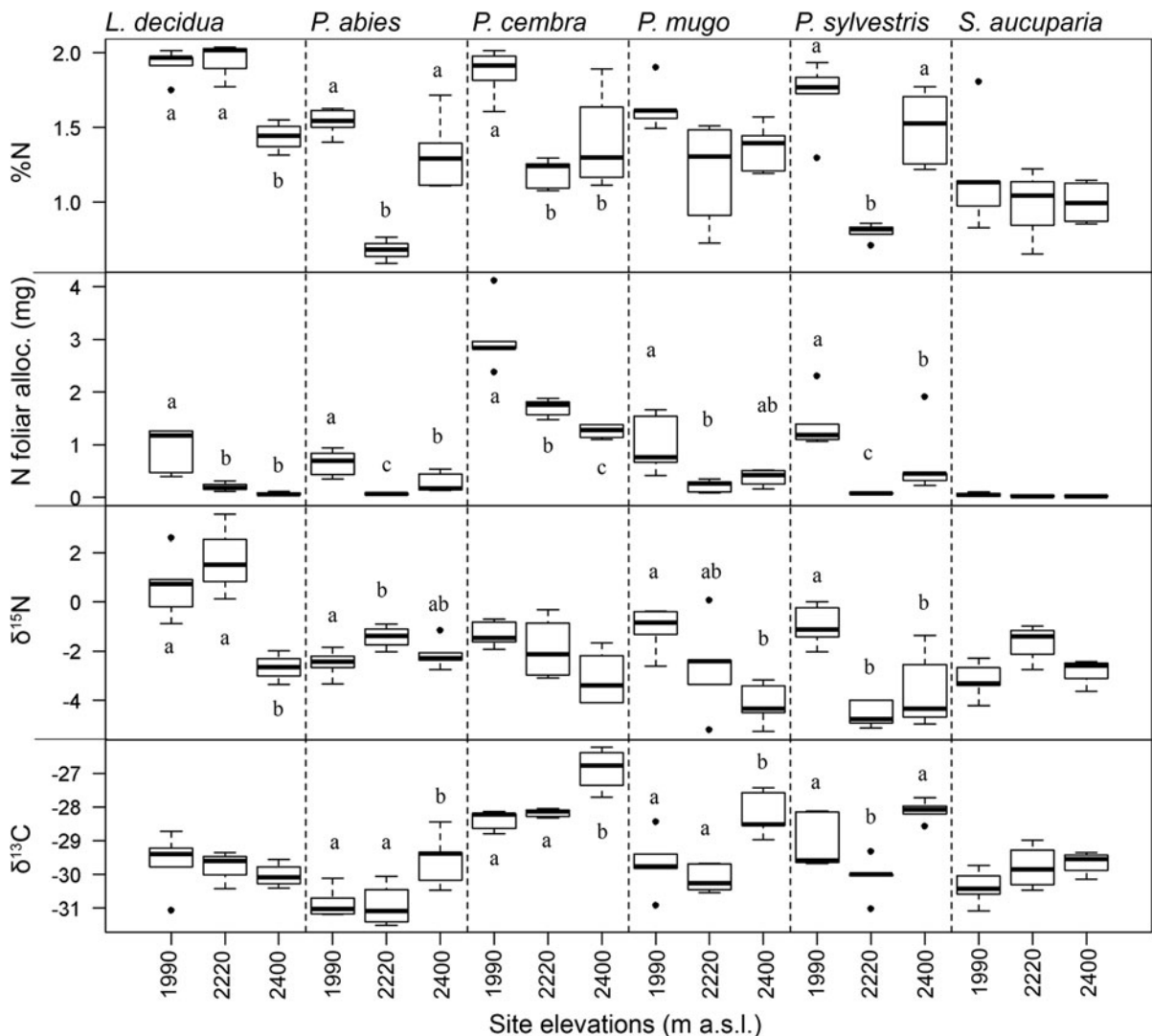
Seedling characteristics

Foliar carbon and nitrogen concentration and isotopes

No significant differences were found among sites for foliar %C (data not shown). The elevational differences in δ^{13}C were not significantly different from the expected mean lapse rate of 1.2 ‰ per 1,000 m (Körner et al. 1991; Körner 2003). Foliar N concentration (%N) and N allocation significantly decreased from the lowest to highest site in *L. decidua* and *P. cembra* (by 0.48 and 0.46 % for %N, and by 0.84 and 1.76 mg for N allocation, respectively), but were lowest at the intermediate site in *P. abies* and *P. sylvestris* (Fig. 1). δ^{15}N significantly decreased with elevation in three species (*L. decidua*, *P. mugo*, *P. sylvestris*; by 3.3, 3.0, and 2.6 ‰, respectively, from the lowest to highest site), but increased by 1.1 ‰ from the lowest to intermediate site in *P. abies*.

Proportions of living seedlings

The proportion of seedlings alive at the end of the experiment (%a39) relative to the number of viable seeds sown (Table 3) was not significantly different among sites except in *P. abies*, where the mean %a39 decreased by 28 % between the lowest and highest site and by 38 % between the lowest and intermediate site (Fig. 2). Changes in proportions of living seedlings over time (Fig. 3) differed markedly among species. *P. cembra* and *S. aucuparia* had low initial germination success, whereas in *A. viridis*, despite high initial germination success at the lowest site, almost all individuals died before the end of the experiment (Table 3; Fig. 3), and the species was excluded from further analysis. All species except *S. aucuparia* and *P. cembra* tended to show larger differences among sites early in the experiment with converging proportions of living seedlings among sites toward the end of the experiment (Fig. 3).

The regression analyses for the yearly %aM indicated that site location and species identity were significant predictors, whereas no relationship with δ^{15}N was found. A significant and species-specific relationship of the proportion of living seedlings with light availability was found 28 months after sowing but not at any other census (Table 4; see also Table S1 and Fig. S1a in Online Resource 1B).

Fig. 1 Seedling characteristics per species (*panels*) and for three sites (*elevations* m a.s.l. on *x*-axis). *Each row* of *panels* shows one variable: Foliar nitrogen concentration relative to dry weight (%N), foliar N allocation, and foliar heavy isotope ratios $\delta^{15}N$ and $\delta^{13}C$. Different letters (*a*, *b*, *c*) indicate significant among-site differences (50–50-MANOVA, Tukey Honest Significant Difference tests, $p < 0.05$; see Online Resource 1B for details). *Labels* were omitted where no significant differences between sites were found. Due to the low initial germination success of *P. cembra* and *S. aucuparia* in the first year of the experiment, pre-germinated seedlings of these two species were added in the second year

Seedling growth

The relative shoot growth rate (RGR_{sh}) decreased from the lowest to highest site in *L. decidua*, *P. abies*, and *P. mugo*, by 0.41, 0.21, and 0.16, respectively (Fig. 2). However, in *P. mugo* the decrease in mean RGR_{sh} from the lowest to intermediate site (0.21) was largest, and in *P. sylvestris* the mean RGR_{sh} at the intermediate site was significantly lower than at both

other sites. In all species except *S. aucuparia*, the mean RGR_{sh} was ≥ 0.2 at all three sites. Absolute seedling height varied among species and among sites, but showed relatively low variability among plots (Table 3).

Mean seedling biomass decreased from the lowest to highest site in *L. decidua*, *P. cembra*, and *P. mugo*, by 88, 37, and 51 %, respectively (Fig. 2). In *P. abies* and *P. sylvestris*, differences between the lowest and

Table 3 Species-specific expected number of viable seeds (based on seed quality assessed in a greenhouse germination experiment; A. Burkhart, personal communication), mean ($\pm$SD) number of surviving seedlings at the end of the experiment (39 months), and mean ($\pm$SD) seedling height of three seedlings in cm

Species	Exp #viable seeds	Mean #seedl 39 months ($\pm$SD)			Mean seedl ht (cm) 39 months ($\pm$SD)		
		Low	Mid	High	Low	Mid	High
Larix decidua	72	24.4 ($\pm$5.2)	7.8 ($\pm$6.1)	14.6 ($\pm$9.3)	9.5 ($\pm$1.2)	4.1 ($\pm$1.4)	2.9 ($\pm$0.7)
Picea abies	72	34.8 ($\pm$5.8)	8.8 ($\pm$5.7)	16.6 ($\pm$5.9)	6.5 ($\pm$1.2)	2.7 ($\pm$0.3)	3.0 ($\pm$1.0)
Pinus cembra	15[a]	7.8 ($\pm$2.5)	8.8 ($\pm$1.3)	7.0 ($\pm$2.7)	7.3 ($\pm$1.0)	6.0 ($\pm$0.2)	5.3 ($\pm$0.8)
Pinus mugo	71	45.4 ($\pm$3.7)	31.4 ($\pm$9.7)	36.0 ($\pm$17.3)	6.1 ($\pm$0.9)	3.2 ($\pm$0.5)	3.3 ($\pm$0.8)
Pinus sylvestris	72	28 ($\pm$5.3)	17.8 ($\pm$7.8)	14.8 ($\pm$10.8)	7.5 ($\pm$1.4)	3.6 ($\pm$0.4)	5.4 ($\pm$0.9)
Sorbus aucuparia	150[a]	47.2 ($\pm$6.1)	51.4 ($\pm$3.7)	34.4 ($\pm$11.4)	5.6 ($\pm$1.7)	3.7 ($\pm$0.1)	2.6 ($\pm$0.5)
Alnus viridis	72	0.8 ($\pm$1.2)	1.6 ($\pm$3.2)	0 ($\pm$0)	(1.0–4.0)[b]	(0.5–1.0)[b]	(0)[b]

Low, mid, and high denote the three elevational sites at 1,990, 2,220, and 2,400 m a.s.l., respectively

[a] Due to low initial germination success, pre-germinated seedlings of these species were added to all plots in the second year of the experiment

[b] Sizes of *A. viridis* are given as ranges (min–max) due to the low number of surviving individuals

intermediate site (-69 and -81 %, respectively) were largest, while those between the lowest and highest site were not significant (Fig. 2).

Biomass allocation to roots (R:S) increased from the lowest to highest site in *L. decidua*, *P. abies*, *P. mugo*, and *P. sylvestris*, by 0.44, 0.23, 0.12, and 0.22, respectively (Fig. 2). This shift in R:S mostly reflected reduced stem biomass relative to root biomass, as the proportion of biomass allocated to foliage (FMR) was not significantly different among sites for most species (*L. decidua*, *P. cembra*, *P. mugo*, *S. aucuparia*), or showed the lowest mean at the intermediate site (*P. abies*). Only *P. sylvestris* had the highest FMR at the lowest site (Fig. 2).

Apart from site and spc, $\delta^{15}N$ was a significant predictor of bm, R:S and FMR, with a positive relationship with bm and R:S, and a negative relationship with FMR. Moreover, the responses to $\delta^{15}N$ were species-specific for bm and FMR (Table 4; see also Table 1 and Fig. S1b–d in Online Resource 1B). Light availability (radC) showed no relationship with growth or biomass.

Discussion

Proportions of living seedlings

Despite some site differences in seedling dynamics (Fig. 3), our first hypothesis that seedling performance should decrease with increasing elevation was not

supported by the proportions of living seedlings. Their means were similar among sites, and higher than expected based on past findings in the European Alps (Hättenschwiler and Körner 1995; Körner 2003), the boreal treeline in Finland (Juntunen and Neuvonen 2006), the Pyrenees (Batllori et al. 2009) and the Snowy Range, Wyoming (Germino et al. 2002). The differences between our results and these previous studies may have resulted from factors that were not studied in our experiment, such as seed dispersal, herbivory, facilitation, or competition (e.g., Moir et al. 1999; Juntunen and Neuvonen 2006; Myster 2009). Apart from soil temperature, spring or summer frosts have a strong impact on seedling survival (Venn et al. 2009), although frost events were mostly absent during our experiment (Rixen et al. 2012). In the absence of such events, the observed high proportions of living seedlings may suggest that the seedlings may only become limited at a later age or size (e.g., Barbeito et al. 2012), or that the local treeline is not in equilibrium with climate.

Likewise, our second hypothesis, that species-specific performance should broadly match their current elevational ranges (Table 1), was also not confirmed by the proportions of living seedlings. The three species currently found at or near the treeline, *L. decidua*, *P. mugo*, and *P. cembra*, showed no significant decrease in their proportions of living seedlings with site elevation. *P. sylvestris* and *P. abies*—common at elevations 400–500 m below the regional treeline—were more successful than
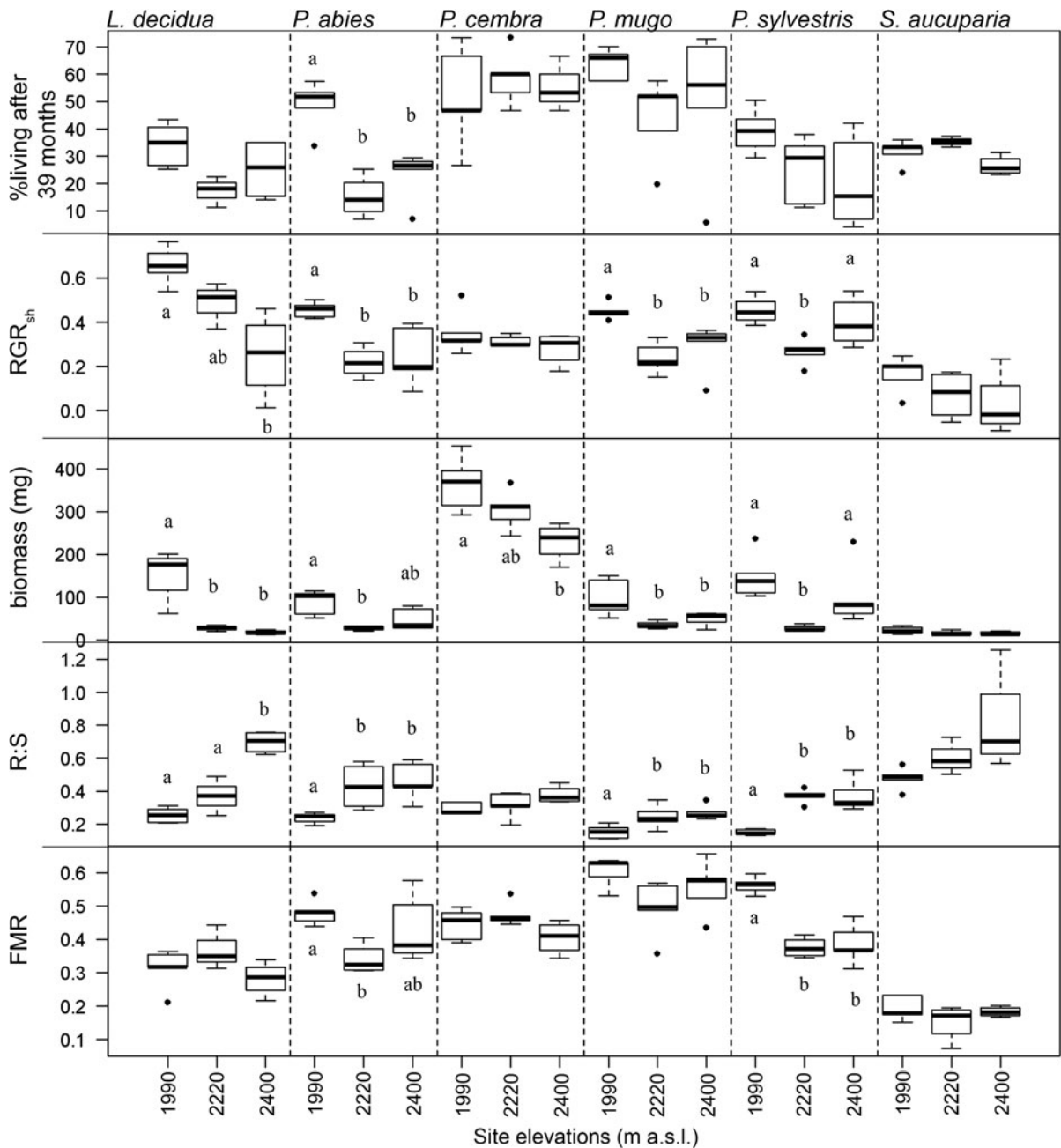
Fig. 2 Seedling characteristics per species (*panels*) and for three sites (*elevations* m a.s.l. on *x*-axis). *Each row* of *panels* shows one variable: Percentage of living seedlings after four vegetation seasons (39 months) relative to the number of viable seeds sown, relative shoot growth rate (RGR_{sh}), total dry weight biomass per seedling, root to shoot biomass ratio (R:S), and foliar mass ratio (FMR). The significant differences between sites are *labeled* as in Fig. 1. Due to the low initial germination success of *P. cembra* and *S. aucuparia* in the first year of the experiment, pre-germinated seedlings of these two species were added in the second year

expected at the highest site 300 m above the current treeline, with >20 % seedlings alive 39 months after sowing in both species. The results for *P. sylvestris* agree with a previous study, which suggested that in the Swiss Alps, this species is more limited by species interactions than by temperature (Hättenschwiler and Körner 1995).

Fig. 3 Percentage of living seedlings relative to viable seeds sown in May 2007, over four vegetation seasons at three sites (1,990, 2,220, and 2,400 m a.s.l.) along an elevational transect across and above the treeline in the Swiss Alps. Mean ± SD (5 replications per site and species) are shown for all censuses at 4–39 months after sowing. Growing seasons (May–September) are *shaded* in gray. Due to the low initial germination success of *P. cembra* and *S. aucuparia* in the first year of the experiment, pre-germinated seedlings of these two species were added in the second year (*dashed lines*)
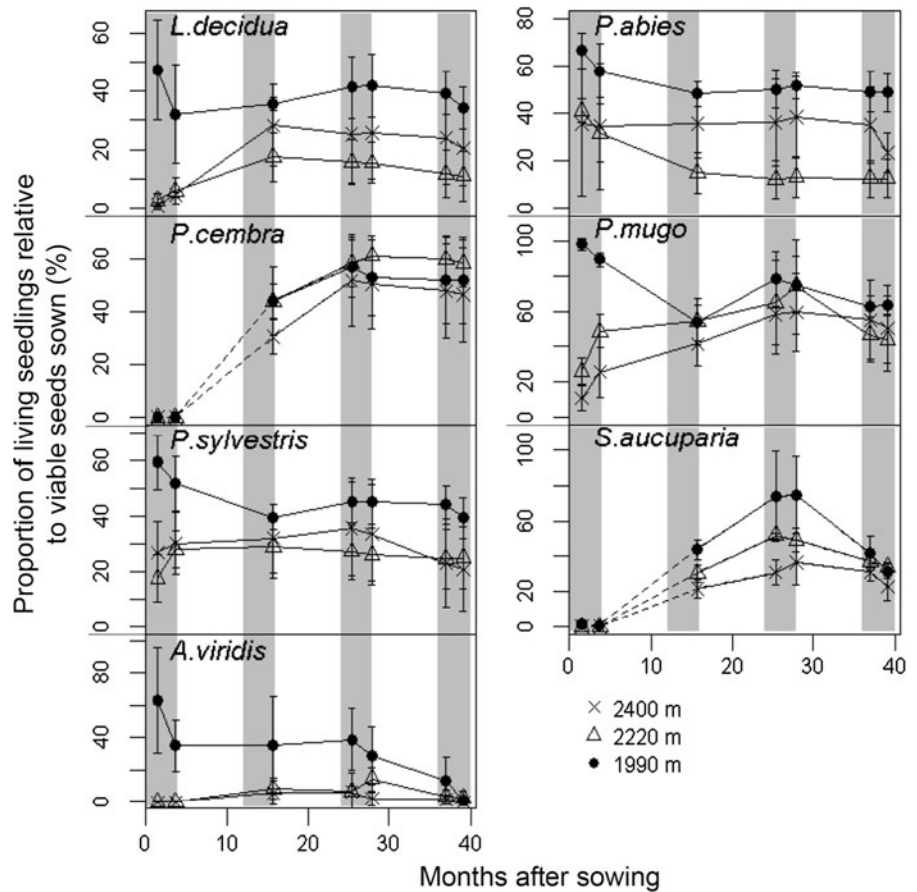
Table 4 Significance (*p* values) of predictor variables (rows) site, species (spc), light availability (radC), and N isotope ratio (δ^{15}N) for the eight variables of interest (columns) percentage of living seedlings (%a4, %a16, %a28, %a39), relative shoot growth (RGR$_{sh}$), dry weight biomass per seedling (bm), root to shoot ratio (R:S), and foliar mass ratio (FMR)

	%a4	%a16	%a28	%a39	RGR$_{sh}$	bm	R:S	FMR
Site	*0.000*	*0.002*	0.171	*0.006*	*0.000*	*0.000*	*0.000*	0.334
spc	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*
radC	0.090	0.061	*0.006*	0.535	0.537	0.056	0.111	0.438
δ^{15}N	0.196	0.344	0.315	0.425	0.086	*0.001*	*0.002*	*0.000*
spc:radC	0.068	0.034	*0.003*	0.414	0.610	0.227	0.095	0.616
spc:δ^{15}N	0.158	0.517	0.319	0.329	0.061	*0.012*	0.063	*0.000*
p_{Ref}	0.0125	0.0125	0.0125	0.0125	0.05	0.05	0.05	0.05
Model R^2	0.85	0.49	0.66	0.65	0.81	0.88	0.86	0.87

The reference *p* values (p_{Ref}) are 0.05 for the growth and biomass variables, and 0.0125 for the four time-series variables, based on a Bonferroni correction (=0.05/4). Significant *p* values are highlighted in italics font. Site and spc were used as factors with 3 and 6 levels, respectively. See the main text and online supplementary material for details of the linear mixed effects model

While *P. cembra* and *S. aucuparia* germinated poorly, their performance in terms of proportions of living seedlings and growth was high after we added pre-germinated seedlings. Based on their current elevational ranges (Table 1), *P. cembra* would be expected to germinate well at least at the two lower sites, while the occurrence of *S. aucuparia* at high elevations may be determined more by vegetative

reproduction. Similarly, even though *A. viridis* is common in Swiss alpine treeline ecotones, our experiment suggests rather poor sexual reproduction success at high elevations. Species-specific seedling dynamics over time (Fig. 3) indicated that different species were likely limited at different stages of their development. Even though the seedling performance data were not significantly correlated with seedling densities, the high seedling densities in our plots may have led to interactions among the seedlings (Moir et al. 1999; Batllori et al. 2009; Venn et al. 2009), and we could not entirely exclude seedling interactions as potential co-drivers of seedling performance.

Our third hypothesis, that proportions of living seedlings should be related to nitrogen or light availability, was only partly confirmed. The proportions of living seedlings were not significantly related to $\delta^{15}N$. Light availability (radC) was a significant and species-specific predictor of the proportion of living seedlings at one point in time (%a28; Table 4, see also Table S1 and Fig. S1a in Online Resource 1B). However, the differences in %a28 did not correspond to the species-specific light requirements, i.e., the relationship of %a28 with radC was not more positive in the more light-demanding species *L. decidua*, *P. sylvestris*, or *P. mugo*, compared to the other species. While light availability may have an influence on early seedling survival (Johnson and Smith 2005), our results suggest that other factors not studied here may have been more important for the species analyzed here during the course of our experiment.

Seedling growth

According to our first hypothesis, seedling RGR_{sh} and biomass decreased with increasing elevation in most species. These results are in agreement with a previous study of four subalpine tree species (*L. decidua*, *P. cembra*, *P. mugo*, and *P. abies*) in the same geographic area (Turner et al. 1975), with laboratory results of *L. decidua* and *P. mugo* (Hoch and Körner 2009), and partly with results of boreal tree species (Peng and Dang 2003).

Contrary to our second hypothesis, the differences in RGR_{sh} among sites provided no evidence for distinct growth potential according to current species-specific elevational ranges, at least in the very early life of germinating seedlings. The high elevations of the seed sources (Table 1) may have partly contributed to higher than expected growth rates, based on

provenance effects (e.g., Castanha et al. 2012). Additionally, species-specific differences in RGR_{sh} may become stronger in later stages of development. Two of the species that occur at comparatively lower elevations, *P. abies* and *P. sylvestris*, did not show significant differences in biomass between the lowest and highest site, while the three species *L. decidua*, *P. cembra*, and *P. mugo* that occur at treeline in our study area experienced strong reductions in total biomass accumulation with increasing elevation. R:S ratios increased significantly with site elevations for all species except *P. cembra* and *S. aucuparia* (Fig. 2), indicating more biomass allocation to the root system while maintaining similar biomass investments in photosynthetic tissues as shown by the rather stable FMR among sites.

Our third hypothesis, that seedling growth and biomass should be related to light or N availability, was partly confirmed. While light availability (radC) was not related to differences in RGR_{sh}, biomass, or biomass allocation, $\delta^{15}N$ was a significant predictor of biomass and biomass allocation. The significant spc:$\delta^{15}N$ interaction in the prediction of biomass and FMR suggested a species-specific pattern of N nutrition, especially in combination with the low overall %N and foliar N allocation in some species (Fig. 1), and the low soil N availability at our research sites (Blaser 1980; Dawes et al. 2011). Species-specific N-uptake traits (Miller and Bowman 2002) could, therefore, lead to the apparent differences in N availability and biomass allocation (Domisch et al. 2002). The elevational decline in foliar $\delta^{15}N$ could arise either from a decreasing soil N availability or declining N uptake, both likely influenced by temperature (Weih and Karlsson 2001; Smith et al. 2009), and N availability may have been additionally influenced by competition with other seedlings or surrounding ground vegetation (Moir et al. 1999).

At relatively low N availability, mycorrhizae-mediated N uptake becomes more likely, which would also be reflected in lower foliar $\delta^{15}N$ (Hobbie et al. 2000; Hobbie and Colpaert 2003). Particularly low $\delta^{15}N$ were found at the highest site in *L. decidua*, *P. sylvestris* and *P. mugo*, which in conjunction with the positive correlation of $\delta^{15}N$ with %N and negative correlations of $\delta^{15}N$ with R:S in these species (Table 3) could indicate that these species are taking up more N via mycorrhizae than the other species. Previous reports have shown the importance of mycorrhization and N uptake in tree seedlings in

culture and field conditions (Hobbie and Colpaert 2003; Hasselquist et al. 2005; Gruffman et al. 2012). Furthermore, the species-specific decrease in R:S and the increase in biomass with increasing foliar $\delta^{15}N$ in our study also support a possible link between belowground C allocation and N availability. In line with this interpretation, fertilization experiments at treeline in the Scandes showed a nitrogen-induced growth enhancement of *Betula pubescens* (Sveinbjörnsson et al. 1992).

Conclusions

We acknowledge the limitations of our case study, along an elevational gradient of one treeline site, for broad generalizations. However, the responses observed here contribute to a better mechanistic understanding of control factors on early performance of germinating seedlings of a wide range of treeline species of the Swiss Alps. Our results showed that tree seedlings overall performed better than expected at and above treeline, and that differences in performance among species could not be predicted based on the species' current elevational range. These results suggest that the early establishment of seedlings of a variety of tree species is not limited by current climatic conditions even beyond their actual upper distribution limit. We further showed that $\delta^{15}N$ as an indicator for N availability and uptake showed a significant and species-specific relationship with seedling biomass and biomass allocation. Nitrogen dynamics may, therefore, be an important co-driver of treeline seedling dynamics in situations where the climatic limit is not reached, and where seed input and herbivory are not limiting.

References

Bansal S, Germino MJ (2010) Variation in ecophysiological properties among conifers at an ecotonal boundary: comparison of establishing seedlings and established adults at timberline. J Veg Sci 21:133–142

Barbeito I, Dawes MA, Rixen C, Senn J, Bebi P (2012) Factors driving mortality and growth at treeline: a 30-year experiment of 92000 conifers. Ecology 93:389–401

Batllori E, Camarero JJ, Ninot JM, Gutierrez E (2009) Seedling recruitment, survival and facilitation in alpine *Pinus uncinata* tree line ecotones. Implications and potential responses to climate warming. Global Ecol Biogeogr 18:460–472

Beniston M, Diaz HF, Bradley RS (1997) Climatic change at high elevation sites: an overview. Climatic Change 36:233–251

Blaser P (1980) Soil as an ecological factor in subalpine afforestations in the timberline zone (Stillberg, Davos). Mitt Eidg Anst Forstl Versuchsw 56:529–611

Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, White JSS (2009) Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol Evol 24:127–135

Brändli UB (1998) Die häufigsten Waldbäume der Schweiz. Ergebnisse aus dem Landesforstinventar 1983–85: Verbreitung, Standort und Häufigkeit von 30 Baumarten, 2nd edn. Eigenössische Forschungsanstalt für Wald, Schnee und Landschaft, Birmensdorf

Byrnes J, Stachowicz JJ, Hultgren KM, Hughes AR, Olyarnik SV, Thornber CS (2006) Predator diversity strengthens trophic cascades in kelp forests by modifying herbivore behaviour. Ecol Lett 9:61–71

Castanha C, Torn MS, Germino MJ, Weibel B and Kueppers LM (2012) Conifer seedling recruitment across a gradient from forest to alpine tundra: effects of species, provenance, and site. Plant Ecol Divers. doi:10.1080/17550874.2012.716087 [online first]

Craine JM, Elmore AJ, Aidar MPM et al (2009) Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. New Phytol 183:980–992

Danby RK, Hik DS (2007) Variability, contingency and rapid change in recent subarctic alpine tree line dynamics. J Ecol 95:352–363

Dawes MA, Hagedorn F, Zumbrunn T, Handa IT, Hättenschwiler S, Wipf S, Rixen C (2011) Growth and community responses of alpine dwarf shrubs to in situ CO_2 enrichment and soil warming. New Phytol 191:806–818

Domisch T, Finer L, Lehto T, Smolander A (2002) Effect of soil temperature on nutrient allocation and mycorrhizas in Scots pine seedlings. Plant Soil 239:173–185

Farquhar GD, Richards RA (1984) Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. Aust J Plant Physiol 11:539–552

Garten CT, Van Miegroet H (1994) Relationships between soil-nitrogen dynamics and natural N15 abundance in plant foliage from Great Smoky Mountains National Park. Can J For Res 24:1636–1645

Germino MJ, Smith WK, Resor AC (2002) Conifer seedling distribution and survival in an alpine-treeline ecotone. Plant Ecol 162:157–168

Gruffman L, Ishida T, Nordin A, Näsholm T (2012) Cultivation of Norway spruce and Scots pine on organic nitrogen improves seedling morphology and field performance. For Ecol Manag 276:118–124

Harsch MA, Hulme PE, McGlone MS, Duncan RP (2009) Are treelines advancing? A global meta-analysis of treeline response to climate warming. Ecol Lett 12:1040–1049

Hasselquist N, Germino MJ, McGonigle T, Smith WK (2005) Variability of *Cenococcum* colonization and its ecophysiological significance for young conifers at alpine-treeline. New Phytol 165:867–873

Hättenschwiler S, Körner C (1995) Responses to recent climate warming of *Pinus sylvestris* and *Pinus cembra* within their montane transition zone in the Swiss Alps. J Veg Sci 6:357–368

Hobbie EA, Colpaert JV (2003) Nitrogen availability and colonization by mycorrhizal fungi correlate with nitrogen isotope patterns in plants. New Phytol 157:115–126

Hobbie EA, Macko SA, Williams M (2000) Correlations between foliar delta N-15 and nitrogen concentrations may indicate plant-mycorrhizal interactions. Oecologia 122:273–283

Hoch G, Körner C (2009) Growth and carbon relations of tree line forming conifers at constant vs. variable low temperatures. J Ecol 97:57–66

Hofgaard A, Dalen L, Hytteborn H (2009) Tree recruitment above the treeline and potential for climate-driven treeline change. J Veg Sci 20:1133–1144

Holtmeier FK (1994) Ecological aspects of climatically caused timberline fluctuations—review and outlook. In: Beniston M (ed) Mountain environment in changing climates. Routledge, London, pp 220–232

Holtmeier FK, Broll G (2005) Sensitivity and response of northern hemisphere altitudinal and polar treelines to environmental change at landscape and local scales. Glob Ecol Biogeogr 14:395–410

Johnson DM, Smith WK (2005) Refugial forests of the southern Appalachians: photosynthesis and survival in current-year *Abies fraseri* seedlings. Tree Physiol 25:1379–1387

Juntunen V, Neuvonen S (2006) Natural regeneration of Scots pine and Norway spruce close to the timberline in northern Finland. Silva Fenn 40:443–458

Kabeya D (2010) Differentiating between the adverse effects of nutrient-limitation and direct-cold-limitation on tree growth at high altitudes. Arct Antarct Alp Res 42:430–437

Körner C (2003) Alpine plant life: functional plant ecology of high mountain ecosystems, 2nd edn. Springer, Berlin

Körner C, Paulsen J (2004) A world-wide study of high altitude treeline temperatures. J Biogeogr 31:713–732

Körner C, Farquhar GD, Wong SC (1991) Carbon isotope discrimination by plants follows latitudinal and altitudinal trends. Oecologia 88:30–40

Landolt E (2010) Flora indicativa. Ökologische Zeigerwerte und biologische Kennzeichen zur Flora der Schweiz und der Alpen, 1st edn. Haupt, Bern

Langsrud O, Jorgensen K, Ofstad R, Naes T (2007) Analyzing designed experiments with multiple responses. J Appl Stat 34:1275–1296

Miller AE, Bowman WD (2002) Variation in nitrogen-15 natural abundance and nitrogen uptake traits among co-occurring alpine species: do species partition by nitrogen form? Oecologia 130:609–616

Moir WH, Rochelle SG, Schoettle AW (1999) Microscale patterns of tree establishment near upper treeline, Snowy Range, Wyoming, USA. Arct Antarct Alp Res 31:379–388

Munier A, Hermanutz L, Jacobs JD, Lewis K (2010) The interacting effects of temperature, ground disturbance, and herbivory on seedling establishment: implications for treeline advance with climate warming. Plant Ecol 210:19–30

Myster RW (2009) Tree seedling survivorship, growth, and allocation in the Cross Timbers ecotone of Oklahoma, USA. Plant Ecol 205:193–199

Peng YY, Dang QL (2003) Effects of soil temperature on biomass production and allocation in seedlings of four boreal tree species. For Ecol Manag 180:1–9

Resco V, Ferrio JP, Carreira JA, Calvo L, Casals P, Ferrero-Serrano A, Marcos E, Moreno JM, Ramírez DA, Sebastià MT, Valladares F, Williams DG (2011) The stable isotope ecology of terrestrial plant succession. Plant Ecol Divers 4:117–130

Rixen C, Dawes MA, 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

Schönenberger W (1975) Site factors influencing afforestation at tree line in the Alps (Stillberg, Davos). Mitt Eidg Anst Forstl Versuchsw 51:358–428

Smith WK, Germino MJ, Hancock TE, Johnson DM (2003) Another perspective on altitudinal limits of alpine timberlines. Tree Physiol 23:1101–1112

Smith WK, Germino MJ, Johnson DM, Reinhardt K (2009) The altitude of alpine treeline: a bellwether of climate change effects. Bot Rev 75:163–190

Sveinbjörnsson B, Nordell O, Kauhanen H (1992) Nutrient relations of mountain birch growth at and below the elevational tree-line in Swedish Lapland. Funct Ecol 6:213–220

Turner H, Rochat P, Streule A (1975) Thermische Charakteristik von Hauptstandortstypen im Bereich der oberen Waldgrenze (Stillberg, Dischmatal bei Davos). Mitt Eidg Anst Forstl Versuchsw 51:95–119

Venn SE, Morgan JW, Green PT (2009) Do facilitative interactions with neighboring plants assist the growth of seedlings at high altitudes in alpine Australia? Arct Antarct Alp Res 41:381–387

Walder UM (1983) Ausaperung und Vegetationsverteilung im Dischmatal. Mitt Eidg Anst Forstl Versuchsw 59:79–212

Weih M, Karlsson PS (2001) Growth response of Mountain birch to air and soil temperature: is increasing leaf-nitrogen content an acclimation to lower air temperature? New Phytol 150:147–155

Wilson C, Grace J, Allen S, Slack F (1987) Temperature and stature: a study of temperatures in Montane vegetation. Funct Ecol 1:405–413