Università degli Studi di Parma

Dipartimento di Bioscienze

Degree of

DOCTOR OF PHILOSOPHY

in Plant Biology

cycle XXVIII

Study of the effects of climate warming on seed germination and seed longevity of snowbed species

Research supervisor:

Dr. Alessandro Petraglia

Candidate:

Giulietta Bernareggi

Research co-supervisors:

Dr. Andrea Mondoni

Dr. Michele Carbognani

Academic Year 2014-2015

Università degli Studi di Parma

Dipartimento di Bioscienze

Degree of
DOCTOR OF PHILOSOPHY
in Plant Biology
cycle XXVIII

Study of the effects of climate warming on seed germination and seed longevity of snowbed species

Research supervisor:

Dr. Alessandro Petraglia

Research co-supervisors:

Dr. Andrea Mondoni

Dr. Michele Carbognani

Candidate:

Giulietta Bernareggi

Academic Year 2014-2015

CONTENTS

ABSTRACT

Understanding how climate change will affect the distribution and the phenology of plants is becoming an increasingly important topic in ecological studies. In response to climate warming, there are documented upward shift and alterations of phenology and physiology of several plant species. Despite this, the effects of climate change on plant regeneration from seeds have largely been neglected. However, regeneration from seeds, a key event in the plant life cycle, could be significantly affected by climate warming. In this regard, we investigated how climatic changes will affect the seasonal dynamics of seed germination and seedling survival in two different alpine context. The first part refers to five species inhabiting a snowbed located at the Gavia pass (Parco Naturale dello Stelvio). Here, plants were exposed, in the field, to natural conditions and to artificial warming using Open Top Chambers proposed by the ITEX (International Tundra Experiment). The germination curves of seeds produced were compared in order to highlight differences in seed germination ecology and in seed physiology induced by the climate warming. In the second part, we considered two tree species that form the treeline in Davos (Switzerland). As a surrogate of climate warming we used the natural thermal gradient driven by the altitude and we compared the germination behavior of the species studied in three sites at three different elevations in order to evaluate the likelihood of treeline shift under the predicted climate warming.

PREFACE

The overall objective of this thesis is to investigate the effect of global warming on the early stages of the plant life cycle. I attempt to study this broad topic in two main sections. Part one refers to the main PhD project developed at the University of Parma (Ialy), while the second part refers to a collateral project developed in collaboration with the SLF Institute for Snow and Avalanches Research, located in Davos (Switzerland).

The introduction chapter gives a broad overview of the background to biodiversity loss, the climatic change in alpine environments and the importance of seeds.

Chapters 1.3, 1.4 and 2.2 have been written as stand-alone scientific papers and are very closely interrelated. Each paper has been written so that it can be read independently. Consequentely, in order to provide the relevant background within each chapter, there is inevitably a small amount of repetition between them.

Chapter 1.3 has been published in Alpine Botany (Springer) and the chapter 1.4 has been submitted to Annals of Botany (Oxford Journals), but they are formatted and presented as for the rest of the thesis for consistency (the published and submitted versions are also provided in the final Appendix A1 and A2).

Chapter 2.2 is in preparation for submission to journals.

Figures and tables are numbered from the beginning within each chapter, while all references are provided at the end of the thesis to avoid repetition.

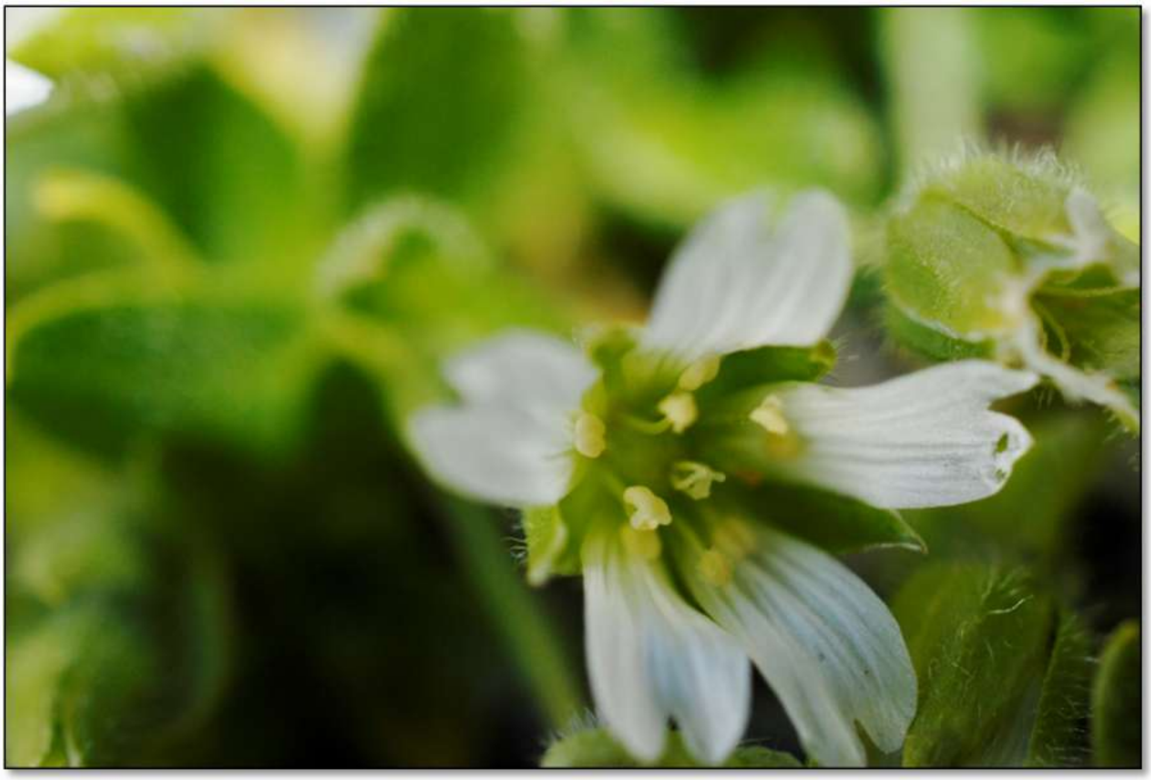
"The mountains are mute masters
who make for silent pupils"

Johann Wolfgang Goethe

"Great things are done when men
and mountains meet"

William Blake

INTRODUCTION



Particular of *Cerastium cerastoides*

(Photo: Giulietta Bernareggi)

Loss of biodiversity in a changing climate

Biodiversity loss is one of the most remarkable environmental problems of the twenty-first century. Current plant extinction is estimated to be 100–1000 times higher than that of the recent geological past (Pimm et al., 1995). One of the main factors affecting biodiversity is climate change. Indeed, the biosphere is currently subjected to a climate warming caused principally by the continued accumulation of anthropogenic greenhouses gases in the atmosphere that would lead to important impacts on natural and human systems (Crowley, 2000; Mann et al., 2003). Carbon dioxide is the most important greenhouse gases, and its level in atmosphere is passed, because of human activities like the use of fossil fuels and deforestation, from preindustrial levels of ~280 ppm to the current ones of ~380 ppm (IPCC 13) and the levels are still rising.

According with some sources (Bali Road Map, UN-Development Program) an increase in the annual mean temperature of 2.5 ° C would lead to the extinction of 20-30% of the species present on the Earth. Although, in the short term, climate change does not represent a major cause of biodiversity loss, in the next decades, it is expected that its effects will assume higher importance in comparison with others threatening factors, like habitat destruction, pollution, invasion of ecosystems by non-native plant and animal species (Thuiller, 2007).

Climate change in alpine environments

The issue of climate change is a matter of great conservation concern, both at descriptive (the analysis of climate alterations can help in the study about plant and animals population dynamic) and predictive (modeling of temporal trends allows predictions for future years) level.

Between 1901 and 2000, in many alpine areas, the increase of the mean annual temperatures has been of 2 °C (Beniston et al., 1997), which is much higher in comparison with the increase of 0.74 °C observed on a global scale (Jones and Morberg, 2003), with a trend that has almost been doubled in the last fifty years (0.13 °C every decade). In the current century, warming is expected to continue and, for the mid latitude European mountain, the increase of the mean temperature, provided by the A1FI and B1 scenario, will be between +2.9 °C and +5.3 °C within 2085 (Nogue´s-Bravo et al., 2007).

It follows that alpine ecosystems, characterized by low temperature, short vegetative seasons (Körner, 2003) and inhabited by species highly specialized to cold climates, are considered particularly threatened by rapid climate change (Christensen et al. 2007). In addition, it is expected that the indirect effects of the global warming on the ecosystems will probably assume an higher importance than the direct ones (Shaver et al., 2000). Indeed, in alpine regions, the provided climate warming will cause the alteration of water regimes, increasing annual precipitation with otherwise changing seasonality (i.e.

reduction between 20 and 40 % of summer rainfalls and soil drying) (*Gobiet* et al.*, 2014), the reduction of the snow cover depth and duration (Valt & Cianfar*ra, 2010) and the fast and alarming retreat of glaciers (minus 50 – 90%) (Haeberli and Beniston, 1998). It is expected that many small glaciers will disappear, while the volume of large glaciers will be reduced by 30 % to 70 % by 2050, with consequent reductions in discharge in spring and summer.

Such environmental alterations are associate with changes in phenology (i.e. advanced of phenological phases) (Wipf and Rixen, 2010; Oberbauer et al., 2013), physiology (i.e. advanced seed germination) (Mondoni et al., 2015) and distribution of alpine species (Grabher et al., 1994; Sandvik et al., 2004; Klanderud and Totland, 2005; Gottfried et al., 2012), which lead to changes in the species composition and in the structure of plant communities (e.g. Elumeeva et al., 2013; Carbognani et al., 2014a; Sandvik and Odland, 2014).

High altitude cenosis are thus considered the most threatened by climate change, since the presence of numerous biodiversity hotspots might rapidly disappear due to the rising of share of lower environments (Dirnböck et al. 2011; Engler et al., 2011), with important consequences for the survival of several alpine/arctic plants (e.g. Theurillat and Guisan 2001) and a biodiversity loss that could reach the 60% in the most pessimistic models (Thuiller et al., 2005).

Alpine snowbed

Within the alpine life zone, snowbed communities are currently considered among the most threatened habitats by climate warming.

Defined by Wahlberg (1813) as "*loci uliginosi frigidi a nive serius relicti*", snowbeds are topographically fragmented and azonal habitats that develop in correspondence of persistent snow accumulation, limiting the duration of the snow-free period from one to four months a year. Alpine snowbed are a unique component of alpine biodiversity, hosting exclusive species and plant communities (Björk & Molau 2007). Such communities, attributable in phytosociological terms to the *Salicetea herbaceae* class, are characterized by a very small vegetation, composed of lichens, bryophytes and vascular plants grasses or low shrubs, which are limited by the length of the snow-free period (Carbognani et al., 2012) and by soil resources (Petraglia et al., 2013, 2014a).

In the context of the upward migration of plants following the climate warming (Parolo and Rossi, 2008), snowbed are, among the alpine habitat, those that will suffer higher restrictions (Theurillat & Guisan 2001), because of their development in low-slope zones, which dramatically decreases towards higher altitudes. In this regard, ongoing changes in snowbed vegetation structure and composition have already been documented (Virtanen et al. 2003; Elumeeva et al. 2013; Sandvik and Odland 2014; Carbognani et al., 2014a) and are expected to lead, at long term, to a reduction or the disappearance of these habitats (Theurillat and Guisan 2001), with the consequent loss of a substantial component of the alpine biodiversity (Björk and Molau 2007).

Moreover, the special sensibility to the future climatic scenarios makes snowbeds suitable natural laboratories to analyse the effects of climate change on alpine vegetation (Björk and Molau 2007; Schöb et al., 2008a).

Snowbeds are worthy of being used as “field laboratories” for analysing the effects of the variability of environmental parameters on plant species living close to the limits of their physiological tolerance (Körner et al., 2005). Moreover, snowbeds are characterized by the occurrence of a broad spectrum of plant growth forms (Wookey et al. 2009), by the variability of the dominant functional type and by a relatively low structural complexity and species diversity. All these features, together with the special sensibility to the future climatic scenarios, are currently regarded as “ideal” for experimental manipulations (Shaver and Chapin 1991) in evaluating the effects of climate change on alpine vegetation (Björk and Molau 2007; Schöb et al., 2008a).

The Treelines

Treelines, represent an abrupt transition in life-form dominance. In arctic and alpine systems, treelines occur where trees reach the limit of their climatic tolerance, marking forest transitions to non-forested ecosystems. Treeline ecotones are important reservoirs of biodiversity, provide wildlife habitat, and important ecosystem services such as carbon storage (G-Tree: Global Treeline Expansion Experiment).

The importance of seeds

Despite the many studies on the overall impact of climate change on plant species (see Pautasso et al., 2010), the effects on plant regeneration from seeds has largely been neglected (Hedhly et al., 2009). Nevertheless, seeds are thought to play an important role in a rapidly changing environment, being the main vehicle for plant migration (Parolo and Rossi, 2008), regeneration (Walck et al., 2011), functional adaptation (e.g. Kochanek et al., 2011; Bernareggi et al., 2015) and persistence (Schwienbacher and Erschbamer, 2001). However, seed success and seedling survival are largely influenced by climate (Lloret et al., 2004), and the alteration of temperature and water supply due to global climate change could preclude, delay or enhance regeneration from seeds (Walck et al., 2011).

In alpine environments, seedling recruitment has long being considered rare because of summer drought and/or winter frost (Körner, 2003). However, in the last years, a number of studies highlighted both high rates of seedling establishment (Schlag and Erschbamer, 2000; Welling and Laine, 2000) and high genotype variability (Jonsson et al., 1996; Gabrielsen, 1998) within alpine plants, arguing the importance of sexual reproduction in the maintenance and in the expansion of plant populations. The impact of climate change on regeneration from seeds is therefore of growing interest (e.g. Shevtsova et al., 2009; Milbau et al., 2009; Mondoni et al., 2012) and changes in phenology, physiology, distribution and seedling establishment in response to climate warming have already been documented (e.g. Grabherr et al., 1994; Sandvik et al., 2004; Klanderud and Totland, 2005; Lenoir et al., 2008; Gottfried et al., 2012; Carbognani et al., 2014a, Bernareggi et al., 2015, Mondoni et al., 2015).

Improving our knowledge about the future of cold adapted species in a rapidly warming climate is become a pressing need for biodiversity conservation and will help to preserve an important part of biodiversity increasingly at risk.

THESIS OUTLINE

The aim of this study is to investigate the effects of climate change on alpine plants, with particular reference to the most critical phases of plant life cycle: seed dispersal and germination.

More specifically, Chapter 1.3 addresses the issue of seed longevity, the ability of seeds to remain viable for long time, that is a crucial prerequisite for seed persistence in the soil and in germplasm banks. We analyzed the effects of a moderately warmer parental growth environment on subsequent seed longevity of four alpine snowbed species using a laboratory-controlled accelerated ageing.

In Chapter 1.4, we investigated how the interplays between pre- and post-dispersal temperatures drives seed dormancy release and seed germination requirements by means of controlled germination tests in laboratory.

The last part, Chapter 2.2, addresses the problem of the shifting of the treelines, aiming to disentangle seed versus substrate limitations on treeline recruitment. The germination of two treelines species were studied in field, with and without seeding, at three different elevations (forest zone, transition zone at the current treeline and alpine zone) and in two consecutive years.

PART 1

CHAPTER 1.1 STUDY AREA AND STUDY SPECIES

The snowbed area at the Gavia Pass

(Photo: Giulietta Bernareggi)

1.1.1 Geography

The study area is located within the Stelvio National Park (46°20′N 10°30′E, 2700 m a.s.l.), in the high Gavia Valley, included in the Ortles-Cevedale Group in the Italian Rhaetian Alps (Eastern Alps). The high Gavia Valley (oriented North-South) includes a plateau that is delimited to the West by Gavia Mount (3223 m), to the East by Tre Signori Horn (3360 m) and to the South by Gavia Pass (2618 m) and Gaviola Mount (3025 m).

The climate of the Ortles-Cevedale Massif is temperate-continental, with cold winters and maximum rainfall in summer and autumn (Albertini, 1955). The nearest meteostation, located at the Forni Glacier at 2180 m of altitude, detects mean annual temperatures of 1.6 °C and mean annual rainfalls of about 700 mm (Geological Monitoring Center, Regione Lombardia, 1980-2002). Regarding the high Gavia Valley, Worldclim data interpolation (Hijmans et al., 2005), at spatial resolution of 30 arc s (1 km) shows a mean annual temperature of 1.6 °C and a mean annual rainfall of about 1150 mm.

The study site is located at the base of a glacial circle, 1 km far from the Gavia Pass, within an area of about 1 ha at around 2700 m of altitude.

1.1.2 Geology

The geological unities units that outcrop in the Gavia Valley, that is located north of the fault system known as Lineamento Insubrico, are part of the Austroalpine domain. The current position of such domain comes from the from the overlap of faults due to the action of compressive forces; translations of Austroalpine faults are both occurred in the Cretaceous (towards West and Northwest) that Eocene (towards North). This structure was further modified by the displacement along the Lineamento Insubrico during the Oligocene; such tectonic line marks the southern boundary of the Austroalpine domain and separates it from Subalpine domain.

The geological formations that outcrop in the study area are related to the medium Ausroalpine, i.e. Languard-Campo-Ortle System, that is the complex of unity between the lower (Margna, Sella, Bernina) and upper(Grosina, Tonale) austroalpine faults. In particular, in the Gavia Valley, the Campo Fault includes two units: one composed of phyllite, structurally superior and of a low metamorphic grade, and another one composed of gneiss and mica-schists, structurally below and of a higher metamorphic grade.

The phyllitic unit, also known as Cevedale Phyllitic Formation, is not lithologically homogeneous: the general mineralogical association of the basic litotype is quartz-muscovite-chlorite, to which are often associated biotite, granet and plagioclase; moreover there are considerable intercalations of amphibolite and chlorite schist (Andreatta 1954). The second unit, named Punta di Pietra Rossa Formation, shows two *facies*: one made of muscovitic-chloritic mica-schists, and another made of biotitic- amphibolitic gneiss. Moreover, there are presences of quartzites and amphibolitic schists (Berruti 1981).

In the study area there are also igneous rocks belonging to the group of the hypo-abysmal rocks, that derive from the intrusion of magmatic materials into the splits. The cooling speed is of thousands of years, that makes it at an intermediate level between the effusive and intrusive extremes. Such rocks usually have a tabular shape, that is evident in the porphyrite outcropping in the area of the Gavia Pass.

1.1.3 Geomorphology

The shape of the reliefs derives from the interaction between the forces within the Earth's crust, which determined the height and the main features, and the unceasing erosion made by the weathering.

The alpine morphology shows signs of the old glacial stages; in the study area the relief has been carved by ice and the subsequent characteristic forms are still present: cirques, truncated spurs, moraines and sheepbacks.

The climatic conditions at high altitudes promote the cryo-clastic breakdown of rocks and slow down the pedogenetic process, that is a system of chemical, physical and biological activities which aimed at the transformation of the lithoid substrate in soil. For these reasons, the soil surface of the high Gavia Valley is often uneven, and bare bedrock or fragmented lithoids represent substantial areas.

The study area is located at an altitude that facilitates a prolonged permanence of snow on the ground, and therefore the presence of snowbed community.

1.1.4 Flora and vegetation

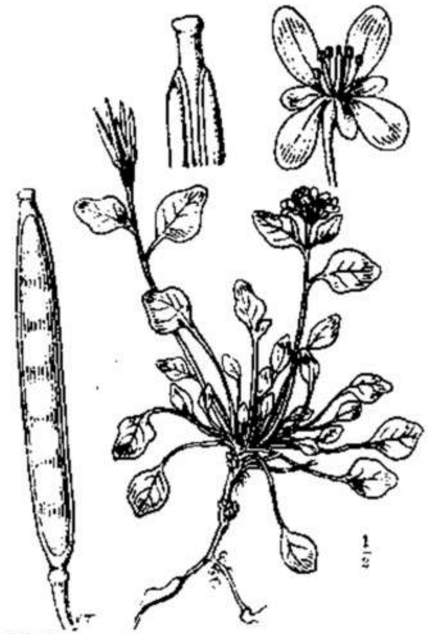
The term flora indicates the set of plant species, traditionally limited to vascular plants, found in an area, while vegetation refers to the system of populations of plant species that grow in line with their settlement sites and that form, together with all other life forms that grow therein, an ecosystem (Westhoff 1970). Species have different forms of growth, denominated biological forms in the system proposed by Raunkiaer (1934), and the range of these forms can be used to compare the floras of different territories. The shape of vegetal organisms, i.e. their *habitus*, is considered a fundamental component of the adaptation process to their settlement environment; therefore, a similarity in the range of biological forms attests an affinity of the ecological factors, climate in particular. The Raunkiaer classification system is based on the morphological shape that plants adopt to overcome the unfavorable season. In particular, the position of winter buds allows to distinguish the vascular species in chamaephytes, hemicryptophytes, cryptophytes and therophytes.

The area of the high Gavia Valley is included in the alpine belt of the altitudinal succession, that corresponds to the alpine ecological environment and that is characterized by a mean annual temperature of about 1 °C. Such altitudinal range, devoid of plant organisms with arboreal *habitus*, is dominated by herbaceous plants, with closed phytocoenosis very rich in species. More precisely, the study area in question falls within the high alpine zone. As mentioned above, the altitude and the topography of the area favour the prolonged permanence of the snow cover and the consequent settlement of the snowbed communities. Such flora is highly selected by the length of the growing season, and it is composed by lichens, mosses, herbaceous plants and prostrate dwarf willows. The Gavia Valley is characterized by a siliceous substrate and, therefore, plant communities are composed predominantly by acidophilic species. According to the phytosociological classification, phytocenoses of snowbeds on siliceous substrates are assigned to the alliance *Salicion herbaceae*.

The different phytocoenoses of snowbed can be differentiated according not only to the lithology of the substrate, but also to other micro-environmental factors, such as the length of the snow-free period or the level of soil moisture. Indeed, a long snow cover (ten or eleven months) is associated with the dominance of bryophytes, such as the hepatic *Anthelia juratzkana* or the moss *Polytrichum sexangulare*. Contrariwise, in the absence of geomorphological and edaphic conditions which determine surface waterlogging, the dominant species in is a tiny prostrate willow, defined by the Swedish botanist Carolus Linnaeus (1707-78) as “minima infra omnia arbor” (The most tiny of all the trees). This *Salix herbacea*, a species widespread throughout the Alps and, sporadically, on the highest peaks of the northern and central Apennines. Least willow forms mixed communities with those bryophytes included in *Polytrichetum sexangularis* and some angiosperms, the majority of which cohabit with the tiny willow throughout the Alps. These include Alpine bittercress (*Cardamine belliflora* subsp*. alpina*), sibbaldia (*Sibbaldia procumbens*), alpine arctic cudweed (*Gnaphalium supinum*), Alpine speedwell (*Veronica alpina*) and ear-like wood-rush (*Luzula alpino-pilosa*). Other angiosperms which coexist with least willow have a more limited distribution. They include cut-leaved lady’s mantle (*Alchemilla pentaphyllea*), which grows from the north-western Alps to the Rhaetian Alps, dwarf snowbell (*Soldanella pusilla*), confined to the central and eastern Alps, and most importantly, pygmy buttercup (*Ranunculus pygmaeus*), recorded in only a few sites in the Swiss Alps and South Tyrol.

1.1.5 Species studied

Cardamine bellidifolia (L.) subsp. *alpina* Willd.



C. *bellidifolia*

© Pignatti

Family: Brassicaceae

Biological form: H scap (herbaceus and perennial plant)

Choritype: Circum-Arctic-Alpine

Flowering: VII-VIII

Altitude: 1900-3000 m

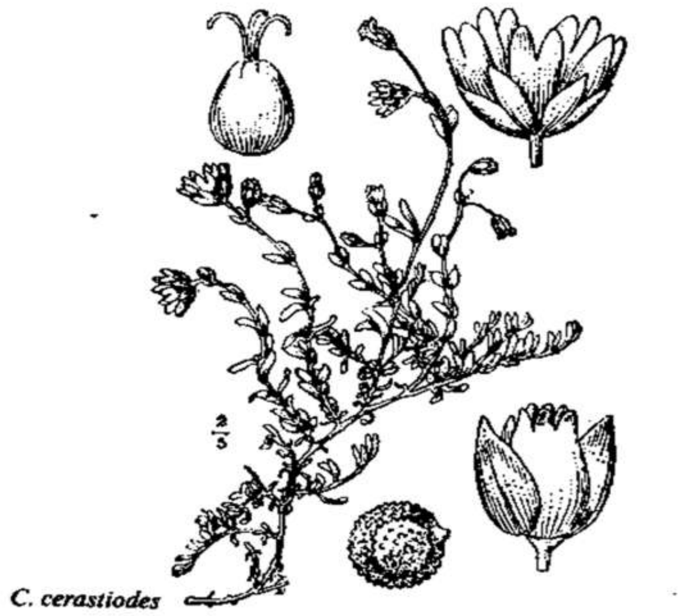
Morphological description: Heigh 2–10 cm. Unbranched and hairless stem. Basal leaves grouped in rosette, with long petiols, undivides, elliptics to suborbiculars, sometimes sinuate-lobed. Cauline leaves 13, undivided, attenuated at the base or short-stalked. Sepals 2 mm long, white petals petals 3–4 mm long. Straight siliques, 10–20 mm long. Pedicel up to max half the length of the silique.

Habitat: Snowbeds, wet rocks, siliceous soils.

Ellenberg's Ecological Index

L	T	C	U	R	N	S
8	1	6	4	6	2	0

Cerastium cerastoides (L.) Britton



© Pignatti

Family: Caryophyllaceae

Biological form: H scap (herbaceus and perennial plant)

Choritype: (Circum.) Arctic-alpine

Flowering: VII-VIII

Altitude: 1800-2900 m

Morphological description: 5–15 cm height. Prostrate or creeping stem, rooting, with fertile branches, glandular at the top. Oblong-lanceolate leaves, hairless, approx. 1 cm long. Peduncles 15–20 mm long, sepals 4–6, white bilobe petals, 1.3–1.5 times longer than sepals. 3–5 styles, 2–5 mm long. Capsule exceeding the calyx, opening with 6 teeth.

Habitat: Snowbeds, wet depressions (long lasting snow). Trampled soils. Calcifuge.

Ellenberg's Ecological Index

L	T	C	U	R	N	S
8	1	X	8	4	7	0

Gnaphalium supinum L.

G. supinum (ic. nova)

© Pignatti

Family: Asteraceae

Biological form: H scap (herbaceus and perennial plant, erect)

Choritype: Arctic - Alpine

Flowering: VII-VIII

Altitude: 2400-3400 m

Morphological description: 2-10cm height. Grey and hairy, forming dense bushes and dwarf, with short and ascending stems. Linear-lanceolate leaves (1-2cm). Flowers grouped in capitulum, in groups of 1-5 on axils of upper leaves. Only tubular flowers, tiny. Achene fruit with short pappus.

Habitat: Snowbeds and scree slopes with long lasting snow cover (siliceous).

Ellenberg's Ecological Index

L	T	C	U	R	N	S
7	1	6	7	3	3	0

Leucanthemopsis alpina (L.) Heyw.

Family: Asteraceae

Biological form: H scap (herbaceus and perennial plant, erect)

Choritype: Orof. SW-Europe

Flowering: VII-IX

Altitude: 2000-3600 m

Morphological description: 5–15 cm height, reddened at the top. Simple and erect stem, monocephalic, glabrous or tomentose, with some little leaves. Basal leaves grouped in rosetta, petiolate, fleshy, pennatifide, with 5 -7 short laciniae. The few leaves of the scape are sessile, linear and undivided. Flowers grouped in capitulum of 2–4 cm in diameter. The disk floret are tubular, yellow, with wingless corolla. The ray floret are ligulate, white and about 12 mm long. Achenes about 3-4 mm long.

Habitat: Landslide slopes and scree, cliffs (pref. siliceous soils)

L. alpina

© Pignatti

Ellenberg's Ecological Index

L	T	C	U	R	N	S
8	1	4	7	2	2	0

Veronica alpina L.

Family: Scrophulariaceae

Biological form: H scap

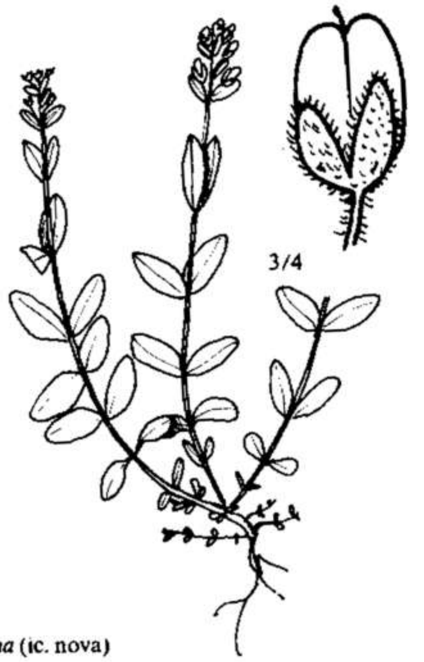
Chorotype: Arctic - Alpine - Eurasian

Flowering: VI-VIII

Altitude: 1500-3000 m

Morphological description: erect undivided stem, sessile leaves, oval to elliptical, obtuse or acute, with scattered and ciliated hairs; racemes with few flowers, lower bracts are usually elongated, opposite and hairy, floral peduncles are shorter than bracts (about half or less), calyx of 3-4 mm, with 4 oblong laciniae, blue with corolla with diameter of 4-7 mm; elliptical capsule higher than the calyx, hairy, bluish when ripe, bearing numerous small and orange seeds.

Habitat: common in snowbed, more rarely in the alpine meadows, in the windy ridges and edges of streams.



V. alpina (ic. nova)

© Pignatti

Ellenberg's Ecological Index:

L	T	C	U	R	N	S
7	1	5	6	X	3	0

CHAPTER 1.2 ARTIFICIAL WARMING

An OTC in field, Gavia Mount on the back

(Photo: Giulietta Bernareggi)

The International Tundra Experiment (ITEX) is a collaborative effort involving scientists from more than 11 countries, including all the Arctic, Subarctic and Alpine nations. The ITEX seeks to examine the response of circumpolar cold adapted plant species and tundra ecosystems to environmental change. Empirical knowledge based on experiments coupled with available evolutionary history, ecology, and genetics was chosen as the best way to predict species response to climate change. The ITEX research uses the OTCs (passive Open Top field Chambers) as a common tool to simulate global warming, since such a system is considered the most suitable for the majority of the tundra environments (Marion et al. 1997). In this study, the warming has been produced using tetragonal OTCs made of polymethylmethacrylate (PMMA, ACRIDITE® ACRISUN, Plastidite, Italy). The chambers were built using a modified version of the ITEX protocol; the dimensions, similarly to other studies (e.g. Sandvik et al. 2004) are: 28 cm high, diameter of 50 cm at the top and 90 cm at the base, 3 mm thick, inclined at 60° and hooked with a total of 8 metal hinges (6×2.5 cm).

The warming treatment started in 2008. From the end of June to the mid of July 2008, at snowmelt dates, 20 plots were randomly located within the study area. The OTCs were placed in 10 randomly selected plots; the other 10 plots were left as natural control. Every year, the OTCs were placed in the field immediately after snow melting and recovered at the end of growing season, at the timing of snowfall. Hereafter, the warming treatment will be referred to “W” and the control (i.e. plants exposed to natural climate) with “C”.

Soil and Air temperatures were measured with a number of data-loggers (HOBO and Tecnosoft) variable between a minimum of 2 and a maximum of 6 for each treatment (C and W) during the years 2008-2015. The loggers were placed in field after the snowmelt and recovered at the end of the growing season. The data, hourly acquired, were expressed as daily mean temperatures.

The data showed that the chambers produce a significant increase of the temperatures of both soil and air; such an increase is consistent with the estimated temperature increase predicted by the thermal models.

The warming produced significant differences between air and soil temperatures during the snow-free period. However, the increase in the soil temperatures was more constant than that of air temperatures, that showed a progressive reduction during the season (in average +2.3, +1.9 and +1.4 °C in July, August and September, respectively).

CHAPTER 1.3 SEED LONGEVITY

Seedling of *Leucanthemopsis alpina* emerged during a laboratory controlled germination test

(Photo: Giulietta Bernareggi)

1.3.1 Introduction

In the last century, Earth's mean global surface temperature increased considerably. Temperatures are expected to further increase in the next years (IPCC 2013), particularly in high-elevation and high-latitude ecosystems, with important consequences for the survival of several alpine/arctic plants (e.g. Theurillat and Guisan 2001). In response to climate warming there are documented changes in phenology, physiology, distribution (as upward shift) and seedling establishment of several alpine species (Grabherr et al., 1994;

Sandvik et al., 2004; Klanderud and Totland, 2005; Lenoir et al., 2008; Gottfried et al., 2012; Mondoni et al., 2015). In this context, seeds are thought to play an important role, being the main vehicle for plant migration (Parolo and Rossi 2008), regeneration (Walck et al., 2011) and persistence (Schwienbacher and Erschbamer 2001). The impact of climate change on regeneration from seeds is therefore of growing interest, especially in arctic (e.g. Shevtsova et al., 2009; Milbau et al., 2009) and in alpine (Mondoni et al., 2012; Hoyle et al., 2013) environments. Despite this, little is known about the effects of global climate change on the ability of seeds to remain viable for a long time. However, the longevity of seeds is a fundamental plant trait, being a prerequisite for seed persistence in the soil and allowing long-term (tens or hundreds of years) *ex situ* plant conservation under seed bank conditions (Walters et al., 2005).

In orthodox seeds (*sensu* Roberts 1973), the longevity of seed is mainly determined by seed moisture content and temperature, with lifespan increasing with decreasing temperature and moisture content (Ellis and Roberts 1980; Murdoch and Ellis 2000; Bewley et al., 2013). By means of experimental alteration of these two factors in laboratory, it is possible to accelerate the ageing process and thereafter seed longevity can be predicted on the base of the viability parameters extrapolated from seed survival curve (Ellis and Roberts, 1980). These parameters, which refer to the initial quality and the rate of deterioration over time of seeds (respectively denoted as K_i and σ^{-1} in the seed viability equation, see below), allow the comparison of seed viability between different seed lots and, consequently, can be used to assess environmental induced changes in potential seed longevity. Moreover, accelerated ageing experiments have been used to predict seed life span under seed bank conditions (Probert et al., 2009) and seed persistence in the field (Long et al. 2008). More recently, results from controlled ageing tests have been considered useful indicators of the development of heat resistance in relation to post-dispersal environmental conditions (Mondoni et al., 2014). In this regard, the climate experienced by plants has been demonstrated to have a large influence on seed longevity. For example, Probert et al. (2009) showed that seeds of species from cool, wet climates are shorter lived than those of species from warm, dry climates. Similarly, in Mondoni et al. (2011) seeds of alpine plants were shorter lived compared to populations or related species from lower elevation (and consequently warmer) habitats. These evidences suggest that a cold and wet post-dispersal environment may contribute to preserve seed from deterioration (see Bewley et al., 2013); therefore, the higher seed longevity of plants from warmer and drier climate might be an adaptation to cope with more stressful post-dispersal environmental conditions, not able to prevent the deterioration of seeds.

Moreover, seed longevity has shown significant transgenerational changes associated with short-term environment-induced effects (Kochanek et al., 2011), suggesting that differences between collections could also be driven by plastic responses to the local environment or seasonality. Indeed, the ability of maternal environment to affect offspring phenotype, through changes in gene expression, may potentially allow progeny adaptation to habitat conditions experienced by the maternal parent (Luzuriaga et al., 2006; Hoyle et al., 2008; Donohue 2009; Nicotra et al., 2010). Functionally adaptive transgenerational effects, elicited by environmental stress, are known to affect several plant traits in the offspring, including germination and seedling growth (Meyer and Allen 1999; Boyko et al., 2010), biomass (Kou et al., 2011), and fruit and seed production (Whittle et al., 2009). More recently, Mondoni et al. (2014) found that when an alpine seed lot is grown in a warmer lowland environment, seed longevity (of the progeny) is greatly enhanced, indicating strong adaptive responses to the local environment. Adaptive adjustments of seed longevity due to transgenerational plasticity may therefore play a fundamental role in survival and persistence of the species in the face of future environmental challenges, such as climate warming. For example, while a warmer climate could modify soil conditions (e.g. temperature) influencing seed preservation (Ooi et al., 2009), the potential increase in seed resistance to heat stress driven by the maternal effects (see above) could buffer seeds against such thermal variations in their environment. However, such an interesting possibility, in the context of climate warming, still needs to be investigated.

Within the alpine life zone, snowbed communities are currently considered among the most threatened habitats by climate warming. Indeed, the ongoing changes in snowbed vegetation structure and composition (Virtanen et al., 2003; Elumeeva et al., 2013; Sandvik and Odland, 2014; Carbognani et al., 2014a) are expected to lead, at long term, to a reduction or the disappearance of these habitats (Theurillat and Guisan, 2001), with the consequent loss of a substantial component of the alpine biodiversity (Björk and Molau, 2007). This especial sensibility to the future climatic scenarios makes snowbeds suitable natural laboratories to analyse the effects of climate change on alpine vegetation (Björk and Molau, 2007; Schöb et al., 2008a).

Here, in order to investigate whether and how global warming could affect seed longevity, four alpine species inhabiting snowbed habitats were exposed to a moderate temperature warming in their growing area. The seeds produced from plants exposed to warming and to natural conditions were used to determine how seed longevity responded to changes in the parental environment. In particular, specific research questions were: do seeds produced by plants under warmer temperatures show higher resistance to ageing? Is such increase consistent across the species studied?

1.3.2 Methods

Study area

See Chapter 1.1 Study Area and Study Species.

Experimental design

See Chapter 1.2 Artificial Warming.

Microclimatic features

In each plot, the snowmelt dates were monitored by direct observations. Air (5 cm height) temperatures of the snow-free period were recorded at hourly interval in four randomly selected plots two plots per treatment (C and W), using four probes in total (Onset, Cape Cod, MA, USA and Tecnosoft, Milan, Italy). In addition, four soil moisture readings (5 cm depth, Onset, Cape Cod, MA, USA) were placed in the same plots; these probes recorded the volumetric water content (by the Time Domain Reflectometry technique) every hour, then transformed in relative water content to saturation (RWC).

Seeds collection

Between 19 Aug. and 07 Sep. 2012, seeds of *Cardamine alpina* Willd. (Brassicaceae), *Leucanthemopsis alpina* (L.) Heywood (Asteraceae), *Gnaphalium supinum* L. (Asteraceae) and *Veronica alpina* L. (Scrophulariaceae) were collected at the time of natural dispersal from both heated and non-heated plots. Such forbs, selected among the most frequent and abundant species in the study site, are characterized by different flowering timings. In particular, the anthesis of *C. alpina* is the earliest in comparison with the other species ("early" flowering class *vs* "intermediate" flowering class, respectively) (Petraglia et al., 2014a). The number of replicates (plots) per species and treatments varied (from 3 to 6) according with the occurrence and abundance of each species/seeds inside the plots (see Table 2a). After collection, seeds were cleaned and held dried at seed bank condition (15 % RH and 15 °C, Diekmann et al., 1994) until use. The high initial viability (> 80 %, Online Resource 1) of fresh seed samples was checked through germination tests at conditions previously found to be optimal for germination (25 °C constant + addition of 250 mg L^{-1} GA_3 at 12-h daily photoperiod) (Table S1).

Experimental ageing

Seed longevity was determined using a standard rapid ageing protocol (Newton et al., 2009). Ageing experiments commenced in Nov. 2012 and were concluded after approximately 5 months. For each seed lot (i.e. the collection of seeds from a single plot), six samples of 50 seeds for *V. alpina* and seven samples of 50 seeds for the other species were transferred to a electrical enclosure box, over a non-saturated solution of LiCl at 60 % RH, placed in a compact incubator (Binder FD53, BINDER GmbH – Headquarters, Tuttlingen, Germany) without light at 45 ± 2 °C. The RH generated by the LiCl in the box was checked with a data logger with LCD display of current readings placed inside the enclosure box (Tinytag View 2 Temperature/Relative Humidity

Logger, Gemini Data Logger, Chichester, UK). The bulk solution was adjusted, if necessary, by adding distilled water, stirring and allowing the solution to equilibrate (Hay et al., 2008; Newton et al., 2009). One sample of 50 seeds for each seed lot was removed after 2, 4, 6, 8, 10, and 15 d for *V. alpina* and 2, 5, 10, 15, 20, 25, and 30 d for the other species for germination testing.

Germination test

Seeds were sown on 1 % distilled water agar held in 50 mm diameter Petri dishes and placed in a temperature and light-controlled incubator (LMS 250A, LMS Ltd, Sevenoaks, UK) at conditions previously found to be optimal for germination (25 °C constant + addition of 250 mg L^{-1} GA_3) and 12-h daily photoperiod (photosynthetically active radiation 40–50 µmol m^{-2} s^{-1}). Plates were checked weekly for germination and seeds scored as germinated once the radicle had reached approximately 2 mm. At the completion of each germination test (4 weeks after sowing), ungerminated seeds were cut-tested to confirm whether there were empty seeds. Ungerminated full seeds were assumed to be killed or weaken by the ageing treatments, since there were no other constrains to germination, while ungerminated empty seeds were excluded from the following analyses.

Data analysis

For each species, a Generalized Linear Model, with binomial error and probit link function, was fitted to the survival data from the germination test (number of seeds germinated, number of seeds sown) performed after the experimental ageing. This analysis was carried out using GenStat Release 15 (VSN International Ltd., Oxford, UK), with storage period as covariate and both treatment (C or W) and replicates (plots) as factors, thereby fitting the viability equation (Ellis and Roberts, 1980; Hay, 2004):

$$\upsilon = K_i - (p/\sigma)$$

This equation states that the probit of percent viability after any storage period (υ, expressed in normal equivalent deviates, NEDs) is equal to the initial viability (K_i, or the intercept on the y-axis, in NEDs) decreased by the seed deaths that can be expected in that time period (p) according to the slope (σ^{-1}) of the line, so that σ is the time (in day) for viability to fall by 1 NED (i.e. 1 standard deviation of the normal distribution of seed deaths over time).

In order to detect differences between species of mean seed longevity (p_{50}) and to test if experimental warming differently affected species p_{50}, two-ways analysis of variance (ANOVA), followed by multiple comparison Tukey HSD post-hoc test, was carried out. Prior to analysis, data were log-transformed to meet linear model assumptions.

In addition, to study the effects of treatment and storage period on the germination timing, the mean time to germinate (MTG) was calculated using the formula:

$$\mathrm{MTG} = \sum_{\mathrm{i,n}} n_i\, t_i \,/\, N$$

where n_i is the number of seeds that emerged within consecutive intervals of time, t_i the time between the beginning of the test and the end of a particular interval of measurement, and N the total number of seeds that emerged. The MTG was calculated using the day of sowing as initial time. The responses of MTG to the different treatments and storage periods were analysed by means of Generalized Linear Model with gamma error.

These latter two analyses were performed using the software R, version 3.1.2 (R Core Team, 2014).

1.3.3 Results

Microclimatic features

The snowmelt time was similar in control (C) and warmed (W) plots (Table 1), with an average date corresponding to 24 Jun. 2012, and the snow-free period lasted until 01 Oct. 2012 (ca. 100 d). Conversely, the time of seed dispersal began earlier (–11 d) in W compared to C (Fig. 1), lasting for about a month in both treatments (29 and 30 d in C and W, respectively).

	Treatment	
Microclimatic features	C	W
Snowmelt time (DOY)	176.3 ± 4.5	176.0 ± 4.0
Beginning of seed dispersal (DOY)	232	221
Air temperature (°C)		
Daily mean	8.61 ± 2.85	10.57 ± 3.12
Day time	11.72 ± 3.79	15.57 ± 4.60
Night time	5.49 ± 2.49	5.57 ± 2.55
Soil moisture (% RWC)		
Daily mean	72.81 ± 5.97	77.53 ± 7.90
Day time	72.69 ± 6.01	77.11 ± 8.01
Night time	73.04 ± 6.28	78.05 ± 7.92

Table 1 Microclimatic features of control (C) and warmed (W) plots from the snowmelt until seed dispersal (18 Jun. – 19 Aug. 2012 and 19 Jun. – 8 Aug. 2012 for C and W, respectively). Values are mean ± SD. Snowmelt time and beginning of seed dispersal are expressed in day of the year (DOY), air temperature in °C, and soil moisture in relative water content (RWC, in % relative to saturation)

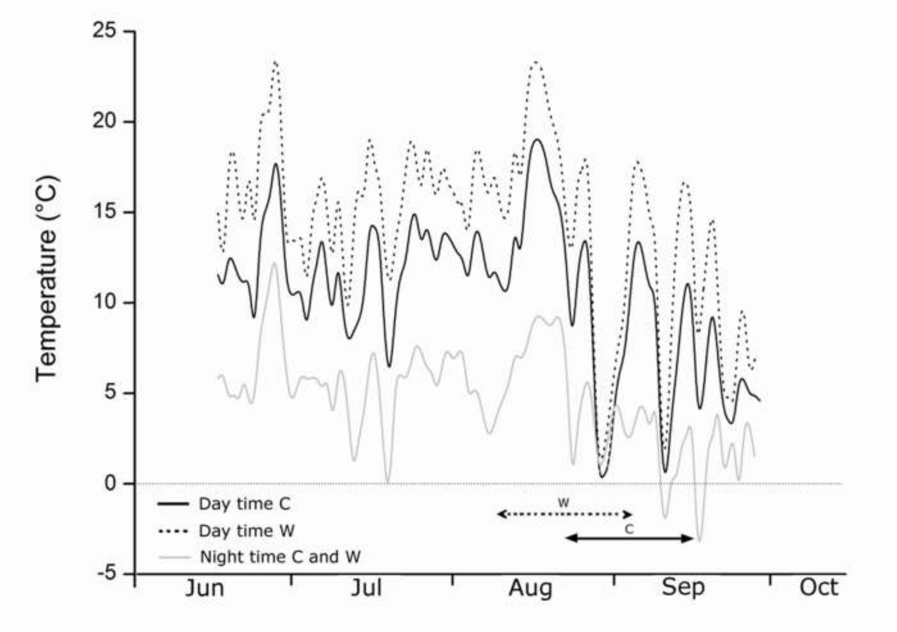


Figure 1 Mean day time (0800–2000 h, black lines) and night time (2000–0800 h, grey lines) air temperature (°C) at 5 cm above the soil surface in control (C, solid lines) and warmed (W, dotted lines) plots during the snow-free period (18 Jun.–01 Oct. 2012). Horizontal arrows indicate the period of seeds dispersal in the control (C, solid line) and warmed (W, dotted line) plots

During the period between the timing of snowmelt and the beginning of seed dispersal, the mean day time air temperature (i.e. 0800 – 2000 h) was higher in W compared to C (on average, +3.8 K), whilst mean night time air temperature (i.e. 2000 – 0800 h) was similar in the two treatments (Fig. 1). The overall mean warming produced by the OTCs in daily air temperature was about 2 K. The relative soil water content showed high values throughout the growing season in both treatments (> 70 % RWC), with slightly higher values in W in comparison with C (on average, +4.7 %), somewhat constant during the diurnal and nocturnal periods.

Seed longevity

Seed viability declined as the period of experimental ageing (45 °C; 60 % RH) increased for all species studied (Fig. 2), with a significant variation in the time taken for viability to fall to 50 % (p_{50}) between species and treatments, but not for the interaction species × treatment (Table 2b). In particular, seeds collected from plants under experimental warming were significantly longer-lived (p_{50} on average from 5.03 d for *V. alpina* to 23.06 d for *G. supinum*) compared to those produced by plants exposed to natural climate (p_{50} on average from 4.87 d for *V. alpina* to 16.80 d for *C. alpina*). However, the increase in p_{50} was not statistically different across the species (Table 2b).

(a)

Species	Treatment	n	K_i (NEDs)	σ^{-1} (days^{-1})	p_{50} (days)
Cardamine alpina	C	5	3.09 ± 0.30	0.18 ± 0.02	16.80 ± 0.56
	W	5	4.44 ± 0.46*	0.22 ± 0.02	20.32 ± 0.52*
Gnaphalium supinum	C	6	2.75 ± 0.26	0.18 ± 0.02	15.06 ± 0.56
	W	6	1.86 ± 0.18*	0.08 ± 0.01*	23.06 ± 1.16*
Leucanthemopsis alpina	C	3	1.91 ± 0.22	0.18 ± 0.02	10.77 ± 0.64
	W	3	1.52 ± 0.18	0.11 ± 0.01*	13.61 ± 0.84*
Veronica alpina	C	5	2.81± 0.33	0.58 ± 0.06	4.87 ± 0.21
	W	5	2.08 ± 0.26	0.41 ± 0.04*	5.03 ± 0.26*

(b)

Source of variation	Df	SS	MS	F-ratio	P-value
Species	3	28.54	9.514	220.37	**<0.001**
Treatment	1	2.35	2.345	54.32	**<0.001**
Species × Treatment	3	0.23	0.075	1.76	0.176
Residuals	30	1.30	0.043		

Table 2 (a) Generalized linear model results on the differences between treatments (C: control, W: warming) of survival curve parameters for each species, including initial viability (K_i, in normal equivalent deviates (NEDs)), time for viability to fall by 1 NED (σ^{-1}, in days^{-1}), time for viability to fall to 50 % (p_{50}, in days). Values are mean ± SE for *n* seed lots. Asterisks indicate a significant ($P<0.05$) difference between C and W, (b) Analysis of variance on effects of species and treatment on p_{50}. Degrees of freedom (df), sum of squares (SS), mean square (MS), F-ratio, and significance level (P-value) are shown. Significant values are highlighted in bold

In *G. supinum*, *L. alpina* and *V. alpina*, the difference in seed longevity was caused by significant ($P < 0.05$) differences in seed deterioration rate (σ^{-1}), highlighting a slower rate of deterioration in W compared to C (Table 2a, Fig. 2). In these species the initial seed viability (K_i) was lower in W compared to C, though statistically significant ($P = 0.005$) only in *G. supinum*. Conversely, in *C. alpina* the difference in seed longevity was due to a significant increase ($P = 0.014$) of initial seed viability (K_i) from warmed plots, while the rate of deterioration (i.e. the slope term, σ^{-1}) did not differ between treatments. Finally, *V. alpina* had a significantly shorter-lived seeds compared to the other species, while seeds of *L. alpina* were shorter-lived compared to those of *C. alpina* and *G. supinum*. These latter species showed similar p_{50}, both in C (on average 16.80 and 15.06 d for *C. alpina* and *G. supinum*, respectively) and in W (on average 20.32 and 23.06 d for *C. alpina* and *G. supinum*, respectively).

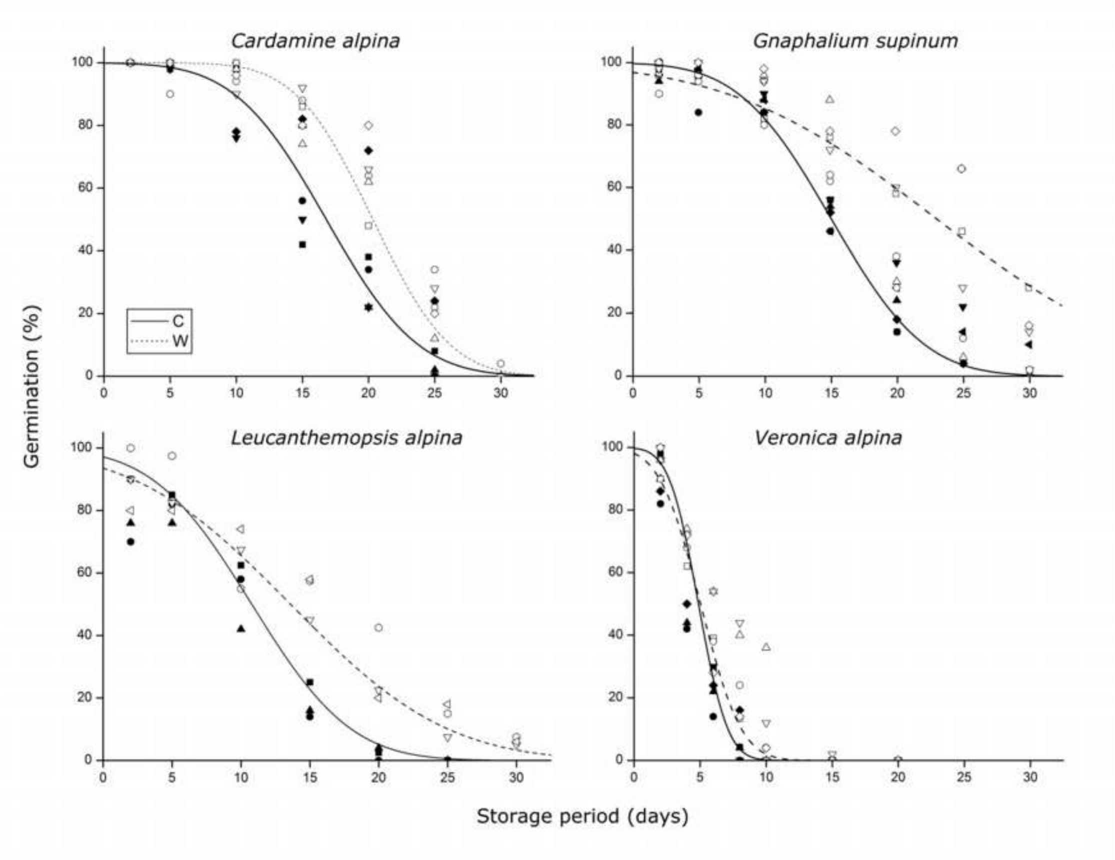


Figure 2 Survival curves fitted using a Generalized Linear Model, with binomial error and probit link function, for seed lots collected from control plots (C, black symbols, continuous lines) and warmed plots (W, open symbols, broken lines), see model parameters in Table 2. Different symbols refer to different seed lots (i.e. plots)

Mean time to germination

Significant differences in the MTG of seeds were found between species ($P < 0.001$) and storage period ($P < 0.001$), but not between treatments ($P = 0.11$). Furthermore, because of the significant species × storage period interaction ($P < 0.001$), species specific analyses were also performed (Table 3; Fig. 3). We found that the treatment had no effect, in any of the species studied. Moreover, the increase in storage period produced a significant increase of the mean time for germination in *C. alpina* and *V. alpina*, but not in *G. supinum* and *L. alpina*. Such increase was similar within the treatments (interaction not significant, $P = 0.37$) in *C. alpina*; conversely, in *V. alpina* it was more pronounced for seeds experiencing current climate than those under warming (Table 3; Fig. 3).

	Df	SS	F-ratio	P-value
Cardamine alpina				
Treatment (Tr)	1	0.29	1.07	0.31
Storage period (St)	1	1.56	57.70	<0.001
Tr × St	1	0.02	0.82	0.37
Gnaphalium supinum				
Treatment (Tr)	1	0.03	0.68	0.41
Storage period (St)	1	0.00	0.00	0.99
Tr × St	1	0.03	0.55	0.46
Leucanthemopsis alpina				
Treatment (Tr)	1	0.00	0.01	0.94
Storage period (St)	1	0.18	3.18	0.08
Tr × St	1	0.01	0.23	0.6
Veronica alpina				
Treatment (Tr)	1	0.01	0.27	0.60
Storage period (St)	1	3.29	73.61	<0.001
Tr × St	1	0.36	8.09	0.01

Table 3 Species specific Generalized Linear Model (with gamma error) results on the effects of treatment (C and W) and storage period (days under ageing conditions) on the seed mean time to germinate

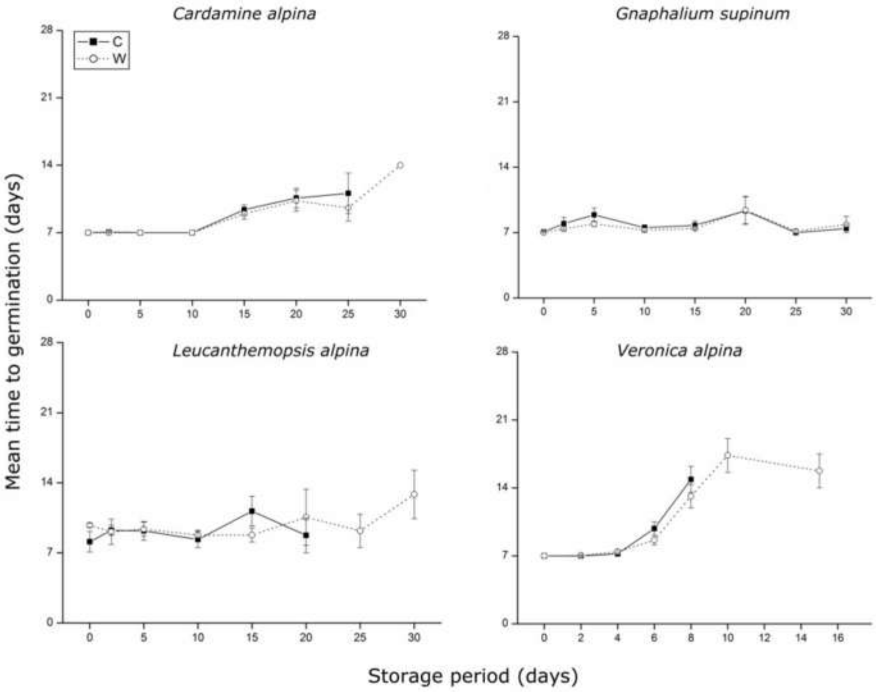


Figure 3 Mean time to germinate (MTG, mean ± SE) of control (full dots and continuous line) and warmed (empty dots and dotted line) seeds of *C. alpina*, *G. supinum*, *L. alpina* and *V. alpina* for different periods of storage

1.3.4 Discussion

The results presented here showed, for the first time, that under a moderate climate warming scenario (i.e. +2 K, IPCC 2013) alpine plants will produce seeds with an extended resistance to heat stress (Table 2a; Fig. 2), indicating a potential adaptive response to a warmer environment. Indeed, despite controlled ageing test at high humidity and temperature (60 % RH, 45 °C) may not reflect all the complex ageing mechanisms occurring in nature, seed life span under such test and persistence in the soil seed bank were found positively related (see Long et al., 2008). Controlled ageing test may therefore have an ecological significance, conceiving (at least) some of the major causes of ageing in nature. In this regard, in seasonal climates, which alternate dry-wet and/or cold-warm periods (even daily) the possibility that seeds buried in the top soil experience conditions similar to the controlled ageing test cannot be ruled out. For example, seed beds of alpine plants on dark humic soils exposed to full sunlight heat up to temperature around 80 °C (Alps of Tirol, Turner, 1985; Australian Snowy Mountains, Korner and Cochrane, 1983). Similarly, in wetter and vegetated soils, like those in snowbeds, leaf temperature of *Salix herbacea* under full solar radiation in midsummer was up to 46.5 °C (Larcher and Wagner, 1976). Furthermore, seed degenerative processes are mostly temperature and humidity dependent and the rule is that the higher is the temperature and/or humidity (at least up to c. 99% RH, when repair processes may be activated), the faster is the deterioration (Bewley et al., 2013). This might explain why species from cold climates show lower selective pressure for seed resistance to heat stress, compared to those from warm climate (Mondoni et al., 2011; Probert et al., 2009). Seeds of alpine plants can anyway persist for many years in soil seed bank (Schwienbacher et al., 2010), likely because the low average temperatures contribute to conserve and protect them from deterioration. Interestingly, our results showed that resistance to heat stress may increase if parent individual experience warmer temperature, suggesting a buffer response against climate change.

Climate has a large influence on seed traits, including longevity, and starts to act also before dispersal time. In particular, the environmental conditions experienced during seed development and maturation are known to affect the longevity of seed. For example, Sinniah et al. (1998) found an improvement in seed longevity when parental plants of *Brassica campestris* were subjected to drought during seed development. Furthermore, Kochanek (2008) found that elevated temperatures during seed maturation in *Wahlenbergia tumidifructa* led to increased seed longevity and, accordingly, our results showed higher longevity under warmer parental growth environment. Seed resistance to ageing is related with the degree of their maturity (Ellis et al., 1993; Ellis and Hong, 1994; Hay and Probert, 1995; Sanhewe and Ellis, 1996), with longevity being highest at the peak of seed maturity (Hay and Smith, 2003). Hence, a possible explanation of the increased resistance to ageing of the seeds produced under warming is that the warmer temperature during development leads to a better seed maturation level. This is consistent with the increase of initial seed viability (*Ki*) of *C. alpina* under warming, but does not explain the lower and the similar *Ki* values in *G. supinum* and the other species, respectively, under the same treatment (Table 2a). Therefore, other mechanisms are likely to be involved in the increased tolerance to ageing stress in seeds produced by plants exposed to warming.

Phenotypic plasticity induced via parental effect is known to affect seed longevity. Kochanek et al. (2011) showed that the conditions that parent plants experience prior to the seed set may alter seed longevity, highlighting that this transgenerational adjustment was passed to the offspring. Phenotypic changes induced to the offspring are expected to be crucial determinants in ensuring plants to cope with future environmental challenges, since they may be adaptations to conditions experienced by the mother plants (Galloway and Etterson, 2007; Herman and Sultan, 2011). In the context of climate warming, maternal induced novel phenotypes will provide a fundamental support in the rapidly changing environment, safeguarding the progeny performance and assisting long-term adaptation (Nicotra et al., 2010). Following this view, the enhanced resistance of alpine seeds to heat stress observed in this study could be a plastic maternal-induced adjustment to cope (or persist) with the new warmer and non-conservative environment.

Within the alpine life zone, the ability of parental plants to produce seeds more resistant to warm temperature is therefore expected to assume a strong adaptive value, crucial for the survival of alpine species, since the longer the seeds are able to survive the more likely the population is buffered against unpredictable changes in the environment (Chesson, 2000; Facelli et al., 2005). However, such adaptive potential may not be enough to ensure an efficient response to the future environmental challenges. Indeed, the plastic response of seed longevity induced by parental environment is locked by the species genetic background (Mondoni et al., 2014), meaning that it cannot fully adapt to environmental alterations. Adaptive adjustments of seed longevity by means of transgenerational plasticity will represent a valid response for alpine species to rapid climatic changes, but the extent to which alpine plants will be able to respond to alterations of greater magnitude and/or for long-term period is still unknown.

The importance that interspecific variations of plant traits plays in driving species coexistence is essential to model the future dynamics of communities (Bolnick et al., 2011; Violle et al., 2012). Our species showed different responses from each other both in the mean seed longevity (p_{50}) and in the mean time to germination (MTG). First, the results revealed that interspecific variations of p_{50} were higher compared with those due to the experimental warming (F-ratio in Table 2b). This high interspecific variation could reflect the different plant reproductive strategy; for example, the larger amount of seeds produced by *V. alpina* (in average, 164.57 seed per individual in comparison with 24.82 of *C. alpina*, 23.53 of *G. supinum* and 25.98 of *L. alpina*) (unpublished data), could be a mechanism to buffer the low lifespan of its seeds, which was the shortest among the species studied. Conversely, the increase in the mean seed longevity (p_{50}) produced by the warming treatment was similar in all the species. However, this effect resulted from changes of different parameters of seed viability equation. In particular, the increase in seed longevity was due to either a higher initial viability of seeds (for *C. alpina*), or a slower seed deterioration rate (for *G. supinum, L. alpina, V. alpina*), highlighting that the response of seed viability to environmental changes can vary at the species level, even from the same habitat. These species-specific responses may be linked to different species phenotype expression and/or to differences in plant phenology. For example, the increase of initial seed viability of *C. alpina* could be somehow related with the early flowering of this species (compared to the other studied, see also Petraglia et al., 2014a), which shifted fruit and seed maturation processes during the warmest part of the growing season. With regard to the MTG, our results showed that it increased with the increasing in the storage period in *C. alpina* and *V. alpina*. Interestingly, seeds of *V. alpina* were the shortest-lived among those of the studied species and they also showed the higher increase in the germination timing during storage, suggesting that the response to MTG may help to differentiate between long- and short-lived species. Here we have shown that seed longevity is affected by environmental cues acting during different stages of seed development. Therefore, the observed potential increase in seed longevity under climate warming is a parameter that should be considered in modelling future vegetation dynamics and responses to climate change. Moreover, the sensibility of the species studied here to a moderate temperature variation suggests that there could be implications for long-term conservation in seed banks, since seed samples may present phenotypical fluctuations to inter annual variations. In this regard, understanding whether and how the longevity of seeds will change in a future climate is crucial also for the effective management of collections held in seed banks. For example, protocols should be overhauled considering the interaction between genetic background and the influence of the climate that parental plants experience. More appropriate regeneration scheduling may therefore ensure collections maintain high levels of viability in dry storage for long-term periods.

1.3.5 Supplementary Material

Table S1 Germination percentage (mean ± SE) of fresh (day 0) and aged seeds (days 2–30 and 2–15) from warmed (W) and control (C) plots, tested at the condition previously found to be optimal for germination (25 °C constant + addition of 250 mg L^{-1} GA_3, with 12-h daily photoperiod)

			Germination (%)							
Species	Treatment	*n*	0d	2d	5d	10d	15d	20d	25d	30d
Cardamine alpina	C	5	100	100	99.2±0.5	90.0±5.3	62.0±8.1	37.6±9.2	6.8±4.54	0
	W	5	99.6±0.4	100	98.0±2.0	95.6±1.7	84.0±3.2	64.0±5.2	23.2±3.7	0.8±0.8
Gnaphalium supinum	C	6	99.0±0.6	97.3±1.0	96.0±2.5	88.0±1.6	51.7±1.9	22.3±3.6	10.3±2.9	2.0±1.6
	W	6	96.5±2.4	97.0±1.5	96.7±1.1	90.7±3.1	73.3±3.9	48.7±8.1	37.3±10.7	10.0±4.6
Leucanthemopsis alpina	C	3	93.6±2.1	78.7±5.9	81.0±2.6	54.2±6.2	18.3±3.4	2.2±1.2	0.0±0.0	0
	W	3	81.7±0.8	90.0±5.8	86.7±5.5	65.5±5.6	53.5±4.3	28.3±7.1	13.5±3.1	6.2±0.7
			0d	2d	4d	6d	8d	10d	15d	
Veronica alpina	C	5	95.04±2.8	89.8±1.9	55.5±4.1	27.0±3.9	5.5±2.4	0.3±0.3	0.0±0.0	
	W	5	97.1±2.3	96.0±1.5	69.8±2.4	37.0±4.7	21.9±4.6	9.5±4.2	1.2±0.8	

1.3.6 Tables and Figures Index

TABLES

FIGURES

CHAPTER 1.4 SEED DORMANCY

Seeds of *Cerastium cerastoides* during the cleaning process in laboratory

(Photo: Giulietta Bernareggi)

1.4.1 Introduction

Climate has a dominant influence on several life-history traits of species. Among plant reproductive phases, seed germination and seedling establishment are probably the most sensitive to variation in climate conditions (Walck et al., 2011) and represent the major bottleneck to species recruitment (Lloret et al., 2004; Fay and Schultz, 2009; Dalgleish et al., 2010). The strong correlation between climate and plant regeneration from seeds has resulted in the evolution of specific germination requirements across many species (Baskin et al., 2000; Fenner and Thompson, 2005), which play a key role for plant distribution and vegetation dynamics (Silvertown and Charlesworth, 2001;

Higgins et al., 2003; Fenner and Thompson, 2005; Neilson et al., 2005; McGill et al., 2006; Pearson, 2006; Thuiller et al., 2008).

In seasonal climates, which are characterized by cyclic variations in temperature and/or rainfall, seed germination is usually synchronized with the changes in environmental conditions, being delayed until the favourable period occurs (Fenner and Thompson, 2005; Baskin and Baskin, 2014). A key mechanism that has enabled the development of such behaviour is seed dormancy, an innate automatism that involves environmental stimuli to avoid seed germination at the time of the year when seedlings emergence would not be successful, since the favourable conditions inducing germination might not persist long enough for seedlings to survive and grow (Vleeshouwers et al., 1995; Geneve, 2003; Finch-Savage and Leubner-Metzger, 2006).

Seed dormancy occurs in all the major angiosperm clades, and the different dormancy types and classes reflect plant adaptation to different climatic and habitat conditions (Finch-Savage and Leubner-Metzger, 2006; Baskin and Baskin, 2014). A commonly accepted hierarchical system of classification distinguishes five classes of seed dormancy: physiological, morphological, morphophysiological, physical, and a combinational dormancy (physical + physiological) (Baskin and Baskin, 2004). Within each class it is possible to distinguish different levels, which provide additional information about dormancy. For example, three levels are distinguished within physiological dormancy: deep, intermediate or non-deep; the latter is the most common kind of seed dormancy. At maturity, seeds with physiological dormancy may be conditionally dormant, and germinate over only a very narrow range of temperatures (Baskin and Baskin, 2014). Indeed, although there are many environmental factors (dormancy-breaking factors), such as moisture, light or chemicals, that contribute to change the dormancy state, temperature is the major driving force for the release or the induction of physiological dormancy (Fenner and Thompson, 2005). Consequently, after perceiving an adequate/accurate thermal stimulus, seeds can forthwith overcome the conditional dormancy and germinate or broaden the range of germination conditions until eventually become non-dormant (Fenner and Thompson, 2005; Baskin and Baskin, 2014). However, temperature can affect the state of dormancy also when it is experienced by seeds during maturation and, in most of the cases, warmer pre-dispersal temperatures result in seeds with lower dormancy and higher germinability (Fenner, 1991; Gutterman, 2000).

This pivotal role of temperature in driving seed dormancy responses highlights the need to understand how changing environmental conditions will affect seed germination patterns. In this regard, a large body of evidence supports the existence of rapid climate change, the main effect of which is the rise in mean global temperatures (IPCC, 2013). This will be particularly evident in high-elevation biomes, making these ecosystems, inhabited by species highly specialized to low temperatures, among the environments most threatened by the predicted climate change (Diaz and Bradley, 1997; Kullman, 2004; Nogués-Bravo et al., 2007). As a consequence, in the last decades, an increasing number of studies has addressed the effects of climate warming on different life-history traits and functions of plants in arctic and alpine tundra ecosystems (Chapin et al., 1995; Hobbie and Chapin, 1998; Arft et al., 1999; De Valpine and Harte, 2001; Dunne et al., 2003; Hollister et al., 2005; Milbau et al., 2009; Shevtsova et al., 2009; Mondoni et al., 2012; Hoyle et al., 2013; Oberbauer et al., 2013; Mondoni et al., 2015).

Seeds of many alpine species are known to be non-dormant or conditionally dormant at dispersal (Amen, 1966; Schwienbacher et al., 2011). Early studies on germination requirements in high elevation environments showed that freshly collected seeds required relatively high temperature (20 – 30 °C) to germinate (Sayers and Ward, 1966; Billings and Mooney, 1968; Chabot and Billings, 1972; Nishitani and Masuzawa, 1996). This requirement has been considered an adaptation to prevent seed to germinate immediately after dispersal, when early frosts could jeopardize seedlings survival (Billings and Mooney, 1968) and when the extremely short growing season could not be enough to allow seedlings to attain a critical mass for withstand the harsh and long-lasting winter conditions (Maruta, 1994). However, spring germination may occur at lower temperature than those required in the late season, since the cold stratification over winter widens the thermal window for germination towards lower values as a result of the loss of physiological dormancy (Baskin and Baskin, 2014). Hence, seedling emergence in alpine species usually occurs after the snowmelt (Körner, 2003), when seeds meet the environmental conditions (e.g. temperatures, moisture, light) required for germination. In response to this general pattern, seed germination of alpine/arctic species have been shown to vary considerably because of climate warming, for example increasing, reducing or shifting in time (see references above) and such variation was likely due to the different species and approach used (Shevtsova et al., 2009).

Nevertheless, all these studies have considered seeds developed and matured under current climate (i.e. not under warming scenarios). However, warming is not a seasonal-selective event, but acts during all the growing season; it is therefore necessary to consider the effect of the rise in temperature not only on dispersed seeds, but also during all the reproductive cycle phases. Temperatures during seed development and maturation could alter the state of dormancy, both in fresh and stratified seeds. In particular, warmer temperatures during seed maturation, due to altitudinal or inter-annual variations, have resulted in reduced seed dormancy, increasing the germinability of both fresh (Fernández-Pascual et al., 2013; García-Fernández et al., 2015) and cold-stratified seeds (Fernández-Pascual and Jiménez-Alfaro, 2014), or decreasing the duration of cold stratification to satisfy pre-germination vernalization requirements (Cavieres and Arroyo, 2000). Thus, seed dormancy seems to be affected by temperature variations both in space, i.e. seeds of populations from lower and warmer elevations being less dormant than those from higher and colder elevations, and in time, i.e. maternal plastic responses to local seasonality, with the general rule “the warmer the climate, the shallower the dormancy”. However, exceptions to this general pattern have been found, e.g. no differences were observed in the germination of fresh seeds of population of *Phacelia secunda* from different elevations (Cavieres and Arroyo, 2000). Moreover, whether and how a warmer parental environment affects the thermal interval for seed germination before and after the cold stratification (i.e. just after seed dispersal and over winter) is still not understood. To this end, the study of climate warming effects on seed dormancy and germination should decouple the effects of pre- and post-dispersal temperatures and analyse their interplay; to the best of our knowledge, no study has yet focused on this purpose.

Understanding the mechanisms affecting the development of seed dormancy plays a crucial role to forecast the future dynamics of alpine plant populations and communities in a warmer climate, since changing in thermal conditions are expected to affect both seed dormancy and germination requirements, which may preclude, delay, or enhance regeneration from seeds (Walck et al., 2011). This is especially true in topographically fragmented and azonal habitats, such as alpine snowbed communities. Snowbeds, which develop in sites characterized by a very short snow-free period, are regarded as especially sensitive to changes in environmental conditions, and, consequently, are retained among the most threatened vegetation types of the alpine life zone under the current climate change (Björk and Molau, 2007). Indeed, a growing body of studies indicates that the environmental conditions of these habitats, such as the length of the growing period, temperatures, and soil nutrients, constrain plant growth and reproduction (e.g. Galen and Stanton, 1995; Huelber et al., 2006; Schöb et al., 2008; Carbognani et al., 2012; Petraglia et al., 2013; Petraglia et al., 2014b; Bernareggi et al., 2015). Consistently, fast alterations in plant community composition and structure have been recently observed (e.g. Elumeeva et al., 2013; Carbognani et al., 2014a; Sandvik and Odland, 2014). The predicted climate change involves that plants colonising snowbeds will experience longer and warmer snow-free periods. Since the phenological development of snowbed plants is strongly controlled by micro-climatic conditions (e.g. Galen and Stanton, 1995; Huelber et al., 2006; Hülber et al., 2010; Petraglia et al., 2014a), it is likely that reproductive phenophases will significantly advance under earlier snowmelt coupled with warmer temperatures.

On these bases, the hypothesis that, in the next decades, seeds of snowbed plants will be dispersed during periods characterized by considerably warmer temperatures cannot be ruled out. Nevertheless, still little is known about the effects (direct and indirect) of climate warming on seed germination in snowbed environments, and even less about the effects on seed dormancy.

Here, we present the first attempt to evaluate the influence of a warmer parental plant environment on seed dormancy and seed germination requirements in an alpine snowbed habitat. To this aim, three alpine species inhabiting snowbeds were exposed to an experimental warming and to natural conditions in the field. Then, the germinability of seeds produced was tested in laboratory after six cold stratification periods combined with four incubation temperatures. In particular, we wanted to answer to the following questions:

a) Does a warmer pre-dispersal environment influence the germination responses of both fresh and cold-stratified seeds by 1) widening the temperatures range for germination and 2) increasing and speeding the final germination?

b) Are such responses further characterized by reduced duration of cold stratification requirements to overcome seed dormancy?

1.4.2 Methods

Study area

See Chapter 1.1 Study Area and Study Species.

Pre-dispersal treatment

See Chapter 1.2 Artificial Warming.

Microclimatic features

In each plot, the snowmelt dates were monitored by direct observations; soil (-5 cm depth) temperatures of the snow-free period from year 2008 to year 2014 were recorded hourly using 12 probes in total, 6 for W plots and 6 for C plots (Onset, Cape Cod, MA, USA and Tecnosoft, Milan, Italy).

Seed collection

Between 11 Sep. and 04 Oct. 2013, seeds of Cerastium cerastoides (L.) Britton (Caryophyllaceae), Leucanthemopsis alpina (L.) Heywood (Asteraceae) and Veronica alpina L. (Scrophulariaceae) were collected at the time of natural dispersal from both W and C plots. The choice of the species was made on the base of their frequency and abundance in the study site. Seeds collected from different plots of the same treatment were pooled together. After collection, seeds were processed (cleaned) and stored at room temperature until the beginning of the experiment, which occurred within one week after the last collection. The high initial viability (> 80 %) of seed samples was checked through germination tests at conditions previously found to be optimal for germination (20 °C constant + addition of 250 mg L–1 GA3, at 12-h daily photoperiod).

Cold stratification and incubation temperatures

Seeds were exposed to six different periods of cold stratification (0, 2, 4, 8, 12, 20 weeks) at 0 °C in complete darkness using a cooled incubator (LMS Ltd, Sevenoaks, UK). After each interval, seeds were incubated for germination at constant temperatures at 5, 10, 15, 20 °C. Constant temperatures were chosen in order to assess thermal influences isolating the effect of determinate temperature levels on seed germination (which would not have been possible under alternating regime). In this regard, preliminary germination tests confirmed that our species did not required the trigger effect of diurnal temperature variation (e.g. Mooney and Billings, 1961; but see Liu et al., 2013), germinating well (i.e. > 90%) under constant regime (data not shown). For each species, pre-dispersal treatment, stratification periods and incubation temperatures, three samples of 20 seeds (C. alpina and L. alpina) or two samples of 25 seeds (C. cerastoides) were sown on agar held in 50-mm diameter Petri dishes and tested for germination. All germination tests were carried out in temperature and light controlled incubators (LMS 250A, LMS Ltd, Sevenoaks, UK) using a 12-h daily photoperiod (photosynthetically active radiation 40 – 50 µmol m-2 s-1). Plates were checked weekly for germination and seeds scored as germinated once radicle protrusion and elongation was more than 2 mm. At the completion of each germination test (4 weeks after sowing), ungerminated seeds were cut-tested to confirm whether there were empty seeds. Ungerminated empty seeds were excluded from the following analyses.

Data analysis

Germination of fresh seeds

Influences of species, pre-dispersal treatments and incubation temperatures on germination of fresh seed (i.e. without cold stratification) were evaluated by means of Generalised Linear Model (GLM) with binomial error and logit link-function. In this GLM, final germination (i.e. number of emerged seedlings out of number of seeds sown) was the response variable, whereas species (as 3-level categorical variable), treatment (as 2-level categorical variable, C and W), temperature (as continuous variable, 5, 10, 15, 20 °C), and their two-way interactions were the fixed effects. In addition, species-specific GLMs were performed with final germination as response variable and pre-dispersal treatment, sowing temperature and their interaction as explanatory variables.

Moreover, to study the effects of warmer incubation temperatures on the speed of seedling emergence, the mean time to germination (MTG) was calculated using the formula:

$$\mathrm{MTG}=\sum_{i,n} n_i\, t_i / N$$

where n_i is the number of seeds that emerged within consecutive intervals of time, t_i the time between the beginning of the test and the end of a particular interval of measurement, and N the total number of seeds that emerged. The MTG was calculated using the day of sowing as initial time. The influences of species, pre-dispersal treatments, incubation temperatures, and their two-way interactions on MTG were analysed by means of GLM with gamma error structure and inverse link-function. Within each combination of species, pre-dispersal treatment, stratification periods, and incubation temperatures, seed lots that failed to germinate more than 1 % were excluded from the analysis, although still present in the figures for comparative purposes.

Germination after cold stratification

Differences in final seed germination between species, incubation temperatures, and stratification periods were assessed using GLM with quasibinomial error structure (due to overdispersion) and logit link-function. In this model, seed germination (i.e. number of emerged seedlings out of number of seeds sown) was the response variable, whereas species (as 3-level categorical variable), treatment (as 2-level categorical variable, C and W), temperature (as continuous variable, 5, 10, 15, 20 °C), stratification (as continuous variable, 0, 2, 4, 8, 12, 20 weeks at 0 °C) and their two-way interactions were the explanatory variables. In addition, in order to gain a better understanding of the influences of variation in incubation temperatures and stratification periods on seed germination, further GLMs were performed at the species level and model selection was carried out comparing residual deviance and AIC of different models. In particular, the relationships between temperature and stratification with germination were evaluated testing the explanatory power of GLMs fitted with different combinations of explanatory variables (incubation temperatures and stratification periods) as linear, log-transformed, or polynomial terms. Then, for each species, minimal adequate model was obtained excluding not significant terms following Crawley (2013).

In these analyses, the experimental units were the Petri dishes (each including 20 – 25 seeds) to which post-dispersal treatments (incubation temperatures and stratification periods) were independently applied. Nevertheless, to check for potential influence due to non-independence of seeds within a Petri (e.g. substrate, neighbour, and incubator effects), all models were re-run including Petri as random factor, with the same structures and selection procedures previously described (with the exception of error structure, since in case of overdispersion, quasibinomial distribution was not required due to the presence of the observation-level random effect). However, in all the cases, the two different approaches produced the same outcomes and, consequently, only the results of the GLMs were reported.

All analyses were performed using the R software (version 3.1.2, R Core Team, 2014).

1.4.3 Results

Microclimatic features

(A)		Soil Temperature (°C)	
Week of the year	Equivalent time of a normal year	C	W
27	02 – 08 July	9.7±0.1	11.0±0.1
28	09 – 15 July	10.2±0.3	11.0±0.2
29	16 – 22 July	8.8±0.2	10.6±0.2
30	23 – 29 July	8.8±0.1	10.3±0.1
31	30 July – 05 August	9.8±0.2	11.3±0.2
32	06 – 12 August	9.4±0.1	10.7±0.1
33	13 – 19 August	8.1±0.3	9.5±0.3
34	20 – 26 August	8.3±0.3	9.8±0.3
35	**27 August – 02 September**	8.1±0.1	**9.5±0.1**
36	**03 – 09 September**	**7.6±0.1**	**9.2±0.2**
37	**10 – 16 September**	**4.9±0.5**	6.1±0.4
38	17 – 23 September	4.7±0.2	6.3±0.2
39	24 – 30 September	5.9±0.2	7.2±0.3
40 – 41	*1 – 14 October*	*Snowfall*	

(B)		2013 Soil Temperature (°C)	
Month	Weeks of the year (2013)	C	W
July	29 – 31	10.8±0.8	12.7±0.7
August	31 – 35	7.7±1.1	9.3±1.1
September	35 – 40	4.9±1.2	6.5±1.1

Table 1 (A) Weekly soil temperatures (mean ± s.e. in °C) during the snow-free period of the years 2008 – 2014 in control (C) and warmed (W) plots. Different styling indicates the significant events of snowmelt (underlined), seed dispersal (bold) and snowfall (italic). (B) Monthly mean soil temperatures of the year 2013

During the period 2008 – 2014, the snowmelt at the experimental site occurred on average during the 27th week of the year (WOY), between the 25th WOY (in 2011) and the 29th WOY (in 2008). The mean weekly soil temperature during this week has been detected between 7.4 °C (in 2014) and 11.4 °C (in 2010) in C and between 8.4 °C (in 2014) and 12.0 °C (in 2010) in W (Table 1A). During the snow free period, which lasted 12 – 13 weeks, the OTCs (W) produced a mean soil warming of 1.5 K. The increase in temperature caused an advanced (of about 10 days) of the phenological phases and, coherently, of seed dispersal timing in W. Indeed, seed dispersal occurred between WOY 35 –

36 in W and between WOY 36 – 37 in C with a corresponding difference of soil temperature of about 3 K (Table 1A). The snowfall usually occurs at WOY 40 – 41, in the first half of October (Table 1A).

Seeds of the studied species were collected in 2013, between the 35th and 36th WOY in W and between the 36th and the 37th WOY in C; the mean monthly temperature of this growing season, consistent with the multi-year monthly mean, is shown in Table 1B.

Germination of fresh seeds

(a) Germination phenology

On fresh seeds (i.e. without cold stratification), significant differences in the final germination were observed among species, whose seeds showed a higher germination proportion under warmer incubation temperatures but were not influenced by the pre-dispersal treatment (Table 2A). Furthermore, the effects of [both] pre-dispersal treatment and incubation temperatures were not consistent among the species studied (i.e. significant interaction terms).

The increase in incubation temperatures resulted in a significant increase in germination percentage in all the species and treatments tested (Table 3). At the coldest temperature (i.e. 5 °C), germination percentage was very low or, in some cases, null in all the species and treatments (Fig. 1A). In particular, seeds of C. cerastoides germinated between 20 and 25 %, the germination of warmed seeds of L. alpina remained less than 1 %, while no emergence was found in control seeds of L. alpina and in V. alpina. At 10 °C, about 60 – 80 % of seeds of C. cerastoides and V. alpina germinated and, under higher incubation temperatures (i.e. 15 and 20 °C), the final germination percentage of these species remained, on average, at high levels. Conversely, germination of L. alpina remained low (< 7 %) until the temperature of 15 °C for warmed seeds and 20 °C for control seeds, after which it showed a significant, but moderate (up to 40 – 50 % in W and to 30 % in C), increase. The pre-dispersal warming had no effects on the germination of C. cerastoides and V. alpina (Table 3), but it determined a higher increase in seed germination of L. alpina (Table 3; Fig.

1A). Despite this, the final germination of this latter species remained lower (< 50 %) in comparison with those of the other two species.

(b) Mean time to germinate

Factor	Df	Dev.	Res. Df	Dev. Res	F-value	*P*-value
(A) *Final germination*						
Species (Sp)	2	276.9	61	711.9	33.30	**<0.001**
Pre-dispersal treatment (Tr)	1	1.2	60	710.7	0.30	0.589
Incubation temperature (T)	1	465.4	59	245.3	111.93	**<0.001**
Sp × Tr	2	26.9	57	218.4	3.24	**0.047**
Sp × T	2	42.4	55	176.0	5.10	**0.009**
Tr × T	1	6.1	54	168.9	1.46	0.232
(B) *Mean time to germinate*						
Species (Sp)	2	0.4	45	8.8	7.47	**0.002**
Pre-dispersal treatment (Tr)	1	0.0	44	8.8	0.15	0.702
Incubation temperature (T)	1	7.6	43	1.2	293.02	**<0.001**
Sp × Tr	2	0.2	41	1.1	3.11	0.056
Sp × T	2	0.1	39	1.0	1.44	0.250
Tr × T	1	0.0	38	1.0	0.02	0.895

Table 2 Results of GLMs on the effects of species (Sp), pre-dispersal treatment (Tr), incubation temperature (T), and their interactions on (A) final germination and (B) mean time to germinate of fresh seeds (i.e. without stratification). The models were performed with binomial error and logit link-function for the final germination and gamma error and inverse link-function for the mean time to germinate. Seed lots that failed to germinate more than 1% were excluded from the analysis of B. Degrees of Freedom (Df), Deviance (Dev.), Residual Degrees of Freedom (Res. Df), Residual Deviance (Dev. Res), *F*- and *P*-value are shown. Significant values are highlighted in bold

Significant differences in the MTG of fresh seeds were found between both species and incubation temperatures tested (with faster germination under warmer temperatures), but no effect of the pre-dispersal warming was found (Table 2B). We found that the increase in incubation temperatures produced a significant reduction of the MTG in all the species, homogeneously between both the pre-dispersal treatments and the species (of about 1 day per 1 K of temperature increase); however, some species-specific patterns were found (Fig. 1B). In particular, at the coldest temperature (i.e. 5 °C) C. cerastoides was the only species able to germinate (values > 5 %) and its seeds kept almost three weeks (20 d) for germinating. Similary, when the other species started to germinate, at 10 °C, the MTG was of 20 – 25 d. With the increase in incubation temperatures the MTG was progressively reduced in all the species, until the lowest value reached at 20 °C.

Factor	Df	Dev.	Res. Df	Dev. Res	F-value	*P*-value
Final germination						
Cerastium cerastoides						
Pre-dispersal treatment (Tr)	1	3.6	14	158.2	0.71	0.417
Incubation temperature (T)	1	93.4	13	64.8	18.52	**0.001**
Tr × T	1	6.5	12	58.3	1.30	0.277
Leucanthemopsis alpina						
Pre-dispersal treatment (Tr)	1	7.6	16	47.9	5.99	**0.028**
Incubation temperature (T)	1	29.2	15	18.7	22.94	**<0.001**
Tr × T	1	0.1	14	18.6	0.07	0.797
Veronica. alpina						
Pre-dispersal treatment (Tr)	1	3.8	16	91.9	1.23	0.286
Incubation temperature (T)	1	51.6	15	40.3	16.85	**0.001**
Tr × T	1	0.1	14	40.2	0.031	0.863

Table 3 Species specific GLMs (with binomial error and logit link-function) results on the influences of pre-dispersal treatment and incubation temperature on fresh seed final germination. Degrees of Freedom (Df), Deviance (Dev.), Residual Degrees of Freedom (Res. Df), Residual Deviance (Dev. Res), *F*- and *P*-value are shown. Significant values are highlighted in bold

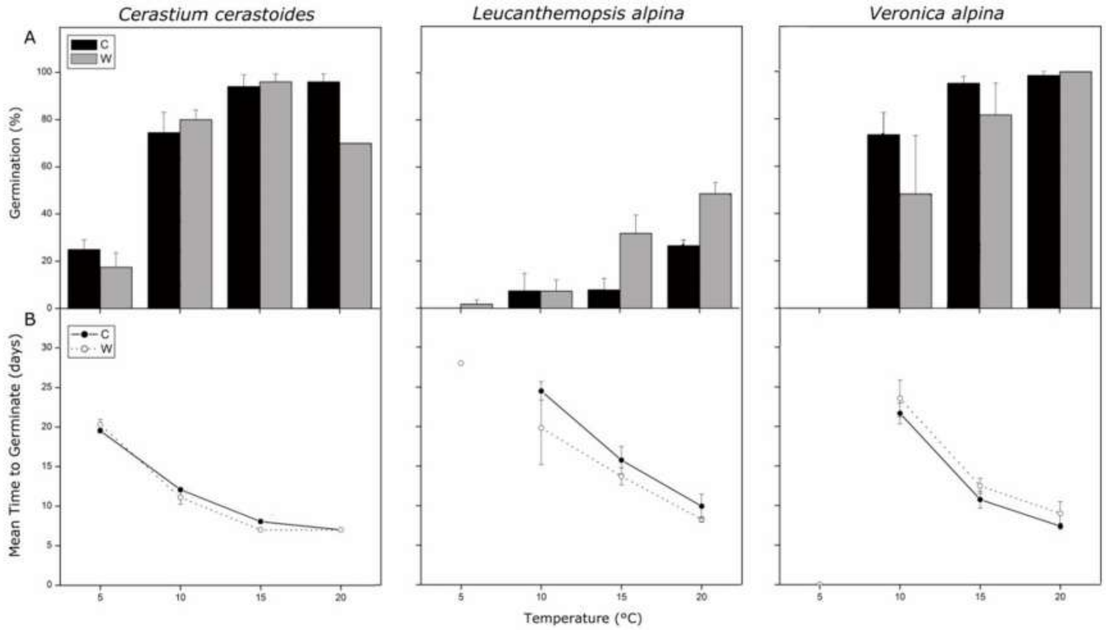


Figure 1 (A) Final germination percentage (mean ± s.e.) of control (C, black bars) and warmed (W, grey bars) seeds and (B) mean time to germinate (in days, mean ± s.e.) of control (full circles and continuous lines) and warmed (empty circles and dotted lines) seeds of C*. cerastoides*, *L. alpina* and *V. alpina* under the four incubation temperatures. Seed lots that failed to germinate more than 1% were excluded from the analyses of the mean time to germination, although still present in the figures for comparative purposes

Germination after cold stratification

Our analyses showed significant differences in germination percentage within all the factors (species, pre-dispersal treatment, incubation temperature, and cold stratification period) tested (Table 4). In particular, germination was on average higher *in C. cerastoides* and *V. alpina* compared *to L. alpina*, in W compared to C seeds, and under warmer incubation temperature and longer cold stratification periods (Fig. 2). Furthermore, significant interactions revealed non-consistent responses of species to all the other factors and non-additive effects of pre-dispersal treatment with different incubation temperatures and stratification periods. Indeed, species-specific GLMs (Table 5) showed that W seeds, compared to C seeds, had higher germination with increasing length of stratification period (in C. cerastoides, Table 5A), with increasing temperature of incubation (in *L. alpina*, Table 5B), or with increasing both the length of stratification period and the incubation temperature (in *V. alpina*, Table 5C).

Factor	Df	Dev.	Res. Df	Dev. Res	F-value	*P*-value
Sp	2	621.1	381	3822.3	69.17	**<0.001**
Tr	1	223.4	380	3598.9	49.75	**<0.001**
T	1	1522.1	379	2076.8	339.00	**<0.001**
Sf	1	50.8	378	2026.0	11.32	**0.001**
Sp × Tr	2	90.6	376	1935.4	10.09	**<0.001**
Sp × T	2	98.6	374	1836.8	10.98	**<0.001**
Sp × Sf	2	81.2	372	1755.7	9.04	**<0.001**
Tr × T	1	70.3	371	1685.4	15.66	**<0.001**
Tr × Sf	1	54.2	370	1631.1	12.08	**<0.001**
T × Sf	1	1.8	369	1629.3	0.40	0.526

Table 4 GLM results on the effects of species (Sp), pre-dispersal treatment (Tr), incubation temperature (Te), cold stratification period (Sf) and their two-way interactions on seed germination proportion. The model was performed with quasibinomial error and logit link-function. Degrees of Freedom (Df), Deviance (Dev.), Residual Degrees of Freedom (Res. Df), Residual Deviance (Dev. Res), *F*- and *P*-value are shown. Significant values are highlighted in bold

The germination of all the three species showed a saturated-pattern in response to increasing incubation temperatures (Table 5), with higher effects of increasing temperature on germination under colder than warmer conditions (i.e. log-transformed temperatures had the best fit to data). However, while *in L. alpina* the effect of the incubation temperatures was consistent under different stratification periods (Table 5B, Fig. 2B), in the other two species seeds incubated at the lowest temperature (i.e. 5 °C) showed different patterns of germination in response to variation in the length of stratification period compared to those of seeds incubated under warmer temperature levels (Table 5A and 5C, Fig. 2A and 2C).

Finally, seed germination of the species studied strongly differed in response to the duration of the cold stratification. In particular, seed germination of *C. cerastoides* (which had “stratification” as polynomial term in the best model) showed an initial increase followed by a decrease of germination percentage with the increasing of the cold stratification period (Table 5A, Fig. 2A). However, the increase of germination after the earliest cold stratification periods was extremely low in seeds produced at the control (C) plots and therefore the general tendency was to lose germinability increasing the duration of stratification in this pre-dispersal treatment (Fig. 2A).

	Estimate	Std. Error	t-value	P-value
(A) *C. cerastoides*				
(Intercept)	-5.6850	0.7610	-7.47	**<0.001**
log(T)	2.8030	0.3237	8.66	**<0.001**
Sf	0.0928	0.0274	3.39	**0.001**
Sf^2	-0.0007	0.0002	-3.81	**<0.001**
Tr	-0.1221	0.2595	-0.47	0.639
log(T) × Sf	-0.0409	0.0117	-3.51	**0.001**
log(T) × Sf^2	0.0003	0.0001	3.43	**0.001**
Sf × Tr	0.0280	0.0044	6.43	**<0.001**
(B) *L. alpina*				
(Intercept)	-4.8571	0.5674	-8.56	**<0.001**
log(T)	1.1410	0.2115	5.39	**<0.001**
Tr	-2.0266	0.7429	-2.73	**0.007**
log(Sf)	0.2996	0.0344	8.72	**<0.001**
log(T) × Tr	1.3783	0.2936	4.69	**<0.001**
(C) *V. alpina*				
(Intercept)	-6.0075	1.4577	-4.12	**<0.001**
log(T)	2.9854	0.5783	5.16	**<0.001**
Sf	0.0734	0.0189	3.88	**<0.001**
Tr	-4.0819	1.2556	-3.25	**0.002**
log(T) × Sf	-0.0283	0.0072	-3.94	**<0.001**
log(T) × Tr	1.6460	0.5109	3.22	**0.002**
Sf × Tr	0.0132	0.0060	2.20	**0.030**

Table 5 Species specific GLM results on the responses of seed germination proportion to the different incubation temperature (T), stratification period (Sf), pre-dispersal treatment (Tr) and their interactions. Estimated coefficient, standard error, t-value and *P*-value are shown. Significant values are highlighted in bold

Differently, *L. alpina* seed germination seemed to have a saturated response to increase in cold stratification length, with more pronounced increases of germination during earlier durations of cold stratification (i.e. log-transformed stratification period had the best fit to data) (Table 5B, Fig. 2B). In the case of *V. alpina*, unlike the previous species, the best relationship between cold stratification period and seed germination was linear, with (after the effects of the others factors) a constant increase of germination with increasing duration of the cold stratification (Tab 5C, Fig. 2C).

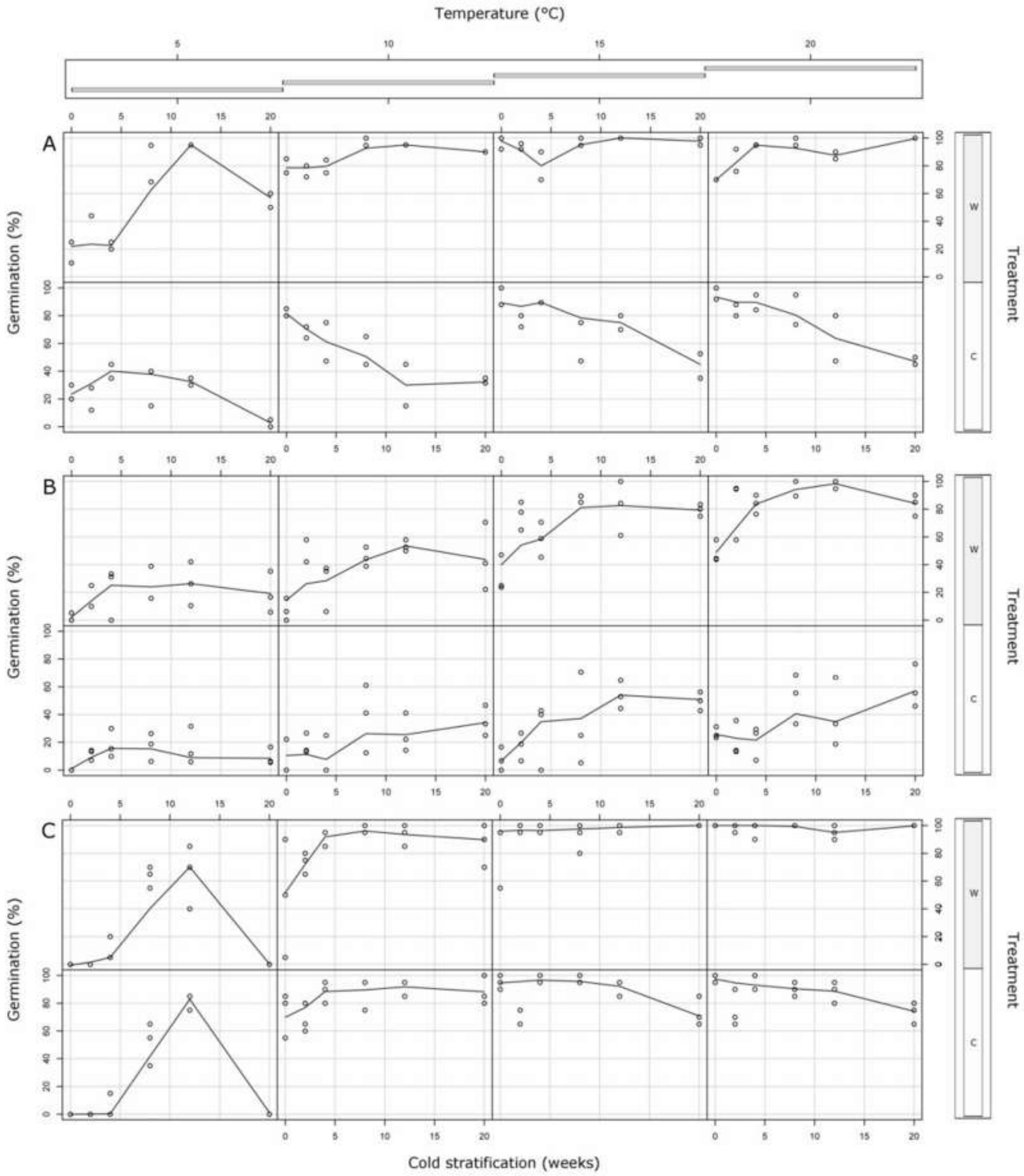


Figure 2 Seed germination (in percentage) of (A) *C. cerastoides*, (B) *L. alpina*, and (C) *V. alpina*. For each pre-dispersal treatment (C: control, W: warmed) the influences on seed germination of the length of cold stratification periods and the four incubation temperatures are shown

1.4.4 Discussion

Germination of fresh seeds

The present study described how pre- and post- seed dispersal climate warming influenced seed dormancy and germination requirements of three alpine species inhabiting snowbeds. Here we have shown that after dispersal germination was similar between seeds produced in the control (C) and under warming (W) at lower incubation temperatures (i.e. 5 and 10 °C) in all species, but that seeds of L. alpina produced in W showed a slight increase in germination under warmer incubation temperatures (i.e. 15 and 20 °C) (Fig. 1A). This alone indicates that germination/dormancy changes on fresh seeds driven by a warmer parental growth environment are limited and may be expected only if seeds experience warm (e.g. > 15 °C) conditions after dispersal. Interestingly, temperatures recorded at the study area at the time of seed dispersal are usually lower (see Table 1) than those able to stimulate an increase of seed germination in warmed seeds of L. alpina (15, 20 °C), indicating that no changes of seedling emergence should be expected in a future warmer climate on fresh seeds of this species. However, the possibility that a warmer parental growth environment (than that used here, about +1.5 K) could have higher effects on germination of fresh seeds cannot be ruled out.

Fresh seeds of all species did not germinate, or germinated very low at temperature (5 °C) similar to those they currently experience after dispersal (Table 1A), but germination increased significantly after the cold stratification (Fig. 2), indicating that seedling emergence of the species studied is likely programmed to occur after the winter season is passed. Seed germination of alpine plants is indeed know to occur in spring, after they have experienced autumn and winter seasons (Körner, 2003). However, the high germination of C. cerastoides and V. alpina (approx. 80 %) found at temperature (10 °C) similar to those occurring under warming during the seed dispersal time (9.5 °C, Table 1), suggesting that germination before the winter season should increase in these species in a warmer climate, which may have important implications for seedling survival and establishment (see Mondoni et al., 2012; 2015).

Interestingly, our data also highlighted that such increase occurred similarly both on seeds produced under W and C, therefore, regardless the climatic scenarios experienced before seed dispersal. Further, here we have shown that an increase of seedlings emergence at the end of the snow-free season is possible only for seeds exposed to approx. 10 °C for at least approx. 10 and 20 days in C. cerastoides and V. alpina respectively (see MTG, Fig. 1B). However, a recent study showed that also a short-term heat event may affect the timing of germination and lead seeds to germinate before the winter season (Orsenigo et al., 2015), suggesting that the early germination could be possible even when the exposure of fresh seeds to warm temperatures is shorter than those shown here.

Considering the warming rate predicted for the next decades in the European mid-latitude mountains (ranging from about +0.3 to 0.5 K per decade, Nogués-Bravo et al., 2007) and the consequent antedating of phenological phases in alpine plants (Wipf and Rixen, 2010; Oberbauer et al., 2013), a significant increase of germination at the end of the snow-free season should be expected at least for C. cerastoides, that is, among the snowbed species at the study site, the earliest seed dispersal (Carbognani et al., unpublished data).

Germination after cold stratification

Seeds of our species showed all a certain degree of non-deep physiological dormancy (sensu Baskin and Baskin 2014), which varied in its depth depending on the species. Indeed, seed germination increased significantly increasing the period of cold stratification in all species, with a wide variation across them (Table 4). In particular, fresh seeds of C. cerastoides germinated at all temperatures, between approx. 20 % (at 5 °C) and 80 – 100 % (at > 10 °C) (Fig. 1A), and its warmed treated seeds showed a significant increase to approx. 90% at 5 °C after three months of cold stratification (Fig. 2A). Indeed, the germination of seeds developed under current climate declined increasing the stratification period, indicating either a loss of viability or the development of a secondary dormancy (see below). Seeds of V. alpina showed similar responses, but did not germinate at 5 °C in absence of cold stratification.

Conversely, seed germination was low in fresh seeds of L. alpina at all temperatures (about 30 – 40 %), increasing mostly after long period cold stratification (i.e. Fig. 2B, Table 5B). On these evidences, although these species should be considered as non-deep physiological dormant, the different temperature windows for germination and durations of cold stratification required to break dormancy, indicate that L. alpina is the strongest (physiologically) dormant species, followed by V. alpina and C. cerastoides. Moreover, the increase in stratification period generally resulted in a significant increase in germination percentage (Table 4, Fig. 2), but the species studied responded differently to the interplay of cold stratification and warming treatment. In seeds of C. cerastoides, the increase in stratification duration modelled opposite responses in seeds produced under W and C, increasing and reducing the germination percentage, respectively, and similar responses were observed in V. alpina (see Sf × Tr, Table 5A, C). Conversely, parental warming did not affect the germination response to cold stratification in L. alpina (Table 5B). Moreover, the significant temperature × treatment interaction found in cold stratified seeds in L. alpina (Table 5) indicates that incubation temperatures had different responses in seeds produced under W and C in this species. These are interesting and novel observations indicating that changes of germination requirements induced by the pre-dispersal warming environment depended on the seed dormancy state, with deeper dormant species showing major changes in response to incubation temperature on both fresh and cold stratified seeds (i.e. L. alpina) and less dormant species in response to stratification (i.e. C. cerastoides and V. alpina) (Table 3 e 5). Moreover, changes of pre-dispersal temperatures affected germination response to both stratification and incubation temperatures (at least on cold stratified seeds) in V. alpina, which showed an intermediate dormancy behavior between C. cerastoides and L. alpina (see above).

As a result, higher seed maturation temperatures consistently increased the germination in all species, although to a different extent across them (Fig. 2). Coming back to the other research questions, warming had no affect the MTG, but widened the suitable temperature range for germination and reduced the cold stratification time to break dormancy in L. alpina. Indeed, at 15 °C germination was extended to approx. 40 % when fresh seeds of this species matured under warming, while it was lower than 10 % on seeds produced in the control. Moreover, seed germination was significantly higher in warmed treated seeds of L. alpina, compared to control ones, just after two weeks of cold stratification (Fig. 2B), indicating that under warming some species will produce seeds with shorter chilling requirement to overcome dormancy. This behaviour occurred at all incubation temperatures, except 5 °C, probably because the cold temperatures constrain the germination (Fig. 2B).

The higher germination responses observed on both fresh and cold stratified seeds produced under warming, compared to those developed under current climate, could be due to changes in the dormancy patterns and/or the seed viability of our species. Warmer maturation conditions are known to lead to better quality of seeds (see reviews by Roach and Wulff, 1987; Wulff, 1995; Gutterman, 2000), and it has been suggested that plants grown in a warmer environment produce longer lived seeds (Bernareggi et al., 2015). In this regard, the different behaviour of warmed and not warmed seeds of C. cerastoides might suggest that current alpine climate is not optimal for seed development and maturation, reducing the survival capacities during the long-lasting winter conditions. Similar conclusions may explain the lower germination of not warmed vs. warmed seeds of L. alpina. However, after 20 weeks of cold stratification seed germination of V. alpina dropped to 0 % at 5 °C, but remained almost complete at the other incubation temperatures, indicating that seeds were still viable. This behaviour reflects the condition of fresh seeds (Fig. 1A, 1C), suggesting that the prolonged permanence at 0 °C could have induced a secondary dormancy state in seeds of this species. Indeed, it is possible that non-dormant seeds that do not meet adequate conditions for germination enter secondary dormancy (Brandel, 2005; Leymarie et al., 2008; Baskin and Baskin, 2014) and, therefore, similar mechanisms may explain also the decrease of germination with increasing cold stratification period in C. cerastoides and the lower germination of not warmed vs. warmed seeds in L. alpina.

Summarizing, this study suggests that climate warming will increase the likelihood of seed germination both just after dispersal and winter season (i.e.

after cold stratification) due to the effects on seed phenology (e.g. advanced seed dispersal), phenology (e.g. advanced seed dispersal) and on environmental conditions (e.g. early snowmelt, warmer temperatures during the snow free period). However, the consequences that these changes will have on plant reproduction success still remains partially investigated. For example, while the germination immediately after seed dispersal has always been considered disadvantageous because constrains seedlings to cope with the harsh winter conditions, a recent study in a glacier foreland showed that a high percentage (about 60 and 75 %) of autumn emerged seedlings can survive through the winter (Mondoni et al., 2015). However, overcoming the winter involve elevated energy consumption (Maruta, 1994), which may reduce the growing capacities in spring. Moreover, the higher germination of warmed treated vs. not warmed seeds also after long periods of stratification, suggest an increase in reproductive success. A persistent soil seed bank in several alpine species is thought to be an ecological adaptation to the low chance of establishment in these environments (Schwienbacher et al., 2010). Hence, higher germination may not necessarily be beneficial for alpine plants, as summer drought and late-winter/early spring soil freeze–thaw events are expected to increase due to climate warming (Wipf and Rixen, 2010; Barriopedro et al., 2011), as already shown in some mountain regions (Rikiishi et al., 2004; Scherrer et al., 2004; Mote et al., 2005), reducing the likelihood of seedlings to survive. In order to ensure a realistic evaluation of the recruitment success in alpine environment, it is necessary to consider the all effects, direct and indirect, that climate warming produces in any time of the year. In this regard, in situ sowing experiments at the Gavia Pass are underway in order to understand whether a longer and warmer growing season could be advantageous for seedling recruitment and establishment in snowbeds.

1.4.5 Tables and Figures Index

TABLES

FIGURES

PART 2

CHAPTER 2.1. THE GLOBAL TREELINE EXPANSION EXPERIMENT

View of the Dicshma Valley and the Jakobshorn, Davos
(Photo: Giulietta Bernareggi)

G-TREE is a globally distributed collaborative project aimed at testing the mechanisms driving treeline position in order to be able to predict the expansion of the treelines in the future.

A field experimental design with seeding and substrate-altering treatments was used in ordet to disentangle seed versus substrate limitations on on range treeline expansion. Participating researchers from Canada, the United States, Europe, Russia, Australia and Venezuela have begun implementing the field experiment in 2013 (follow an identical field protocol, Brown et al., 2013) at arctic and alpine treeline sites, covering both latitudinal and altitudinal treelines. This initiative will provide empirical data on where and under what circumstances treeline expansion can occur globally.

In the Swiss Alps, a G-TREE site was set up in the Dischma valley, Davos.

CHAPTER 2.2. THE SWISS G-TREE EXPERIMENT: SEED LIMITATION AT AN ALPINE TREELINE

View of the Dicshma Valley from the Schwarzhorn, Davos

(Photo: Giulietta Bernareggi)

2.2.1 Introduction

Global mean temperatures have increased over the past century and future warming is predicted to be most pronounced in high latitudinal and mountain ecosystems (Solomon et al., 2007). Recent warming induced range shifts as species track their optimal climate (Parmesan and Yohe, 2003). Increased shrub abundance in Arctic tundra ecosystems worldwide provides evidence for latitudinal range shifts (Sturm et al., 2005, Myers-Smith et al., 2011; Elmendorf et al., 2012). Upward shifts of shrubs and trees have also been observed in mountain regions (Theurillat and Guisan, 2001; Tinner and

Ammann, 2005; Kullmann, 2002, Moiseev and Shiyatov, 2003; Esper and Schweingruber, 2004; Devi et al., 2008). Treeline position is widely considered temperature sensitive (Mikola, 1952; Koerner, 2007) and thus potentially responsive to climate warming (Holtmeier and Broll, 2005, Körner, 2012; Körner and Paulsen, 2004). However, treeline advances are not yet a worldwide phenomenon, because they lag behind rates of climate change (Chapin et al., 2005; Loarie et al., 2009). Nevertheless, a global meta-analysis revealed considerable seedling recruitment above treeline consistent with observed rates of recent warming implying the possibility of future treeline advances (Harsch et al., 2009). Although seedling recruitment is not directly linked to treeline shifts (Graumlich et al, 2005), upper treeline positions depend on natural regeneration in the long-term (Körner, 2012).

Besides climate, numerous other abiotic factors and biotic interactions are limiting tree seedling recruitment. One pre-requisite for successful seedling recruitment is the availability of viable seed (Turnbull et al., 2000), which commonly declines with elevation (Körner, 2012; Holtmeier, 2009). Seed limitation at treeline is mainly caused by decreasing quality and quantity of available seed such as extended production time intervals (reviewed in Holtmeier, 2009). Seed dispersal may also be a limiting factor with short wind-mediated dispersal distances restricting dispersal beyond treeline (Kullman, 2005; Mazepa, 2005; Kuoch, 1965). However, other studies contradict the importance of seed limitation at treeline (Körner, 2012).

Successful recruitment also strongly depends on the availability of suitable microsites for establishment and survival. A multitude of factors determines the quality of such microsites, such as light, the presence of competitors, climate and herbivory (Leck et al., 2008). Dense vegetation cover may impede recruitment by shading and competition for moisture and nutrients (Hobbie and Chapin III, 1998; Schönenberger, 1975; Weih and Karlsson, 1999), but also facilitate recruitment by sheltering seedlings from adverse climate effects (Holtmeier, 2009; Haettenschwiler and Smith, 1999). Along environmental stress gradients, biotic interactions are predicted to change from competition to facilitation at high and low stress levels respectively (Callaway, 2002). Treeline ecotones are characterized by more open spaces exposing tree seedlings to similar beneficial life conditions as low stature alpine vegetation (Körner et al., 2003). Such open microsites are warmer than seedbeds in montane forests due to surface heating effects that are maintained over the growing season (Scherrer and Koerner, 2010). Many tree species need open and thus warmer micro-habitats for successful germination and establishment (Bullock and Clarke, 2000; Leck et al., 2008). Seed germination rates of the most common tree species, e.g. *Larix decidua* and *Picea abies*, peak at temperatures above 20 °C (Black and Bliss, 1980; Nather, 1958; Tranquillini, 1979; Sveinbjornsson et al., 1996). In contrast to these positive thermal effects, open microsites with exposure to high solar radiation may also negatively affect seedling recruitment by increasing low-temperature and water stress (Germino et al. 2002; Bader et al., 2007; Maher et al., 2005). Moreover, the lack of shelter increases the susceptibility of seedlings to herbivores. In fact, herbivory, together with drought, are the most common causes for seedling mortality in these environments (Moles and Westoby 2004; Anschlag et al. 2008).

Only few studies focus on individual abiotic and biotic factors affecting seedling recruitment in treeline ecotones (e.g. reviewed in Holtmeier 2009; Zurbriggen et al. 2013), while there is even more need for research on interactions among multiple factors in treeline ecotones (but see Brown et al., 2013; Brown and Vellend, 2014; Tingstad et al., 2015). In this study we investigated the effects of climate factors and biotic interactions, as well as their interactions, on seedling recruitment of two common tree species, European Larch (*Larix decidua* MILL.) and Norway spruce (*Picea abies* (L.) KARST.) at three different elevations below, at and above the current treeline in the Swiss Alps. In half of the plots, seeds of local low and high elevation provenances were sown into intact vegetation and bare-ground plots in two consecutive years and with herbivore exclusion from half of the plots. Specifically, we addressed the following research questions:

(i) Is seedling recruitment along an elevational climate gradient across treeline limited by the availability of viable seeds?

(ii) Is seedling recruitment influenced by biotic interactions (i.e. seed bed openness, herbivory)?

(iii) If so, do biotic interactions vary along an elevational climate gradient across treeline and over time?

(iv) Are there species- and provenance specific differences in recruitment responses to interacting abiotic and biotic factors?

2.2.2 Methods

Study area

The Swiss G-TREE experiment was set up along an elevational gradient in an alpine treeline ecotone located in the Dischma valley, Davos, Switzerland (Table 1). The forest site (the lowest) is located at 1,930 m a.s.l., in a subalpine larch-spruce forest (*Larici-Picetum*) neat to the regional upper treeline limit. The underwood is consists in tall and large-leafed herbaceous vegetation (predominantly *Adenostylion*). The treeline site (mid elevation) is located at 2,090 m a.s.l., at the current treeline, and is dominated by dense dwarf shrub vegetation (predominantly *Rhododendro-Vaccinietum*). The alpine site (the highest) is located at 2,410 m a.s.l., approximately 300 m above treeline, in an alpine meadow (*Caricetum curvulae*).

Site	Longitude [°N]	Latitude [°E]	Elevation [m a.s.l.]	Mean annual soil Temperature [°C]
forest	46.777	9.868	1930	5.7±4.0
treeline	46.774	9.866	2100	5.4±3.7
alpine	46.769	9.862	2390	5.3±5.2

Table 1 Position, elevation and mean annual temperature at the forest, treeline, and alpine G-TREE site

Studied species

Larix decidua Mill. (European Larch)

© Pignatti

Family: Pinaceae

Biological form: P scap

Choritype: Orof. Centro-Europ.

Flowering: IV-VI

Altitude: 0-2400 m

Morphological description: Heigh 8–25 (-55) cm. Gray-brown sclay bark, with grooves of max 1.5 cm. Pendulous branches with longitudinal striations are of about 3 mm in diameter. Brachiblasts are oval (3 × 5 mm) with 30-40 soft and capillary needle-like leaves of 1 × 8-18 mm. The cones are erect, ovoid-conic, 2 × 3 cm.

Habitat: mountain and sub-alpine woods.

Ellenberg's Ecological Index

L	T	C	U	R	N	S
8	3	9	4	0	3	0

Distribution

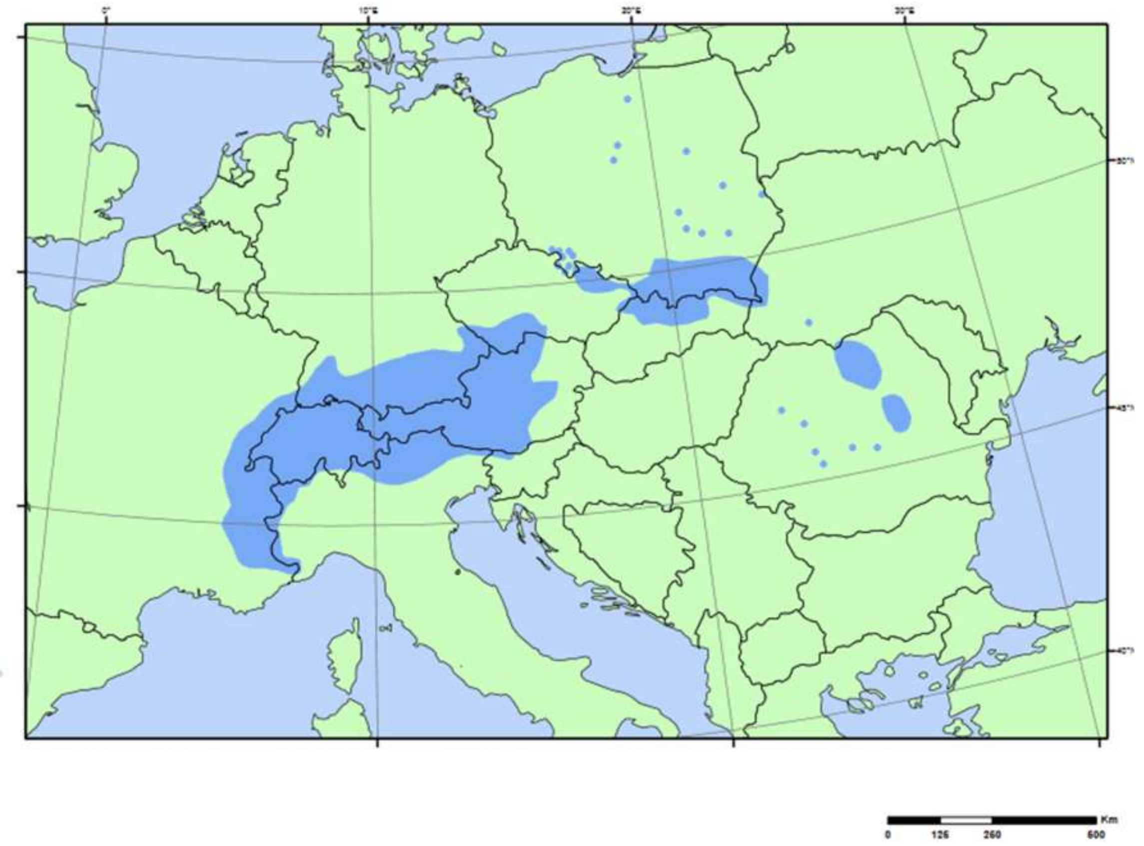
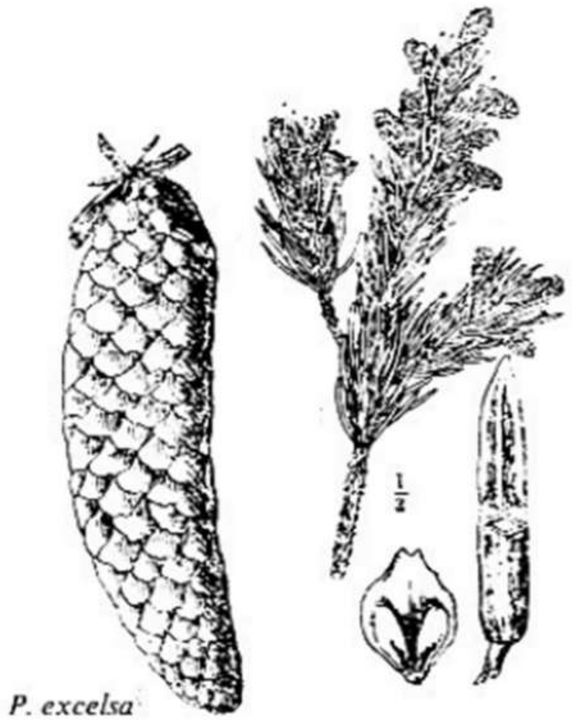


Figure 1 Distribution map of *Larix decidua* (European Larch) (Source: EUFORGEN)[1]

Picea abies (= *Picea excelsa* L.) KARST (Norway spruce)



© Pignatti

Family: Pinaceae

Biological form: P scap

Choritype: Eurosib.

Flowering: IV-V

Altitude: 0-2200 m

Morphological description: Heigh 8–35 (-65) cm. Gray-brown bark, scaly on old trees with plates 5mm thick and a reddish-brown underlying layer. Branches of 1cm in diameter, reddish-brown bark with longitudinal scales of 1 × 3 mm. twigs orange-brown, usually glabrous. Buds reddish brown, 5–7 mm, apex acute. Niddle-like leaves 1 × 8-12 (-25) mm, rigid, light to dark green. Seed cones 3–4 × 8–15 cm; scales diamond-shaped.

Habitat: subalpine coniferous forest.

Ellenberg’s Ecological Index

L	T	C	U	R	N	S
5	3	6	0	0	0	0

Distribution

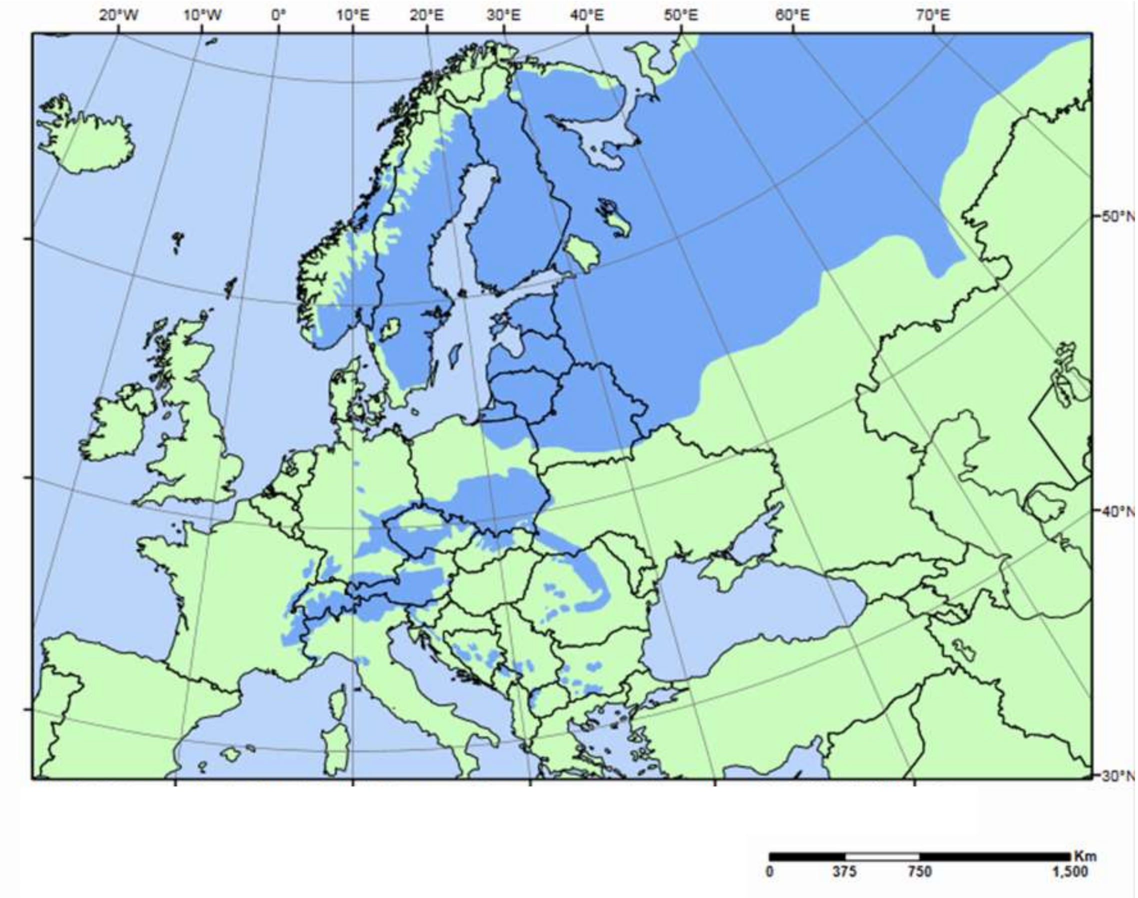


Figure 3 Distribution map of *Picea abies* (Norvay spruce) (Source: EUFORGEN)[2]

In Switzerland, the elevation range of L. decidua is 600 to 2,100 m a.s.l., whereas P. abies has a slightly lower elevation range of 500 to 1,800 m a.s.l.83. For each species, we used seeds from a low- and a high-elevation provenance, located within 30 km of the study area (Table 2). Seeds were procured from the Swiss Federal Institute for Forest, Snow and Landscape Research WSL (Birmensdorf, Switzerland) and stored at 5 °C prior to sowing. Seed viability was determined by direct germina-tion tests under laboratory conditions (Frey et al., 2018).

Provenance	Longitude [°N]	Latitude [°E]	Elevation [m a.s.l.]	Aspect	TGW [g]	Viability [%]	Elevation range of species [m a.s.l.]
LL	46.699	9.709	1350	SW	1995	28	600-2100
LH	46.509	9.849	1760	SE	1970	11	600-2100
PL	46.917	9.785	1000	S	1985	74	500-1800
PH	46.734	9.849	1960	SW	1983	61	500-1800

Table 2 Locations of the seed provenances. TGW: seed mass in thousand grain weight. Viability: seed viability. LL: low-elevation provenance of *L. decidua*; LH: high-elevation provenance of *L.decidua*; PL: low-elevation provenance of *P. abies*; PH: high-elevation provenance of *P. abies*

Experimental design

In summer 2013, the three experimental sites were set up following the standard protocol of the global GTREE-initiative (Brown et al., 2013). A scarification treatment was applied using a hand-cultivator to remove surface plant litter, as well as mosses and lichens, but leaving vascular plants rooted in the soil. Seeds of the two studied species were equally spread on half of the subplots in the first year (2013). The other half of the subplots was seeded at the beginning of the following growing season in spring 2014. Immediately after seeding, half of the subplots were covered with durable and stable metallic cages (45 cm x 28 cm) to exclude herbivores (“split subplot”). Each metal cage covered two adjacent subplots. These cages are expected to exclude mice, ungulates, mountain hares, and birds, whereas burrowing animals, such as voles, are probably not prevented from entering (M. Schütz, personal communication 2013). Fences were installed at all three sites to protect experimental plots from grazing and trampling by cattle and horses.

In this full factorial experimental design, seeding and substrate treatments were applied at plot level. Treatment combinations were randomly assigned to 20 plots per site, which were 2.24 m long and 0.45 m wide each. This resulted in 5 replications of each treatment combination at each of the three experimental sites. Each of these plots was subdivided into 16 sub-plots (22.5 cm x 28 cm each) to which all possible combinations of the four other experimental factors (species, provenance, herbivory exclosure, and year of seeding) were randomly assigned. Overall, this resulted in 960 subplots.

Seedling emergence and survival were assessed by counting the seedlings in each subplot at the beginning and end of the growing season in 2013, 2014 and 2015. All seedlings were marked to avoid double counts. The height of the seedlings was measured using a hand ruler at the end of growing seasons 2014 and 2015. In subplots with more than ten seedlings, ten arbitrarily chosen seedlings were measured.

At each site, temperature loggers (iButton; Maxim Integrated Products, Sunnyvale, CA, USA) recorded soil temperature at a depth of 5 cm (Table S3). Daily minimum, mean and maximum climate data (air temperature, precipitation, snow depth, wind) was provided by a climate station at 2100 m a.s.l. that is located in a distance of approx. 50 m from the mid-elevation site.

Statistical Analyses

Linear and generalized linear mixed-effects models (LMMs and GLMMs; Faraway, 2006; Pinheiro and Bates, 2000) were used to analyse the response of seedling recruitment (emergence, winter survival and growth) to the investigated abiotic and biotic factors as well as their interactions. Due to the (almost complete) lack of natural germination unseeded plots were omitted from these analyses and the forest site was excluded from survival and growth trait models because of the very low seedling recruitment at this site. GLMMs for seedling emergence and first winter survival contained site, substrate, species, provenance, year of seeding, and herbivore exclosure treatment as fixed effects. Because of the small data set for second winter survival, it was not possible to test the influence of substrate treatment, herbivore exclosure and interactions including these factors. All three GLMMs used binomial distributions. Seedling height was modelled using an LMM that contained the fixed effects site, species, provenance, and year of seeding. This data set was too small to analyse interactions between model factors. All models accounted for spatial correlation among subplots by including plot and two subplot-structures as random effects. Significances of model factors were determined by likelihood ratio tests and non-significant fixed effects were eliminated from the final models. All models were fit using standard procedures for model diagnostics (Zuur et al., 2009) with the lme4-library (Bates et al., 2012) in R 3.1.3 (R Core Team, 2014).

2.2.3 Results

Seedling emergence

Natural recruitment was observed only at the forest site where 15 *L. decidua* seedlings and seven *P. abies* seedlings were found. In the seeded plots, a total of 768 *L. decidua* seedlings and 959 *P. abies* seedlings were counted at the end of the first growing season in all 60 plots. Emergence was significantly higher for *L. decidua* (6.3±0.7 % of viable seeds per subplot; mean ± standard error) than for *P. abies* (2.9±0.4 %; species $P < 0.001$) (Fig. 4).

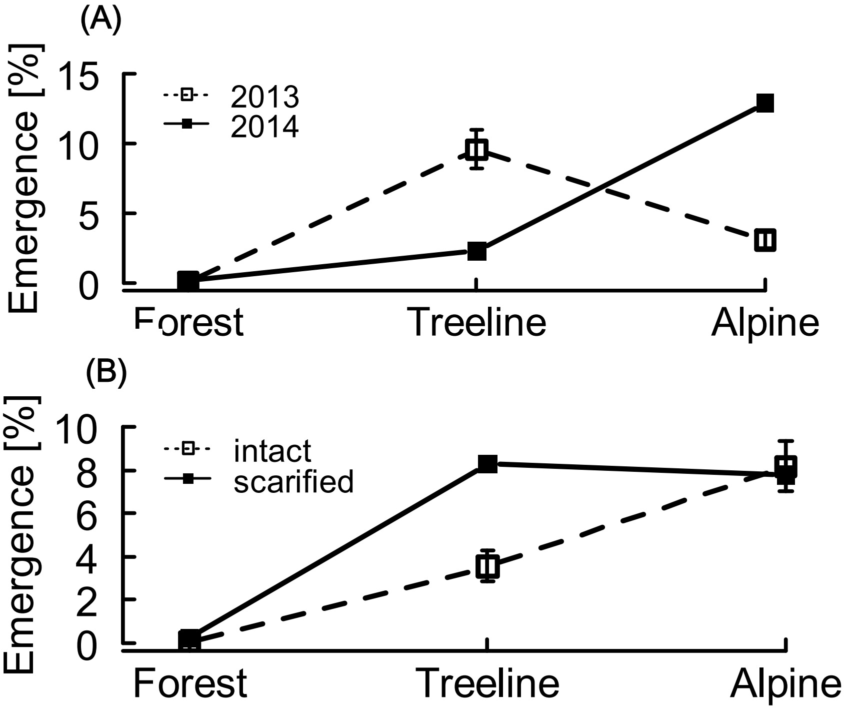


Figure 2 Seedling emergence as percentage of viable seeds in dependence of experimental site for the two seeding years 2013 and 2014 (A) and for the two substrate treatments (B). Error bars indicate standard errors of trait means

Only 12 seedlings (0.1±0.04 % of viable seeds) emerged at the forest site. Seedling emergence was significantly higher at the treeline and alpine sites, where 734 (5.8±0.8 %) and 981 (8.0±0.8 %) seeds emerged, respectively (site $P = 0.013$). Total emergence was similar in both years 2013 and 2014 (year P = 0.87). However, the distribution of emergence among sites varied between the two seeding years (year x site P < 0.001; Fig. 4a). It was more than three times higher at the treeline site than at the alpine site in 2013 (9.5 % vs. 3.0 %). Conversely, in 2014, emergence at the alpine site was more than six times higher as compared to the treeline site (13.1 % vs. 2.0 %).

Factor	χ^2	Df	Pr(> χ^2)	Significance (α = 0.05)
year	0.025264229	1	0.873710431	
site	6.181764048	1	0.012907353	*
scarified	2.883306059	1	0.08950209	
species	24.54276987	1	7.27E-07	***
provenance	36.7375166	1	1.35E-09	***
cage	15.38009863	1	8.79E-05	***
year:site	93.02452267	1	5.17E-22	***
year:scarified	3.225830347	1	0.072485027	
year:species	1.08038928	1	0.298610488	
year:provenance	0.897816263	1	0.343367927	
year:cage	4.624882191	1	0.031511425	*
site:scarified	6.463003202	1	0.011014334	*
site:species	0.0216611	1	0.882992232	
site:provenance	0.478995865	1	0.488877501	
site:cage	0.011326132	1	0.915245713	
species:scarified	0.454900164	1	0.500017083	
provenance:scarified	0.354472221	1	0.551592367	
scarified:cage	0.043812323	1	0.834203175	
species:provenance	19.58046866	1	9.65E-06	***
species:cage	0.956126895	1	0.328164637	
provenance:cage	7.029602793	1	0.008017313	**

Table 2 Effects of seeding year, experimental site, scarification treatment (scarified), species, provenance and herbivore exclosure treatment (cage), as well as their interactions, on seedling emergence. Values and symbols are χ2-values and significances, respectively, from likelihood ratio tests of mixed-effect models. Significance levels: (*)P < 0.1; *P < 0.05; **P < 0.01; ***P < 0.001

Emergence of the two *P. abies* provenances differed only slightly (3.3 % for low vs. 2.5 % for high provenance). In *L. decidua*, however, low provenance seeds (10.6 %) emerged about five times more frequently than high provenance seeds (2.1 %; species x provenance P < 0.001). Substrate scarification enhanced emergence at the treeline site but not at the alpine site (site x scarified P = 0.01) in both species and years (Table 2; Fig. 4b). The protection against herbivores resulted in a general increase in emergence from 4.0 % to 5.2 % (cage P < 0.001). This effect was slightly stronger in 2013 than 2014 (year x cage P = 0.03) and the increase was more pronounced for low than for high provenance seedlings (P = 0.008).

Early stage seedling establishment

The fraction of seedlings that survived the first winter was significantly lower at the alpine site (8.5±2.0 %) than at the treeline site (51.5±4.4 %, site P < 0.001; Fig. 5, Table 3).

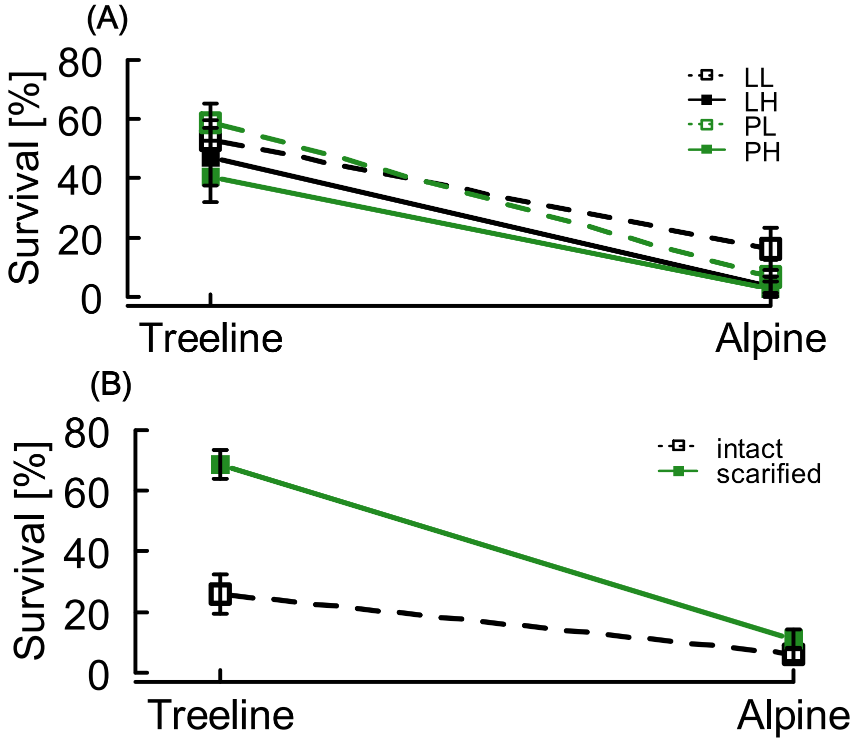


Figure 3 Seedling survival rates after the first winter in dependence of experimental site for species and provenances (A) and for the two substrate treatments (B). LL: *L. decidua* low elevation provenance, LH: *L. decidua* high elevation provenance, PL: *P. abies* low elevation provenance, PH: *P. abies* high elevation provenance. Error bars indicate standard errors of trait means.

Factor	χ^2	Df	Pr(> χ^2)	Significance

				(α = 0.05)
site	27.05833918	1	1.97E-07	***
scarified	18.54736328	1	1.66E-05	***
species	3.365937057	1	0.066557709	(*)
provenance	4.330422172	1	0.037436959	*
cage	0.473961241	1	0.491170435	
site:scarified	3.739132591	1	0.053152031	(*)
site:species	5.810687129	1	0.015929069	*
site:provenance	0.109503799	1	0.740709762	
site:cage	1.166019207	1	0.280220699	
species:scarified	1.191007313	1	0.275126464	
provenance:scarified	3.697858298	1	0.054482359	(*)
scarified:cage	1.997239877	1	0.157585941	
species:provenance	0.010684409	1	0.917672962	
species:cage	0.536497429	1	0.463887941	
provenance:cage	0.200002353	1	0.654718947	

Table 3 Effects of experimental site, scarification treatment (scarified), species, provenance and herbivore exclosure treatment (cage), as well as their interactions, on first winter survival. Values and symbols are $\chi 2$-values and significances, respectively, from likelihood ratio tests of mixed-effect models. Significance levels: (*)P < 0.1; *P < 0.05; **P < 0.01; ***P < 0.001.

Survival was generally higher on scarified plots than on plots with an intact vegetation cover (scarified P < 0.001), but the difference was more pronounced at the treeline than at the alpine site (site x scarified P = 0.053; Fig. 5b). All other interactions were not significant (P > 0.05). Low provenance seedlings of both species had slightly higher survival rates after the first winter as compared to high provenance seedlings (provenance P = 0.037). Differences between the two species were only marginal (species P = 0.067) and herbivore exclosure did not significantly influence winter survival. Absolute numbers of surviving seedlings after the first and second winter can be found in Table S4.

Similar to first winter survival, survival after the second winter was lower at the alpine site (25.9±10.3 % of the seedlings observed at the end of the previous growing season, Table 4) than at the treeline site (79.2±6.0 %; site P = 0.023; Fig. 6). Low provenance *L. decidua* seedlings had a higher survival rate during the second winter than their high provenance counterparts, whereas in *P. abies* survival was higher for high than for low provenance seedlings (species x provenance P = 0.007). Species and provenance individually, as well as their interactions with site were not significant.

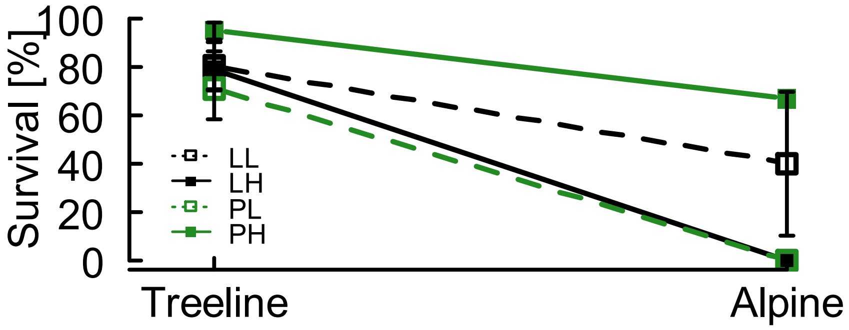


Figure 4 Seedling survival rate after the second winter in dependence of experimental site for species and provenances. LL: *L. decidua* low elevation provenance, LH: *L. decidua* high elevation provenance, PL: *P. abies* low elevation provenance, PH: *P. abies* high elevation provenance. Error bars indicate standard errors of trait means

Factor	χ^2	Df	Pr(> χ^2)	Significance (α = 0.05)
site	5.15882155	1	0.02312859	*
species	1.00374778	1	0.31640535	
provenance	0.55284162	1	0.45715893	
site:species	0.1418914	1	0.70640789	
site:provenance	0.15861445	1	0.69043538	
species:provenance	7.36905897	1	0.00663556	**

Table 4 Effects of experimental site, species and provenance, as well as their interactions, on second winter survival. Values and symbols are χ2-values and significances, respectively, from likelihood ratio tests of mixed-effect models. Significance levels: (*)P < 0.1; *P < 0.05; **P < 0.01; ***P < 0.001

After the second growing season, seedlings had grown to an average height of 3.3±0.1 cm. They were significantly taller at the treeline (3.8±0.1 cm) than at the alpine site (2.6±0.1 cm; site P < 0.001; Fig. 7, Table 5). However, there were no differences in seedling height with respect to year of seeding, species and provenance (P > 0.1 for all three factors).

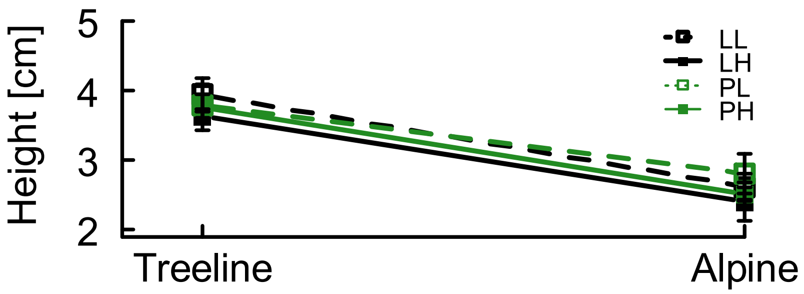


Figure 5 Seedling height in dependence of experimental site for species and provenances. LL: *L. decidua* low elevation provenance, LH: *L. decidua* high elevation provenance, PL: *P. abies* low elevation provenance, PH: *P. abies* high elevation provenance. Error bars indicate standard errors of trait means

Factor	χ^2	Df	Pr(> χ^2)	Significance (α = 0.05)
year	0.00026151	1	0.98709767	
site	20.9631683	1	4.68E-06	***
species	1.69618866	1	0.19278717	
provenance	2.48948283	1	0.11460938	

Table 5 Effects of seeding year, experimental site, species and provenance on seedling height. Values and symbols are χ2-values and significances, respectively, from likelihood ratio tests of mixed-effect models Significance levels: (*)P < 0.1; *P < 0.05; **P < 0.01; ***P < 0.001

Climate

The average mean air temperature for summer (i.e. June, July and August) over the period 1981 – 2010 measured at the climate station at 2100 m a.s.l. was 9.4 °C (Table S3). Summer 2013 with a mean temperature of 10.6 °C was clearly above average, whereas the following summer 2014 with a mean temperature of 8.7 °C was slightly below average. Summer temperature in 2015 was the second highest ever measured (11.8 °C). Measurements at the nearby MeteoSwiss station Davos (1594 m a.s.l.) show that the summers of 2013 (265 mm) and 2015 (261 mm) both had significant precipitation deficits with only 64 % of the normal precipitation sum (410 mm in the 1981 – 2010 period). Conversely, precipitation in summer 2014 (492 mm) was 20 % above average (MeteoSwiss, 2015).

2.2.4 Discussion

Limitation by the availability of viable seeds

This study provides experimental evidence for seed source limitation in an alpine treeline ecotone with almost no natural recruitment. While seed limitation has been reported for different forest ecosystems (e.g. Turnbull et al., 2000; Eriksson & Ehrlén, 1992; Tilman, 1994; 1997), it seems to be particularly common for treeline ecotones (Kuoch, 1965; Holtmeier, 1999; 2009). This can be partly explained by decreasing seed production with increasing elevation of treeline species such as *P. abies* and *L. decidua*, resulting in good seed crop years only once a decade (Tschermak, 1950). In addition, seed ripening may be decelerated and the amount of empty, non-viable seeds may be increased due to the shorter growing period at treeline (Körner, 2012). As a consequence, natural regeneration from seeds is very critical in the treeline ecotone (Holtmeier, 1993;1995a; 1999). The extremely low recruitment at the forest site is expected to be caused by the dense cover of competing understorey vegetation that prevents seedling emergence by strongly reduced light availability at the soil surface. Imbeck and Ott (1987) studied *P. abies* regeneration in a similar subalpine spruce forest rich in tall forbs (*Piceto-Adenostyletum*) near our study site in the Swiss Alps and reported less than 5% of natural recruitment on sites with dense vegetation cover. Our findings also agree with the reduced survival in one-year-old transplanted *P. mariana* seedlings in shaded plots (Cranston and Hermanutz, 2013) and the negative correlation between survival and canopy cover in *L. decidua* and *Picea* spp. (Manson et al., 2004). Another important aspect is the asynchronous phenological timing of the understorey forbs and seedling emergence (Imbeck and Ott, 1987). In spring, the understorey vegetation at our forest site is still very short-grown enabling seeds to emerge, as shown by the few naturally emerged seedlings at the start of the experiment in June 2013 that did not survive the first summer. A similar situation applies for the treeline site with the dense tall dwarf-shrub layer limiting seedling recruitment despite single seed bearing trees nearby (Grau et al., 2013; Cranston and Hermanutz, 2013) whereas seed bearing trees are quite far from the uppermost site that is located approx. 300 m above the natural treeline. However, recent studies showed that seedlings of *L. decidua* and *P. abies* have higher altitudinal distribution limits than adult trees of the same species (Lenoir et al., 2009). According to Körner (2012), this difference in range limits between seedlings and adult trees might be due to the enhanced temperatures close to the ground from which seedlings profit whereas adult trees are exposed to lower air temperatures. However, tree seedling occurrence above treeline has to be considered carefully and is not directly linked to climate warming (Körner, 2012). Nevertheless, it shows the potential for an upward range expansion of trees in the Alps in the context of future climate warming (Vitasse et al. 2012).

Biotic and abiotic interactions as recruitment limitations

Substrate limitation

Suitable substrate as seedbed is decisive for seedling establishment by allowing the germination and successive thrive of those seeds that reach the soil surface. The higher emergence on scarified plots at the treeline site, in comparison with the alpine site, is likely influenced by the dense dwarf-shrub and thick moss layer covering the soil surface and indicating substrate limitation for seedling emergence in this site (Table 2; Fig. 4). Indeed, dense vegetation cover may prevent seeds from reaching a suitable seed bed by competition for moisture and nutrients, as well as shading effects (Weih and Karlsson, 1999). Moreover, Hunziker and Brang (2005) showed that a deep and dense moss mat impeded germination of *P. abies* seeds by preventing seedlings from reaching the soil surface (but see Motta et al., 1994; Munier et al., 2010). Conversely, seedbed at our alpine site was not limiting for germination probably because of the less dense vegetation cover with a relatively high proportion of rock and bare ground. Indeed, reduced competition and exposed mineral soil provide a particularly favourable seedbed for seed recruitment (Munier et al., 2010; Tingstad et al., 2015).

Similar to emergence, seedling survival after the first winter was higher on scarified plots than on intact vegetation in particular at the treeline site (Table 3; Fig. 5b). In contrast, Wheeler et al. (2011) observed lower survival in one year old *Picea mariana* seedlings on bare ground than on moss covered substrate. The authors relate their findings to the protective effect of the moss covered substrate against adverse climate conditions, whereas competing effects of dense moss cover and shading dwarf-shrubs seem to outweigh positive sheltering effects in our study system. Hence, the presence of bare soil generally enhanced seedling recruitment, indicating substrate limitation and suggesting how substrate disturbance may contribute to reduce the competing effect of vegetation.

Seedling predation

Post-dispersal predation is known to be an important constraint on seedling recruitment and several experiments about post-dispersal seed and seedling predation in *Picea* and *Abies* species showed that seeds might be eaten by different groups of animals, i.e. invertebrates, rodents, birds, small and large mammals (Côté et al., 2003; 2005; Castro et al., 1999; Itô and Hino, 2004; Dulamsuren et al., 2013). The protection against herbivores in our study resulted also in an increased seedling emergence (Table 1) supporting the evidence that herbivores can be an important constraint on seedling recruitment at treeline. However, the overall impact of post-dispersal seed and seedling predation might have been reduced in our study system by the exclusion of invertebrates and digging animals, such as voles, mice and small rodents that seem to have a major influence on seed predation.

Effects of inter-annual climate variability and species-provenances on seedling recruitment

Emergence

The present study provides evidence for the fact that inter-annual climate variability affects seedling emergence (Table 2). Growing season temperatures and precipitation varied considerably among the three study years. The warm and dry summer of 2013 was followed by a cold and wet summer in 2014 and an exceptionally warm summer in 2015 (Fig. S2). In 2013, highest seedling emergence occurred at treeline, whereas in 2014, highest emergence was observed at the alpine site (Table 2; Fig. 4a). This result may be explained by stronger limitations due to summer drought at high elevation, where no tall-growing vegetation, such as dwarf-shrubs, give protection against strong solar radiation and prevent drought related to the early seedling mortality. Indeed, several studies provide evidence for negative effects of high solar radiation on seedling recruitment (Germino et al., 2002; Bader et al., 2007; Maher et al., 2005). However, our result is in contrast to the finding of Castanha et al. (2012), who observed highest germination at an alpine site in an exceptionally warm summer (2009) in a study on seedling recruitment of *Pinus flexilis* and *Picea engelmanni* across a treeline ecotone in the Rocky Mountains (Colorado, USA). The authors relate their findings to the fact that higher temperatures extended the alpine growing season due to earlier snow melt, while they induced emergence constraining drought at the lower forest site (Castanha et al., 2012).

On plots with intact vegetation, seedling emergence from seeds sown in 2014 was higher than emergence from seeds sown in 2013 (Table 2). This possibly reflects the cold and wet conditions of summer 2014 and suggests that the main constraint was not water availability, but water surplus leading to rotting of seeds. The effect of the surrounding vegetation may have been positive in summer 2014, due to higher interception, absorption and evaporation (Hibbert, 2007) in contrast to the low water supply in summer 2013, where reduced competition for water on scarified plots may have facilitated seedling emergence. The influence of inter-annual climate variability can thus hardly be generalized, and it is important to consider the timing and duration of drought periods, but also the timing of spring snowmelt and potential water surplus effects.

Early Seedling Establishment

In our experiment, seedlings that survived the first winter generally survived till the end of the second vegetation period (Table S4). This finding coincides with Batllori et al. (2009) who observed 100 % summer survival after the first winter for *Pinus uncinata* in a seedling transplant experiment in the Pyrenees. While this confirms the hypothesis that first winter is one of the major bottle necks for seedling recruitment, as it bears the greatest risk to survival (Körner, 2012), we have to take into account that, depending on climate, mortality waves are also possible in later stages of seedling recruitment. Indeed, our findings of generally high seedling mortality during the second winter that was more pronounced at the alpine than the treeline site (Table 4,S4; Fig. 6) may be related to more adverse autumn climate conditions at high elevation (Fig. S1). Likewise, earlier research in our study area showed that seedling survival was high during the first three years of observation for *P. abies*, *L. decidua* and other coniferous species, but was strongly reduced after the third winter for the broadleaved species *Alnus viridis* and *Sorbus aucuparia* (Zurbriggen et al., 2013). This shows the need for studying seedling recruitment also in later stages and that these effects may be strongly species specific and even affected by provenance differences.

Species and Provenance Effects

In our study, low provenance seedlings of *L. decidua* emerged better than high provenance seedlings of the same species (Table 2). This result may be seen as a contradiction to several investigations and practical experiences with low survival rates of lowland provenances in higher altitudes near treeline (Nather, 1987, Schönenberger et al., 1990). However, while most of these investigations and experiences with lowland provenances are based on afforested trees and showed that a particular susceptibility to frost damage during a later stage of seedling development was increased for lowland provenances, this was obviously not yet a critical factor for the one- and two-year old seedlings in our study. The better emergence of low provenance seedlings of *L. decidua* in our experiment might possibly be because those seeds were considerably heavier compared to the seeds of the high elevation provenance and this larger seed sizes may have favoured germination. In fact, under higher environmental stress, larger seed size may favour germination even when such a relationship is absent under less extreme conditions (Castro et al, 1999). The role of provenance is thus likely to have diverging effects at different stages of seedling establishment near treeline.

Seedling establishment varies also among species. In our treeline ecotone, we observed higher survival of *L. decidua* than *P. abies* at the alpine site, whereas the inverse pattern occurred at treeline (Table 3). This finding is in line with the current range limits of the two species in the Central Swiss Alps (Brändli et al, 1998, Zurbriggen et al, 2013). While it is well known that *L. decidua* is better adapted to the cold environment of treeline sites in the continental Alps compared to the evergreen and less frost tolerant species *P. abies* (Tranquillini, 1979), it is less clear if the first years of seedling establishment contribute already to this difference. Other studies in our study area provide evidence that later stages in the seedling development (years 5-15) are also critical bottle necks for tree survival in and above the treeline ecotone and that the effect of elevation on seedling survival is relatively small during the first years, but increasing in later years of seedling development (Barbeito et al, 2012, Zurbriggen et al., 2013). In order to understand and disentangle the importance of the different treeline limiting factors, it is thus important to study these effects in different stages of seedling development and to take advantage of long term observations.

Conclusions

Our results provide experimental evidence for seed source limitation and the importance of a multitude of interacting abiotic and biotic factors in determining seedling recruitment in an alpine treeline ecotone in the Swiss Alps. Seedling emergence and establishment are generally not temperature limited but rather depend on the availability of viable seeds and suitable seedbed conditions. More open alpine microsite conditions promote seedling emergence in contrast to dense subalpine dwarf shrub or forest understorey vegetation. Although elevation dependent temperature differences are not limiting, inter-annual climate variation seems to have an important influence on early seedling recruitment in this alpine treeline ecotone. In addition to this, natural regeneration at treeline is potentially delayed because of, for example, longer masting year cycles or irregular predator outbreak cycles, which further emphasizes the need for longer term studies on treeline recruitment. Continued research and combining our findings with a global research initiative will allow to disentangle the importance of different treeline limiting factors and their interacting effects worldwide and will contribute to a widely supported prediction of future treeline expansion.

2.2.5 Supplementary Materials

TABLES

Table S1 Location of the experimental sites

Site	Longitude [Swiss Grid]	Latitude [Swiss Grid]	Elevation [m a.s.l.]
forest	2785500	1183500	1930
treeline	2785340	1183220	2100
alpine	2785090	1182570	2390

Table S2 Characteristics of seed provenances

Species	Provenance	Elevation [m a.s.l.]	Location	Aspect [°]	Collection year	TGW [g]	Viability [%]
L. decidua	low	1350	Wiesen	225	1995	8.5	28
L. decidua	high	1760	Celerina	0	1970	7.3	11
P. abies	low	1000	Küblis	0	1985	6.8	74
P. abies	high	1960	Sertig	90	1983	6.8	61

TGW: thousand grain weight.

Table S3 Soil temperatures (monthly mean ±standard deviation) for the experimental sites from July 2013 to August 2015

Year	Month	Soil temperature at forest site [°C]	Soil temperature at treeline site [°C]	Soil temperature at alpine site [°C]
2013	July	10.4±1.0	9.9±0.8	14.2±1.9
	August	9.7±1.4	9.2±0.9	11.2±2.2
	September	7.0±1.6	6.8±1.2	6.6±2.5
	October	4.8±1.9	4.8±1.3	3.2±2.2
	November	1.4±1.0	2.1±0.5	0.7±0.1
	December	0.4±0.1	0.8±0.2	0.4±0.1
2014	January	0.4±0.0	0.8±0.0	0.3±0.0
	February	0.4±0.0	0.8±0.0	0.3±0.0
	March	0.4±0.0	0.8±0.0	0.3±0.0
	April	0.5±0.2	0.7±0.0	0.3±0.0
	May	4.8±2.4	3.3±2.0	0.7±0.7
	June	8.7±1.5	8.6±1.4	9.2±3.7
	July	8.8±1.1	9.4±1.1	9.9±2.5
	August	8.6±1.3	9.0±1.3	9.4±1.8
	September	7.3±1.3	7.2±1.3	6.9±1.7
	October	5.2±2.4	4.9±1.6	3.8±2.1
	November	1.9±0.7	1.9±0.3	0.6±0.1
	December	0.3±0.6	0.6±0.4	0.4±0.0
2015	January	0.2±0.1	0.3±0.1	0.3±0.0
	February	0.2±0.0	0.4±0.0	0.2±0.0
	March	0.2±0.0	0.4±0.0	0.2±0.0
	April	0.3±0.0	0.4±0.0	0.3±0.0
	May	4.6±2.2	3.0±2.2	2.7±3.1
	June	9.1±1.5	9.5±1.3	11.1±3.0
	July	11.5±1.2	12.4±1.1	16.3±2.3
	August	10.8±1.4	11.2±1.4	13.3±2.7

Soil temperatures were measured with i-buttons in 5 cm soil depth.

Table S4 Number of seedlings that survived the first (a) and the second winter (b) grouped by species and provenance for site, substrate scarification and seeding year

(a) Survival 1st winter

	total	Larix	Picea	Larix low	Larix high	Picea low	Picea high
Treeline site	348	164	184	148	16	147	37
Alpine site	60	51	9	50	1	5	4

	total	Larix	Picea	Larix low	Larix high	Picea low	Picea high
scarified	344	173	171	157	16	131	40
intact	64	42	22	41	1	21	1

	total	Larix	Picea	Larix low	Larix high	Picea low	Picea high
seeded 2013	302	132	170	121	11	135	35
seeded 2014	106	83	23	77	6	17	6

(b) Survival 2nd winter

	total	Larix	Picea	Larix low	Larix high	Picea low	Picea high
Treeline site	227	104	123	96	8	94	29
Alpine site	9	7	2	7	0	0	2

	total	Larix	Picea	Larix low	Larix high	Picea low	Picea high
scarified	229	104	125	96	8	94	31
intact	7	7	0	7	0	0	0

FIGURES

Figure S1 Soil temperatures for the alpine (A), treeline (T) and forest site (F) from July 2013 to August 2015 measured by i-buttons in 5 cm soil depth

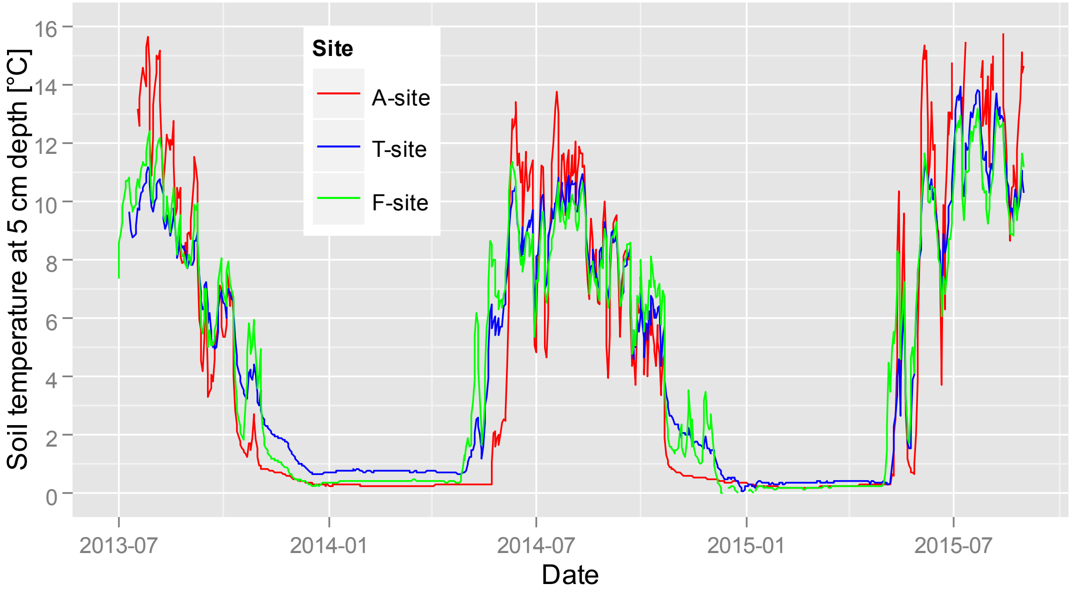
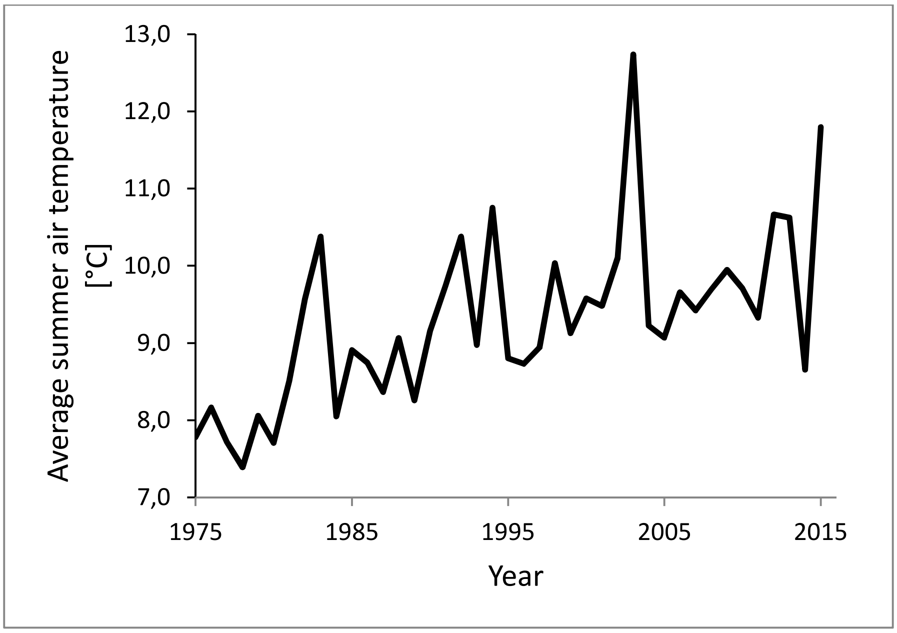


Figure S2 Annual mean summer air temperatures from 1975 – 2015 from the Stillberg climate station in the research area at 2100m (longitude: 46.773573/ latitude: 9.86716). Growing season temperatures were calculated from monthly averages of the months June, July and August.



2.2.6 Tables and Figures Index

TABLES

FIGURES

SUPPLEMENTARY MATERIALS

Tables

Figures

SYNTHESIS

Sunset in the Alps, Bormio, Italy

(Photo: Giulietta Bernareggi)

This study investigated the effects of climate change on the early stages of alpine plant life cycle, namely seed dispersal and germination. We focused on two different environments, an alpine snowbed and a treeline ecotone, considering different typology of plant species, herbaceous and trees.

This chapter synthesises the main findings from the thesis, puts the objectives in a global perspective and discusses future research directions and practical implications.

WHAT WE HAVE LEARNED: GLOBAL EFFECTS

In high-elevation alpine environment, under the current climate, the snowmelt usually occurs between the first and the second week of July; afterwards, pants take about one month to flowering and to start the seed production, which is completed at the end of the summer and followed by seed dispersal during the first half of September. 15 days later, at the beginning of October, the snowfall is expected; thus, seeds spend the winter season under the snow and prepare themselves, by breaking the dormancy, to germination, that will be trigged after the snowmelt the following summer. In such a context, the influence of climate warming can affect a variety of biotic and abiotic elements, whose effects can act in direct or indirect ways and altering the normal seasonal vegetative cycle of alpine species.

The first of the direct effects is the rising of temperatures during the growing season. The recent researches mainly focused on the effects on fresh seeds that, subjected to higher temperatures after dispersion, can alter they dormancy status and being trigged to germination before the winter season, as we also demonstrate in Chapter 1.4. However, the rise in temperatures is not selective for the Autumn, but acts during all the growing season, affecting other phases of the seed production process. Indeed, we showed how a warmer parental environment enhances (a) the seed resistance to ageing (i.e. seed longevity, Chapter 1.3), (b) the seed resistance to long winter seasons (Chapter 1.4), (c) affects the seed dormancy by further widening towards lower values the temperatures for germination (Chapter 1.4) and (d) improves the seed quality which, in turn, emphasizes all the precedent responses. Moreover, a warmer growing season can also speed up the plant phenological rhythms, advancing the phenological phases by 15 days, in average. It follows that, even if the temperatures during the current seed dispersal time do not increase to a significant extent, the speeded phenology would anyway cause an early seed dispersal, in a time of the year where the temperatures are actually higher and leading, also in this case, to increased seedling emergence before the winter season.

Among the indirect effects of the climate warming, the reduction of the snow cover duration (anticipated snowmelt and delayed snowfall) is the one that causes the most important consequences on the plant life cycle. Indeed, the snowmelt time marks the beginning of the vegetative season; it follows that an advanced snowmelt inevitably leads to an early start and the consequent advance of the phenological phases, including seed dispersal, thus laying, also in this case, dispersed seeds to warmer temperatures. Moreover, the short duration of the snow cover can threaten the survival of the early seedlings emerged soon after the snowmelt from the vernalized seeds. Indeed, early absence of snow is often associated with top soil frost events, that can damage or kill the new born seedlings. On the other hand, delays in snowfalls imply important consequences on the fresh seeds dispersed at the end of the growing season. Although, normally, the temperatures at this time of the year are not warm enough to stimulate the germination of fresh seeds, the possibility that the prolonged persistence at these condition before the snowfall allows seeds to reach heating degree-day for germination cannot be ruled out. This thought is supported by another indirect effect that must be considered: the heat waves. A recent study (Orsenigo et al., 2015) demonstrated that a short but extreme heating in the late season triggers the germination of early dispersed seeds.

PRATICAL IMPLICATION OF OUR RESULTS

In Chapter 1.3 we demonstrated, for the first time, that under moderate climate warming (about +2 K) alpine snowbed species produced seeds with an extended resistance to heat stress, indicating an effective and potentially adaptive rapid response to the warmer environment. Such result, showing the key role of plastic responses for survival and persistence of alpine species facing climate change, highlights the need to prove and scientifically correlate the results of the ageing test with the effective ability of seeds to persist in the soil. Moreover, this study opens the way for a new management of the *ex situ* conservation: our result show that an accurate scheduling for seed storing can occurs only if it is taken into account not only the plant provenance but also the condition in which seeds have developed.

Chapter 1.4 described how pre- and post- seed dispersal climate warming influence seed dormancy and germination requirements in alpine snowbeds species. First of all, our results suggest that, in a warmer climate, regardless the climatic scenarios experienced before seed dispersal, germination at the end of the growing season should increase. In line with the recent studies about the heat waves and in view of the important implications for seedling survival and establishment, also here we have showed how understanding which short term heating periods can enhance seed germination immediately after seed dispersal is becoming increasingly urgent.

Moreover, this study highlights that the current alpine climate can have unfavourable impacts on seed survival capacities, pointing out that not only the seedlings, but also the seeds can be damaged by long-lasting winter conditions. Following these observations, a crucial future challenge will be to understand which could be the minimum periods and/or the minimum thermal thresholds that a seedling must reach in order to survive the winter season.

Finally, this chapter shows interesting and novel observations indicating that changes of germination requirements induced by the pre-dispersal warming environment could depend on the seed dormancy state. In particular, deeper dormant species shows major changes in response to incubation temperature on both fresh and cold stratified seeds and less dormant species in response to stratification. Further studies are therefore needed in order to confirm whether this could be assumed as a general trend for the alpine species.

In the context of alpine ecotones, Chaper 2.2, we focused on the limiting factors for the treeline expansion under climate warming. Our findings suggest that tree seedlings can establish above the current treeline, with a high evidence for seed limitation and moderate evidence for substrate limitation. It follow that, despite the main role of temperature in driving seed germination, the emergence and early establishment of seedlings seem less limited by temperature than by multiple interacting abiotic and biotic factors, thus leading the treeline advance to leg behind rates of climate change.

CONCLUSIONS

This PhD research wants primarily emphasize the necessity to consider the all effects, direct and indirect, that climate warming produces in any time of the year. Indeed, the omission/non-consideration of even only one indirect effect can drastically change the outcome of a research on the direct ones. Therefore, in the research about the seed germination and the early establishment of seedlings, it is extremely important to always take into account the complex interrelation between the climate warming effects on seed phenology (e.g. advanced seed dispersal), and on environmental conditions (e.g. early snowmelt, warmer temperatures during the snow free period), only in this way a realistic evaluation of the recruitment success in alpine environment can be ensured.

Secondly, but related, we want to point out the crucial role that plastic adjustments can have in the future plant adaptation to the rapid climate change. The potentiality of such transgenerational adjustment and extent that can reach in front of different magnitudes of alteration is still unknown.

Thirdly, we want to highlight the importance that interspecific variations of plant traits plays in driving species coexistence. In modelling the future dynamics of communities, it will be essential know the specie-specific responses to the alteration of each studied parameter. Different responses in species that currently inhabit the same areal will inevitably alter the mechanisms of dominance or coexistence that drive the community composition, subjecting the species to new selective forces that will push towards the evolution.

This thought directly connects with the last conclusion: the need of long term observations. Not only the germination, but also the survival of seedling during the early stages of development is a key factor in determining the future evolution of both erbaceous plant communities in high-elevation environment and tree plants forming the treelines.

ACKNOWLEDGEMENTS

I thank all the people that have supported and helped me during my studies and researches, it has been a great privilege to do a PhD.

In particular, I want to thank my supervisor Dr. Alessandro Petraglia for possibility to get this PhD, for the huge amount of opportunities he gave me and, above all, for the sincere trust that he always shown me. He has been a patient teacher and an important guide during these 3-years route.

I want to extend a special thank also to Michele Carbognani, colleague and friend, always present, that has accompanied, supported, educated and criticized me, sharing good and bad experiences, in work and in life. I'm very grateful to him.

And I cannot forget to thank Dr. Andrea Mondoni, the first that drove me towards research. It has always been a safe and important reference point for any necessity, a teacher, a frien, again, in work and in life. He was the person

with whom I spent most of the experiences during the early years of the PhD, and I’m very grateful to him for all the things that he shared with me.

I am grateful to all of them, for opinions exchanges and fruitful discussion of results, that led to new ideas on present and future researches!

I’m also extremely grateful to Christian Rixen and Peter Bebi for giving me the possibility to join the Swiss G-Tree Projet, for welcoming me in their research group, for giving me an accommodation in Davos and for having trusted me during the work. The Swiss period has been a precious experience.

Thanks also to all the colleagues that during these years have shared with me the efforts and satisfactions of daily work and the joys and difficulties of the field work, both in Italy: Thomas, Simone, Lino, Daniele, Nicola, Elena, Maurizia, Silvia, Paolo… and in Swiss: Janet, Chiara, Aino, Chelsea, Rachel, Francesca, Ueli, Moritz, Gregy, Alessio, Sammy, Dave and many others. Each of them taught me something and showed me that together we achieve more!

I’m also grateful to all the people that I met during my trips in Alps, in Pravasivo and in Santa Caterina, with whom I shared chats, experiences and daily life… some have been important achievements!

Finally special thanks to my family, to my parents, that never doubted about me and are always the first to encourage me in any new adventure, and to You, the happyest discovery of this PhD.

REFERENCES

Albertini R. 1955. *Contributo alla conoscenza della morfologia crionivale del Gruppo Ortles-Cevedale*. Studi sui fenomeni crionivali delle Alpi Italiane, Fondazione per i problemi montani dell'Arco Alpino, n.11, Parma-

Amen RD. 1966. The Extent and Role of Seed Dormancy in Alpine Plants. *The Quarterly Review of Biology* **41**: 271–281.

Anschlag K, Broll G, Holtmeier F. 2008. Mountain birch seedlings in the treeline ecotone, subarctic Finland: variation in above- and below-ground growth depending on microtopography. *Arctic Antarctic and Alpine Research* **40**:609–616.

Arft AM, Walker MD, Gurevitch J, *et al.* 1999. Responses of tundra plants to experimental warming: meta-analysis of the International Tundra Experiment. *Ecological Monographs* **69**: 491–511.

Bader MY, 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 MA, Rixen C, Senn J and Bebi P. 2012. Factors driving mortality and growth at treeline: a 30-year experiment of 92 000 conifers. *Ecology* **93**: 389–401.

Barriopedro D, Fischer EM, Luterbacher J, Trigo RM, García-Herrera R. 2011. The hot summer of 2010: redrawing the temperature record map of Europe. *Science* **332**: 220–224.

Baskin CC, Baskin JM. 2014. *Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination*, 2nd edn. London: Academic Press.

Baskin JM, Baskin CC. 2004. A classification system for seed dormancy. *Seed Science Research* **14**: 1–16.

Baskin JM, Baskin CC, Li X. 2000. Taxonomy, ecology, and evolution of physical dormancy in seeds. *Plant Species Biology* **15**: 139–152.

Bates DM, Maechler M, Bolker B. 2014. *lme4: Linear mixed-effects models using Eigen and S4*. R package version 1.0-4, http://CRAN.R-project.org/package=lme4.

Batllori E, Camarero JJ, Ninot JM, Gutieérrez 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.

Bebi P, Hagedorn F, Martin M, Rixen C, Senn J, Wasem U. 2009. Langfristige Waldgrenzen-Forschung am Stillberg – vor lauter Bäumen den Wald noch sehen. Forum für Wissen, 87–92.

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

Bernareggi G, Carbognani M, Petraglia A, Mondoni A. 2015. Climate warming could increase seed longevity of alpine snowbed plants. *Alpine Botany* **125**: 69–78.

Bewley JD, Bradford KJ, Hilhorst HWM, Nonogaki N. 2013. *Seeds: Physiology of development, germination and dormancy*, 3rd edn. Springer, New York Heidelberg Dordrecht London.

Billings WD, Mooney HA. 1968. The ecology of arctic and alpine plants. *Biological Reviews* **43**: 481–529.

Björk RG, Molau U. 2007. Ecology of alpine snowbeds and the impact of global change. *Arctic, Antarctic and Alpine Research* **39**: 34–43.

Black RA, Bliss LC. 1980. Reproductive ecology of *Picea mariana* (Mill.) BSP., at tree line near Inuvik, Northwest Territories, Canada. *Ecological Monographs* **50**: 331–354.

Bolnick DI, Amarasekare P, Araújo MS, *et al.* 2011. Why intraspecific trait variation matters in community ecology. *Trends in Ecology and Evolution* **26**: 183–19.

Boyko A, Blevins T, Yao Y, ***et al.*** **2010.** Transgenerational adaptation of Arabidopsis to stress requires DNA methylation and the function of dicer-like proteins. *PLoS One* 5: e9514. doi:10.1371/journal.pone.0009514.

Brandel M. 2005. The effect of stratification temperatures on the level of dormancy in primary and secondary dormant seeds of two *Carex* species. *Plant Ecol*ogy **178**: 163–169.

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

Brown CD, Johnstone JF, Mamet SD, Trant AJ. 2013. Global Treeline Range Expansion Experiment Field Protocols.

Brown CD, Vellend M. 2014. Non-climatic constraints on upper elevational plant range expansion under climate change. *Proceeding of the royal society* **doi**: 10.1098/rspb.2014.1779.

Bullock JM, Clarke RT. 2000. Long distance seed dispersal by wind: measuring and modelling the tail of the curve. *Oecologia* **124**: 506–521.

Callaway RM, Brooker RW, Choler P*, et al.* **2002** Positive interactions among alpine plants increase with stress. *Nature* **417**: 844–848.

Carbognani M, Petraglia A, Tomaselli M. 2012. Influence of snowmelt time on species richness, density and production in a late snowbed community. *Acta Oecologica* **43**: 113–120.

Carbognani M, Tomaselli M, Petraglia A. 2014*a*. Current vegetation changes in an alpine late snowbed community in the south-eastern Alps (N-Italy). *Alpine Botany* **124**: 105–113.

Carbognani M, Petraglia A, Tomaselli M. 2014*b*. Warming effects and plant trait control on the early-decomposition in alpine snowbeds. *Plant and Soil* **376**: 277–290.

Castanha C, Torn MS, Germino MJ, Weibel B, Kueppers LM. 2012. Conifer seedling recruitment across a gradient from forest to alpine tundra: effects of species, provenance, and site. Plant Ecology and Diversity **6**: 307–318.

Castro J. 1999. Seed mass versus seedling performance in Scots pine: maternally dependetn train. *New Phytologist* **144**: 153–61.

Cavieres LA, Arroyo MTK. 2000. Seed germination response to cold stratification period and thermal regime in *Phacelia secunda* (Hydrophyllaceae): Altitudinal variation in the mediterranean Andes of central Chile. *Plant Ecology* **149**: 1–8.

Chabot BF, Billings WD. 1972. Origins and Ecology of the Sierran Alpine Flora and Vegetation. *Ecological Monographs* **42**: 163–199.

Chapin FS III, Shaver GR, Giblin AE, Nadelhoffer KJ, Laundre JA. 1995. Responses of arctic tundra to experimental and observed changes in climate. *Ecology* **76**: 694–711.

Chapin FS III, Sturm M, Serreze MC, *et al.* 2005. Role of Land-Surface Changes in Arctic Summer Warming. *Science* **310**: 657–660.

Chesson P. 2000. Mechanisms of maintenance of species diversity. *Annual Review of Ecology, Evolution and Systematics* **31**: 343–366.

Christensen JH, Hewitson B, Busuioc A, *et al.* 2007. *Regional climate projections , Climate Change, 2007: The Physical Science Basis*. Contribution of Working group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, University Press, Cambridge, Chapter 11, ISBN: 978-0-521-88009-1 .

Côté M Ferron J, Gagnon R. 2003. Impact of seed and seedling predation by small rodents on early regeneration establishment of black spruce. *Canadian Journal of Forest Research* **33**: 2362–2371.

Côte M Ferron J, Gagnon R. 2005. Invertebrate predation of postdispersal seeds and juvenile seedlings of black spruce (*Picea mariana*) in the boreal forest of eastern Canada. *Canadian Journal of Forest Research* **35**: 674–681.

Cranston BH, Hermanutz L. 2013. Seed–seedling conflict in conifers as a result of plant–plant interactions at the forest-tundra ecotone. *Plant Ecology and Diversity* **6**: 319–327.

Crawley MJ. 2007. *The R book*, Chichester: Chichester : Wiley.

Crowley TJ. 2000. Causes of climate over the past 1000 years. *Science* **289**: 270–277.

Crawley MJ. 2013. The R Book. Chichester: John Wiley & Sons.

Dalgleish HJ, Koons DN, Adler PB. 2010. Can life-history traits predict the response of forb populations to changes in climate variability? *Journal of Ecology* **98**: 209–217.

De Valpine P, Harte J. 2001. Plant responses to experimental warming in a montane meadow. *Ecology* **82**: 637–648.

Devi N, Hagedorn F, Moiseev P, Bugmann H, Shiyatov S, Mazepa V, Rigling A. 2008. Expanding forests and changing growth forms of Siberian larch at the Polar Urals treeline during the 20th century. *Global Change Biology* **14**: 1581–1591.

Diaz HF, Bradley RS. 1997. Temperature variations during the last century at high elevation sites. *Climatic Change* **36**: 253–279.

Diekmann M, Frison EA, Putter T. 1994. FAO/IPGRI *Technical Guidelines for the Safe Movement of Small Fruit Germoplasm*. Food and Agriculture Organisation of the United Nations, Rome / International Plant Genetic Resource Institute, Rome.

Dirnböck T, Essl F, Rabitsch W. 2011. Disproportional risk for habitat loss of high-altitude endemic species under climate change. *Global Change Biology* **17**: 990–996.

Donohue K 2009. Completing the cycle: maternal effects as the missing link in plant life histories. *Philosophical Transactions of the Royal Society B* **364**: 1059–1074.

Dulamsuren C, Hauck M and Leuschner C. 2013. Seedling emergence and establishment of Pinus sylvestris in the Mongolian forest-steppe ecotone. *Plant Ecology* **214**: 139-152.

Dunne JA, Harte J, Taylor KJ. 2003. Subalpine meadow flowering phenology responses to climate change: Integrating experimental and gradient methods. *Ecological Monographs* **73**: 69–86.

Ellis RH, Roberts EH. 1980. Improved equations for the prediction of seed longevity. *Annals of Botany* **45**: 13–30.

Ellis RH, Hong TD, Jackson MT. 1993. Seed production environment, time of harvest, and the potential longevity of seeds of three cultivars of rice (*Oryza sativa* L.). *Annals of Botany* **72**: 583–590.

Ellis RH, Hong TD. 1994. Desiccation tolerance and potential longevity of developing seeds of rice (*Oryza sativa* L.). *Annals of Botany* **73**: 501–50.

Elmendorf SC, Henry GHR, Hollister RD, *et al.* 2012. Plot-scale evidence of tundra vegetation change and links to recent summer warming. *Nature Climate Change* **2**: 453–457.

Elumeeva T, Onipchenko VG, Egorov AV, *et al.* 2013. Long-term vegetation dynamic in the Northwestern Caucasus: which communities are more affected by upward shifts of plant species? *Alpine Botany* **123**: 77–85.

Engler R, Randin CF, Thuiller W, *et al.* 2011. 21st century climate change threatens mountain flora unequally across Europe. *Global Change Biology* **17**: 2330–2341.

Eriksson O, Ehrlén J. 1992. Seed and microsite limitation in plant populations. *Oecologia* **91**: 360–364.

Esper J, Schweingruber FH. 2004. Large-scale treeline changes recorded in Siberia. *Geophysical Research Letters* **31**: 1–5.

Facelli JM, Chesson P, Barnes N .2005. Differences in seed biology of annual plants in arid lands: a key ingredient of the storage effect. *Ecology* **86**: 2998–3006.

Fay PA, Schultz MJ. 2009. Germination, survival, and growth of grassland forb seedlings: effects of soil moisture variability. *Acta Oecologica* **35**: 679–684.

Fenner M. 1991. The effects of the parent plant environment on seed germinability. *Seed Science Research* **1**: 75–84.

Fenner M, Thompson K. 2005. *The ecology of seeds*. Cambridge: Cambridge University Press.

Fernández-Pascual E, Jiménez-Alfaro B, Caujapé-Castells J, Jaén-Molina R, Díaz TE. 2013. A local dormancy cline is related to the seed maturation environment, population genetic composition and climate. *Annals of Botany.* **112**: 937–945.

Fernández-Pascual E, Jiménez-Alfaro B. 2014. Phenotypic plasticity in seed germination relates differentially to overwintering and flowering temperatures*.* *Seed Science Research* **24**: 273–280.

Finch-Savage WE, Leubner-Metzger G. 2006. Seed dormancy and the control of germination. *New Phytologist* **171**: 501–523.

Gabrielsen TM, Brochmann C. 1998. Sex after all: high levels of diversity detected in the arctic clonal plant *Saxifraga cernua* using RAPD markers. *Molecular Ecology* **7**: 1701–1708.

Galen C, Stanton ML. 1995. Responses of snowbed plant-species to changes in growing-season length. *Ecology* **76**: 1546–1557.

Galloway LF, Etterson JR. 2007. Transgenerational plasticity is adaptive in the wild. *Science* **318**: 1134–1136.

García-Fernández A, Escudero A, Lara-Romero C, Iriondo JM. 2015. Effects of the duration of cold stratification on early life stages of the Mediterranean alpine plant *Silene ciliata*. *Plant Biology* **17**: 344–350.

Geneve RL. 2003. Impact of Temperature on Seed Dormancy. *Hort Science* **38**: 336 –341.

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

Gobiet A, Kotlarski S, Beniston M, Heinrich G, Rajczak J, Stoffel M. 2014. 21st century climate change in the European Alps – A review. *Science of The Total Environment* **493**: 1138–1151.

Gottfried M, Pauli H, Futschik A, et al. 2012. Continent-wide response of mountain vegetation to climate change. *Natural Climate Change* **2**: 111–115.

Grabherr G, Gottfried M, Pauli H. 1994. Climate effects on mountain plants. *Nature* **369**: 448–448.

Grau O, Ninot JM, Cornelissen JHC, Callaghan TV. 2013. Similar tree seedling responses to shrubs and to simulated environmental changes at Pyrenean and subarctic treelines. *Plant Ecology and Diversity* **6**: 329–342.

Graumlich LJ, Waggoner LA, Bunn AG. 2005 Detecting global change at alpine treeline: coupling paleoecology with contemporary studies. In: *Huber UM, Bugmann KM, Reasoner MA(eds) Global change and mountain regions. An overview of current knowledge*. Springer, Dordrecht, pp. 501–508.

Gutterman Y. 2000. Maternal effects on seeds during development. In: Fenner M, ed. *Seeds: The Ecology of Regeneration in Plant Communities*, 2nd edn. Wallingford: CABI Publishing, 59–84.

Haeberli W, Beniston M. 1998. Climate Change and Its Impacts on Glaciers and Permafrost in the Alps. *Ambio* **27**: 258–265.

Haettenschwiler S, Smith WK. 1999. Seedling occurrence in alpine treeline conifers: a case study from the central Rocky Mountains, USA. *Acta Oecologica* **20**: 219–224.

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

Hay F. 2004. The seed viability equations. http://data.kew.org/sid/viability/SeedViabilityEquationsFHDec04.pdf. Accessed 19 August 2013.

Hay FR, Probert RJ. 1995. Seed maturity and the effects of different drying conditions on desiccation tolerance and seed longevity in foxglove (*Digitalis purpurea* L.). *Annals of Botany* **76**: 639–647.

Hay FR, Smith RD. 2003. Seed maturity: when to collect seeds from wild plants. In: Smith RD, Dickie JB, Linnington SH, Pritchard HW, Probert RJ (eds) *Seed conservation: turning science into practice.* London: Royal Botanic Gardens, Kew, pp 97–133.

Hay FR, Adams J, Manger K, Probert RJ. 2008. The use of non-saturated lithium chloride solutions for experimental control of seed water content. *Seed Science Technology* **36**: 737–746.

Hébert F, Boucher JF, Bernier PY, Lord D. 2006. Growth response and water relations of 3-year-old planted black spruceand jack pine seedlings in site prepared lichen woodlands. *Forest Ecology and Management* **223**: 226–236.

Hedhly A, Hormaza JI, Herrero M. 2009. Global warming and sexual plant reproduction. *Trends in Plant Science* **14**: 30–36.

Herman JJ, Sultan SE. 2011. Adaptive transgenerational plasticity in plants: case studies, mechanisms, and implications for natural populations. *Frontiers in Plant Science* **2**: 102. doi:10.3389/fpls.2011.00102.

Hibbert, AR. 2007. Water yield improvement potential by vegetation management on western rangelands. *Journal of the American Water Resources Association* 19: 375–381.

Higgins SI, Clark JS, Nathan R, *et al*. 2003. Forecasting plant migration rates: managing uncertainty for risk assessment. *Journal of Ecology* 91: 341–347.

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25: 1965–1978.

Hobbie SE, Chapin FS III. 1998. The response of tundra plant biomass, aboveground production, nitrogen, and CO_2 flux to experimental warming. *Ecology* 79: 1526–1544.

Hollister RD, Webber PJ, Tweedie CE. 2005. The response of Alaskan arctic tundra to experimental warming: differences between short- and long-term responses. *Global Change Biology* 11: 525–536.

Holtmeier FK. 1993a. Der Einfluß der generativen und vegetativen Verjüngung auf das Verbreitungsmuster der Bäume und die ökologische Dynamik im Waldgrenzbereich. *Beobachtungen und Untersuchungen in Hochgebirgen Nordamerikas und den Alpen. Geoökodynamik* XIV: 153–182.

Holtmeier FK. 1993. Timberlines as indicators of climatic changes: Problems and research needs. *Paläoklimaforschung* 9: 211–222.

Holtmeier FK. 1995a. Waldgrenze und Klimaschwankungen – Ökologische Aspekte eines vieldiskutierten Phänomens. *Geoökodynamik* XVI: 1–24.

Holtmeier FK. 1995*b*. European Larch in Middle Europe with special reference to the Central Alps. In: *Ecology and management of Larix forests: A look ahead.* In: Proc. int. symp., Whitefish, MT, October 5–9, 1992 (Schmidt, W. C. and McDonald, K. J., eds.): 41–49, USDA For. Serv., Intermtn. Res. Stat., Gen. Techn. Rep. GTR-INT-319

Holtmeier FK. 1999. Timberline dynamics in view of climate change. In: A terrestrial transect for Scandinavia/Northern Europe: Proc. int. Scantran conference (Turunen, M., Hakkinen, J., Heal, O. W., Saelthun, R. and Holten, J., eds.), Eur. Comm. *Ecosystems research report* 31: 53–55

Holtmeier FK. 2009. *Mountain timberlines : ecology, patchiness, and dynamics*, Dordrecht: [Dordrecht] : Springer Science+Business Media B.V.

Holtmeier FK, Broll G. 2005. Sensitivity and response of northern hemisphere altitudinal and polar treelines to environmental change at landscape and local scales. *Global Ecology and Biogeography* 14: 395–410.

Hoyle GL, Venn SE, Steadman KJ, *et al.* 2013. Soil warming increases plant species richness but decreases germination from the alpine soil seed bank. *Global Change Biology* 19: 1549–1561.

Hoyle GL, Steadman KJ, Daws MI, Adkins SW. 2008. Pre- and post-harvest influences on seed dormancy status of an Australian Goodeniaceae species, *Goodenia fascicularis*. *Annals of Botany* 102: 93–101.

Huelber K, Gottfried M, Pauli H, Reiter K, Winkler M, Grabherr G. 2006. Phenological Responses of Snowbed Species to Snow Removal Dates in the Central Alps: Implications for Climate Warming. *Arctic, Antarctic, and Alpine Research* 38: 99–103.

Hülber K, Winkler M, Grabherr G. 2010. Intraseasonal climate and habitat-specific variability controls the flowering phenology of high alpine plant species. *Functional Ecology* 24: 245–252.

Hunziker U and Brang P. 2005. Microsite patterns of conifer seedling establishment and growth in a mixed stand in the southern Alps. *Forest Ecology and Management* 210: 67–79.

Imbeck H, Ott E. 1987. Verjüngungsökologische Untersuchungen in einem hochstaudenreichen subalpinen Fichtenwald, mit spezieller Berücksichtigung der Schneeablagerung und der Lawinenbildung.

IPCC (International Panel on Climate Change). 2013. Summary for policymackers. In: Stocker TF, Qin D, Plattner G-K, *et al.*, eds. *Climate change 2013: the physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge: Cambridge University Press, 3–29.

Itô H and Hino T. 2004. Effects of deer, mice and dwarf bamboo on the emergence, survival and growth of Abies homolepis (Piceaceae) seedlings. *Ecological Research* 19: 217–223.

Johnstone J, Boby L, Tissier E, Mack M, Verbyla D, Walker X. 2009. Postfire seed rain of black spruce, a semiserotinous conifer, in forests of interior Alaska. *Canadian Journal of Forest Research* 39: 1575–1588.

Jones PD, Moberg A. 2003. Hemispheric and Large-Scale Surface Air Temperature Variations: An Extensive Revision and an Update to 2001. *Journal of Climate* 16: 206–223.

Klanderud K, Totland Ø. 2005. Simulated climate change altered dominance hierarchies and diversity of an alpine biodiversity hotspot. *Ecology* 86: 2047–2054.

Kochanek J. 2008. Parental Growth Environment Modulates Seed Longevity: Investigations Using Australian Native Species. PhD Thesis, University of Queensland, Australia.

Kochanek J, Steadman KJ, Probert RJ, Adkins SW. 2011. Parental effects modulate seed longevity: exploring parental and offspring phenotypes to elucidate pre-zygotic environmental influences. *New Phytologist* 191: 223–233.

Körner C, Cochrane P. 1983. Influence of plant physiognomy on leaf temperature on clear midsummer days in the Snowy Mountains, south-eastern Australia. *Acta Oecologica Oecol Plant* 4: 117–124.

Körner C. 2003. Alpine plant life: functional plant ecology of high mountain ecosystems. 2nd edn. Berlin: Springer–Verlag.

Körner C. 2003a. Limitation and stress - always or never? Journal of Vegetation Science **14**: 141–143.

Körner C, Paulsen J. 2004. A world-wide study of high altitude treeline temperatures. *Journal of Biogeography* **31**: 713-732.

Körner C, Ohsawa M, Spehn S, et al. 2005. Mountain Systems. Chapter 24. In: *Millennium Ecosystem Assessment, 2005. Current State and Trends: Findings of the Condition and Trends Working Group. Ecosystems and Human Well-being*, Vol. 1. Island Press, Washington, DC, USA.

Körner C. 2007. Climatic treelines: Conventions, global patterns, causes. *Erdkunde* **61**: 316–324.

Körner C. 2012. Alpine treelines : functional ecology of the global high elevation tree limits, Basel: Basel : Springer.

Kou HP, Li Y, Song XX *et al.* 2011. Heritable alteration in DNA methylation induced by nitrogen-deficiency stress accompanies enhanced tolerance by progenies to the stress in rice (*Oryza sativa* L.). *Journal of Plant Physiology* **168**: 1685–1693.

Kullman L. 2002. Rapid recent range-margin rise of tree and shrub species in the Swedish Scandes. *Journal of Ecology* **90**: 68–77.

Kullman L. 2004. Long-term geobotanical observations of climate change impacts in the Scandes of West-Central Sweden. *Nordic Journal of Botany* **24**: 445–467.

Kullman L. 2005. Wind-conditioned 20th century decline of birch treeline vegetation in the Swedish Scandes. *Arctic* **58**: 286–294.

Kuoch R. 1965. Der Samenanflug 1963/1964 an der oberen Fichtenwaldgrenze im Sertigtal. *Schweizerische Anstalt für das forstliche Versuchswesen, Mitteilungen* **41**: 63–85.

Larcher W, Wagner J. 1976. Temperaturgrenzen der CO2-Aufnahme und Temperaturresistenz der Blätter von Gebirgspflanzen im vegetationsaktiven Zustand. *Oecological Plant* 11:361–374.

Leck MA, Parker VT, Simpson RL. 2008. *Seedling Ecology and Evolution*, Cambridge: Cambridge : Cambridge University Press.

Lenoir J, Gégout JC, Marquet PA, de Ruffray P, Brisse H. 2008. A significant upward shift in plant species optimum elevation during the 20th century. *Science* **320**: 1768–1771.

Lenoir J, Gégout JC, Pierrat JC, Bontemps JD, Dhôte JF. 2009. Differences between tree species seedling and adult altitudinal distribution in mountain forests during the recent warm period (1986-2006). *Ecography* **32**: 765–777.

Leymarie J, Robayo-Romero ME, Gendreau E, Benech-Arnold RL, Corbineau F. 2008. Involvement of ABA in induction of secondary dormancy in barley (*Hordeum vulgare* L.) seeds. *Plant and Cell Physiology* **49**: 1830–1838.

Liu K, Baskin JM, Baskin CC, Bu H, Du G, Ma M. 2013. Effect of Diurnal Fluctuating versus Constant Temperatures on Germination of 445 Species from the Eastern Tibet Plateau. PLoS ONE **8**: e69364. doi: 10.1371/journal.pone.0069364.

Lloret F, Peñuelas J, Estiarte M. 2004. Experimental evidence of reduced diversity of seedlings due to climate modification in a Mediterranean-type community. *Global Change Biology* **10**: 248–258.

Loarie SR, Duffy PB, Hamilton H, Asner GP, Field CB, Ackerly DD. 2009. The velocity of climate change. *Nature* **462**: 1052–1055.

Long RL, Panetta FD, Steadman KJ, *et al.* 2008. Seed persistence in the field may be predicted by laboratory-controlled aging. *Weed Science* **56**: 523–528.

Luzuriaga AL, Escudero A, Pérez-García F. 2006. Environmental maternal effects on seed morphology and germination in *Sinapis arvensis* (Cruciferae). *Weed Research* **46**: 163–174.

Maher EL, Germino MJ, Hasselquist NJ. 2005. Interactive effects of tree and herb cover on survivorship, physiology, and microclimate of conifer seedlings at the alpine tree-line ecotone. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere* **35**: 567–574.

Mann ME, Jones PD. 2003. Global surface temperatures over the past two millennia. *Geophysical Research Letters* **doi**:10.1029/2003GL017814

Manson WL, Edwards C, Hale SE. 2004. Survival and early seedling growth of conifers with different shade tolerance in a Sitka spruce spacing trial and relationship to understorey light climate*. Silva Fennica* **38**: 357–370.

Marion GM, Henry GHR, Freckman DW, *et al*. 1997. Open-top design for manipulating field temperature in high-latitude ecosystems. *Global Change Biology* **3**: 20–32.

Maruta E. 1994. Seedling establishment of *Polygonum cuspidatum* and *Polygonum weyrichii* var. *alpinum* at high-altitudes of Mt Fuji. *Ecological Research* **9**: 205–213.

Matras J, Pâques L. 2008. EUFORGEN Technical Guidelines for genetic conservation and use for European Larch (*Larix decidua*).

Mazepa V. 2005. Stand density in the last millennium at the upper tree-line ecotone in the polar Ural Mountains. *Canadian Journal of Forest Research* **35**: 2082–2091.

McGill BJ, Enquist BJ, Weiher E, Westoby M. 2006. Rebuilding community ecology from functional traits. *Trends in Ecology & Evolution* **21**: 178–185.

MeteoSwiss. 2015. Monthly homogenized values, Davos. Online: http://www.meteoschweiz.admin.ch/product/output/climate-data/homogenous-monthly-data-processing/data/homog_mo_DAV.txt (retrieved on 09.01.2016).

Meyer SE, Allen PS. 1999. Ecological genetics of seed germination regulation in Bromus tectorum L. II. Reaction norms in response to a water stress gradient imposed during seed maturation. *Oecologia* **20**: 35–43.

Mikola P. 1952. The effect of recent climatic variations on forest-growth in Finland. *Fennia* **75**: 69–76.

Milbau A, Graae BJ, Shevtsova A, Nijs I. 2009. Effects of a warmer climate on seed germination in the subarctic. *Annals of Botany* **104**: 287–296.

Moiseev P, Shiyatov S. 2003. Vegetation dynamics at the treeline ecotone in the Ural Highlands, Russia. *Ecological Studies* **167**: 423–435.

Moles AT, Westoby M. 2004. What do seedlings die from, and what are the implications for evolution of seed size? *Oikos* **106**: 193–199.

Mondoni A, Probert RJ, Rossi G, Vegini E, Hay FR. 2011. Seeds of alpine plants are short lived: implications for long-term conservation. *Annals of Botany* **107**: 171–179.

Mondoni A, Rossi G, Orsenigo S, Probert RJ. 2012. Climate warming could shift the timing of seed germination in alpine plants. *Annals of Botany* **110**: 155–64.

Mondoni A, Orsenigo S, Donà M, *et al.* 2014. Environmental-induced transgenerational changes in seed longevity: maternal and genetic influence. *Annals of Botany* **113**: 1257–1263.

Mondoni A, Pedrini S, Bernareggi G, *et al.* 2015. Climate warming could increase recruitment success in glacier foreland plants*. Annals of Botany* **116**: 907–916.

Mooney HA, Billings WD. 1961. Comparative Physiological Ecology of Arctic and Alpine Populations of *Oxyria digyna*. *Ecological Monographs* **31**: 1–29.

Mote PW, Hamlet AF, Clark MP, Lettenmaier DP. 2005. Declining mountain snowpack in western North America*. Bulletin of the American Meteorological Society* **86**: 39–49.

Motta R, Brang P, Frehner M, Ott E. 1994. Copertura muscinale e rinnovazione di abete rosso (*Picea abies* L.) nella pecceta subalpina di Sedrun (Grigioni, Svizzera). *Monti e Boschi* **45**: 49-56.

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 Ecology* **210**: 19–30.

Murdoch AJ and Ellis RH, 2000. Dormancy, Viability and Longevity. In Fenner M (ed) *Seeds: The Ecology of Regeneration in Plant Communities*, 2nd edn. CABI Publishing, New York, pp 183–214.

Myers-Smith IH, Forbes BC, Wilmking M, *et al.* 2011. Shrub expansion in tundra ecosystems: dynamics, impacts and research priorities. *Environmental Research Letters* **6** 045509.

Nather H. 1958. Zur Keimung der Zirbensamen*. Zentralbl GesForst* **75**: 61–70.

Nather J. 1987. Der Erfolg bei der Aufforstung ist von vielen Faktoren abhängig. *Österr. Forstzeigung* **3**: 8–9.

Neilson RP, Pitelka LF, Solomon AM, *et al.* 2005. Forecasting regional to global plant migration in response to climate change. *Bioscience* **55**: 749–759.

Newton R, Hay F, Probert R. 2009. Protocol for comparative seed longevity testing. Technical Information Sheet_01, Royal Botanic Gardens Kew, UK. http://www.kew.org/sites/default/files/1_ppcont_014163_Protocol%20for%20comparative%20seed%20longevity%20testing_0.pdf. Accessed 8 November 2013

Nicotra AB, Atkin OK, Bonser SP, *et al.* 2010. Plant phenotypic plasticity in a changing climate. *Trends in Plant Science* 15: 684–69.

Nishitani S, Masuzawa T. 1996. Germination Characteristics of Two Species of *Polygonum* in Relation to Their Altitudinal Distribution on Mt. Fuji, Japan. *Arctic and Alpine Research* **28**: 104–110.

Nogués-Bravo D, Araújo MB, Errea MP, Martínez-Rica JP. 2007. Exposure of global mountain systems to climate warming during the 21st Century. *Global Environmental Change* **17**: 420–428.

Oberbauer SF, Elmendorf SC, Troxler TG, *et al.* 2103. Phenological response of tundra plants to background climate variation tested using the International Tundra Experiment. *Philosophical Transaction Royal Society B* 368: 20120481. doi: 10.1098/rstb.2012.0481.

Ooi MJ, Auld T, Denham AJ. 2009. Climate change and bet-hedging: interactions between increased soil temperatures and seed bank persistence. *Global Change Biology* **15**: 2375–2386.

Orsenigo S, Abeli T, Rossi G, *et al.* 2015. Effects of Autumn and Spring Heat Waves on Seed Germination of High Mountain Plants. PLoS ONE **10**: e0133626. doi: 10.1371/journal.pone.0133626.

Parmesan C, G Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* **421**: 37–42.

Parolo G, Rossi G. 2008. Upward migration of vascular plants following a climate warming trend in the Alps. *Basic Applied Ecology* **9**: 100–107.

Paulsen J, Weber UM, Korner C. 2000. Tree growth near treeline: abrupt or gradual reduction with altitude? *Arctic Antarctic and Alpine Research* **32**: 14–20.

Pautasso M, Dehnen-Schmutz K, Holdenriede O, *et al.* 2010. Plant health and global change – some implications for landscape management. *Biological Reviews* **85**: 729–755.

Pearson RG. 2006. Climate change and the migration capacity of species. *Trends in Ecology & Evolution* **21**: 111–113.

Petraglia A, Carbognani M, Tomaselli M. 2013. Effects of nutrient amendments on modular growth, flowering effort and reproduction of snowbed plants*. Plant Ecology & Diversity* **6**: 475–486.

Petraglia A, Tomaselli M, Petit Bon M, Delnevo N, Chiari G, Carbognani M. 2014*a*. Responses of flowering phenology of snowbed plants to an experimentally imposed extreme advanced snowmelt*. Plant Ecol*ogy **215**: 759–768.

Petraglia A, Tomaselli M, Mondoni A, Brancaleoni L, Carbognani M. 2014*b*. Effects of nitrogen and phosphorus supply on growth and flowering phenology of the snowbed forb *Gnaphalium supinum* L. *Flora* 209: 271–278.

Pimm SL, Russell GJ, Gittleman JL, Brooks TM. 1995. The future of biodiversity. *Science* 269: 347–350.

Pinheiro JC, Bates DM. 2000. *Mixed-effects models in S and S-PLUS*, New York: Springer.

Probert RJ, Daws MI, Hay FR. 2009. Ecological correlates of ex situ seed longevity: a comparative study on 195 species. *Annals of Botany* 104: 57–69.

R Core Team. 2014. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.

Raunkiaer C.1934. *The life forms of plants*. Clarendon, Oxford.

Rikiishi K, Hashiya E, Imai M. 2004. Linear trends of the length of snow-cover season in the Northern Hemisphere as observed by the satellites in the period 1972–2000. *Annals of Glaciology* 38: 229–237.

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

Roach DA, Wulff RD. 1987. Maternal Effects in Plants. *Annual Review of Ecology and Systematics* 18: 209–235.

Roberts EH. 1973. Predicting the storage life of seeds. *Seed Science Technology* 1: 499–514.

Sandvik SM, Heegaard E, Elven R, Vandvik V. 2004. Responses of alpine snowbed vegetation to long-term experimental warming. *Ecoscience* 11: 150–159.

Sandvik SM, Odland A. 2014. Changes in alpine snowbed-wetland vegetation over three decades in northern Norway. *Nordic Journal of Botany* **32**: 377–384.

Sanhewe AJ, Ellis RH. 1996. Seed development and maturation in *Phaseolus vulgaris*. I. Ability to germinate and to tolerate desiccation. Journal of Experimental Botany **47**: 949–958.

Sayers RL, Ward RT. 1966. Germination Responses in Alpine Species. *Botanical Gazette* **127**: 11–16.

Scherrer SC, Appenzeller C, Laternser M. 2004. Trends in Swiss alpine snow days: the role of local- and large-scale climate variability. *Geophysical Research Letters* **31**: L13215, doi: 10.1029/2004GL020255.

Scherrer D, Koerner C. 2010. Infra-red thermometry of alpine landscapes challenges climatic warming projections. *Global Change Biology* **16**: 2602–2613.

Schlag RN, Erschbamer B. 2000. Germination and establishment of seedlings on a glacier foreland in the Central Alps, Austria. *Arctic, Antarctic, and Alpine Research* **32**:270–277.

Schöb C, Kammer PM, Kikvidze Z, Choler P, Veit H. 2008a. Changes in species composition in alpine snowbeds with climate change inferred from small-scale spatial patterns. *Web Ecology* **8**: 142–159.

Schöb C, Kammer PM, Choler P, Veit H. 2008b. Small-scale plant species distribution in snowbeds and its sensitivity to climate change. *Plant Ecology* **200**: 91–104.

Schönenberger W, Frey W, Leuenberger F.1990. Ökologie und Technik der Aufforstung im Gebirge: Anregungen für die Praxis WSL-Berichte **325**: 1–58.

Schütt P, Lang K, Schuck HJ. 1984. Nadelhölzer in Mitteleuropa : Bestimmung, Beschreibung, Anbaukriterien, Stuttgart: Fischer.

Schütt P, Weisgerber H, Lang U, Roloff A, Stimm B. 2006. Enzyklopädie der Holzgewächse: Handbuch und Atlas der Dendrologie.

Schwienbacher E, Erschbamer B. 2001. Longevity of seeds in a glacier foreland of the Central Alps – a burial experiment. *Bulletin of the Geobotanical Institute ETH* **68**: 63–71.

Schwienbacher E, Marcante S, Erschbamer B. 2010. Alpine species seed longevity in the soil in relation to seed size and shape – A 5-year burial experiment in the Central Alps. *Flora* **205**: 19–25.

Schwienbacher E, Navarro-Cano JA, Neuner G, Erschbamer B. 2011. Seed dormancy in alpine species. *Flora* **206**: 845–856.

Shaver GR, Chapin III FS. 1991. Production: Biomass Relationships and Element Cycling in Contrasting Arctic Vegetation Types. *Ecological Monographs* **61**:1–31.

Shaver GR, Canadell J, Chapin III FS, ***et al.*** **2000**. Global Warming and Terrestrial Ecosystems: A Conceptual Framework for Analysis. BioScience **50**: 87–882.

Shaver GR, Street LE, Rastetter EB, Van Wijk MT, Williams M. 2007. Functional convergence in regulation of net CO2 flux in heterogeneous tundra landscapes in Alaska and Sweden. *Journal of Ecology* **95**: 802–817.

Shevtsova A, Graae BJ, Jochum T, ***et al.*** **2009.** Critical periods for impact of climate warming on early seedling establishment in subarctic tundra. *Global Change Biology* **15**: 2662–2680.

Silvertown JW, Charlesworth D 2001. *Introduction to plant population biology.* 4th edn. London: Blackwell.

Sinniah UR, Ellis RH, John P. 1998. Irrigation and seed quality development in rapid-cycling Brassica: seed germination and longevity. *Annals of Botany* **82**: 309–314.

Skrøppa, T. 2003. EUFORGEN Technical Guidelines for genetic conservation and use for Norway spruce (*Picea abies*).

Solomon S, Qin D, Manning M, ***et al.*** **2007.** Technical Summary. http://www.ipcc.ch/ipccreports/ar4-wg1.htm. Accessed 15 November 2015.

Sturm S, Schimel J, Michaelson G, ***et al.*** **2005.** Winter Biological Processes Could Help Convert Arctic Tundra to Shrubland. *BioScience* **55**: 17–26.

Sveinbjornsson B, Kauhanen H, Nordell O. 1996. Treeline ecology of mountain birch in the Tornetroeask area. Ecological Bulletins **45**: 65–70.

Theurillat JP, Guisan A. 2001. Potential impact of climate change on vegetation in the European Alps: a review. *Climatic Change* **50**: 77–109.

Thuiller W, Lavorel S, Arauji MB, Skyes MT, Prentice IC. 2005. Climate change threats to plant diversity in Europe. *PNAS* **102**: 8245–8250.

Thuiller W. 2007. Biodiversity: Climate change and the ecologist. *Nature* **448**:550–552

Thuiller W, Albert C, Araújo MB, ***et al.*** **2008.**. Predicting global change impacts on plant species' distributions: Future challenges. *Perspectives in Plant Ecology, Evolution and Systematics* **9**: 137–152.

Tilman, D. 1994. Competition and biodiversity in spatially structured habitats. *Ecology* **75**: 2–16.

Tilman D. 1997. Community invasibility, recruitment limitation, and grassland biodiversity. *Ecology* **78**: 81–92.

Tinner W, Ammann B. 2005. Long-term responses of mountain ecosystems to environmental changes: resilience, adjustments, and vulnerability. In: *Global Change and Mountain Regions: an overview of current knowledge* (eds. U. Huber, H. Bugmann, M. Reasoner). Springer, Dordrecht: 133-144

Tingstad L, Olsen SL, Klanderud K and V. Vandvik 2015. Temperature, precipitation and biotic interactions as determinants of tree seedling recruitment across the tree line ecotone. *Oecologia* **179**: 599–608

Tranquillini W. 1979. Physiological ecology of the alpine timberline. Tree existence at high altitudes with special references to the European Alps. Ecological studies 31, Springer, Berlin Heidelberg New York

Trant AJ, Hermanutz L. 2014. Advancing towards novel tree lines? A multispecies approach to recent tree line dynamics in subarctic alpine Labrador, northern Canada. *Journal of Biogeography* **41**: 1115–1125.

Trevisan R, Mariani P, Rascio N, Venturini MG, Baldan B. 1990. Seed Germination in *Larix decidua*. *Phyton Annales Rei Botanica* **30**: 1–13.

Tschermak L. 1950. Waldbau auf pflanzengeographisch-ökologischer Grundlage. Wien

Turnbull LA, Crawley MJ, Rees M. 2000. Are plant populations seed-limited? A review of seed sowing experiments. *Oikos* **88**: 225–238.

Valt M, Cianfarra P. 2010. Recent snow cover variability in the Italian Alps. *Cold Region Science* Technology **64**: 146–157.

Violle C, J. Enquist BJ, McGill BJ, *et al.* 2012. The return of the variance: intraspecific variability in community ecology. *Trends in Ecological Evolution* **27**: 244–252.

Virtanen R, Eskelinen A, Gaare E. 2003. Long-term changes in alpine plant communities in Norway and Finland. In: Nagy L, Grabherr G, Körner C, Thompson DBA (eds). *Alpine Biodiversity in Europe*. Springer-Verlag Berlin Heidelberg, pp 411–422.

Vitasse Y, Hoch G, Randin CF, Lenz A, Kollas C, Körner C. 2012. Tree recruitment of European tree species at their current upper elevational limits in the Swiss Alps. *Journal of Biogeography* **39**: 1439–1449.

Vleeshouwers LM, Bouwmeester HJ, Karssen CM. 1995. Redefining seed dormancy: an attempt to integrate physiology and ecology. *Journal of Ecology* **83**:1031–1037.

Walck JL, Hidayati SN, Dixon KW, Thompson K, Poschlod P. 2011. Climate change and plant regeneration from seed. *Global Change Biology* **17**: 2145–2161.

Walder UM. 1983. Ausaperung und Vegetationsverteilung im Dischmatal. Eidgenössische Anstalt für das forstliche Versuchswesen Mitteilungen, 79–212.

Walters C, Wheeler LM, Grotenhuis JM. 2005. Longevity of seeds stored in a genebank: species characteristics. *Seed Science Research* **15**: 1–20.

Walther GR, Post E, Convey P, *et al.* 2002. Ecological responses to recent climate change. *Nature* **416**: 389–395.

Weih M, Karlsson PS. 1999. The nitrogen economy of mountain birch seedlings: Implications for winter survival. *Journal of Ecology* **87**: 211–219.

Welling P, Laine K. 2000. Characteristics of the seedling flora in alpine vegetation, subarctic Finland, I. Seedling densities in 15 plant communities. *Annales Botanici Fennici* **36**: 69–76.

Westhoff V. 1970. Vegetation study as a branch of biological science. *Misc. Papers Landbouwhogeschool Wageninegn* **5**: 11–30.

Wheeler JA, Hermanutz L, Marino PM. 2011. Feathermoss seedbeds facilitate black spruce seedling recruitment in the forest–tundra ecotone (Labrador, Canada). *Oikos* **120**: 1263-1271.

Whittle CA, Otto SP, Johnston MO, Krochko JE. 2009. Adaptive epigenetic memory of ancestral temperature regime in *Arabidopsis thaliana*. *Botany* **87**: 650–657.

Wipf S, Rixen C. 2010. A review of snow manipulation experiments in Arctic and alpine tundra ecosystems. *Polar Research* **29**: 95–109.

Wookey PA, Aerts R, Bardgett RD, et al. 2009. Ecosystem feedbacks and cascade processes: understanding their role in the responses of Arctic and alpine ecosystems to environmental change. *Global Change Biology* **15**: 1153-1172.

Wulff RD. 1995. Environmental maternal effects on seed quality and germination. In: Kigel J, Galili G, eds. *Seed development and germination*. New York: Marcel Dekker, 491–506.

Zurbriggen N, Hättenschwiler S, Frei ES, Hagedorn F, Bebi P. 2013. Performance of germinating tree seedlings below and above treeline in the Swiss Alps. *Plant Ecology* **214**: 385–396.

Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM. 2009. *Mixed effects models and extensions in ecology with R*, New York, N.Y: New York, N.Y. : Springer.

APPENDIX I

Article 1

Bernareggi G, Carbognani M, Petraglia A, Mondoni A. (2015).

Climate warming could increase seed longevity of alpine snowbed plants

Alpine Botany **125**: 69–78.

Article 2

Bernareggi G, Carbognani M, Mondoni A, Petraglia A. (2015).

Seed dormancy and germination changes of snowbed species under climate warming: the role of pre- and post-dispersal temperatures

Annals of Botany Under revision.

Original article

Seed dormancy and germination changes of snowbed species under climate warming: the role of pre- and post- dispersal temperatures.

Giulietta Bernareggi[1,★], Michele Carbognani[1,★], Andrea Mondoni[2,*] and Alessandro Petraglia[1]

[1] Università di Parma, Dipartimento di Bioscienze, Parco Area delle Scienze 11/A, 43124 Parma, Italy and [2] Università di Pavia, Dipartimento di Scienze della Terra e dell'Ambiente, Via S. Epifanio 14, 27100 Pavia, Italy

** For correspondence. E-mail andrea.mondoni@unipv.it*

★contributed equally to this work and should be considered co-first authors

Running title: Seed dormancy changes of snowbed species under climate warming

andrea.mondoni@unipv.it

Abstract

Background and Aims Climate warming has major impacts on seed germination of several alpine species, hence on their regeneration capacity. Despite most studies investigated the effects of warming after seed dispersal, little is known about the effects that a warmer parental environment may induce on germination and dormancy in the seed progeny. Nevertheless, temperatures during seed development and maturation could alter the state of dormancy, affecting the timing of emergence and seedlings survival. Here, we investigated the interplays between pre- and post-dispersal temperatures driving seed dormancy release and germination requirements of alpine plants.

Methods Three plant species inhabiting alpine snowbeds were exposed to an artificial warming (i.e. +1.5 K) and to natural conditions in the field. Seeds produced were exposed to six different periods of cold stratification (0, 2, 4, 8, 12, 20 weeks at 0 °C), followed by four incubation temperatures (5, 10, 15, 20 °C) for germinating testing.

Results A warmer parental environment produced either no or a significant increase of germination, depending on duration of cold stratification, incubation temperatures and their interaction. In contrast, the speed of germination was less sensitive to changes in the parental environment. Moreover, the effects of warming appeared to be linked to the level of (physiological) seed dormancy, with deeper dormant species showing major changes in response to incubation temperatures and less dormant species in response to cold stratification periods.

Conclusions Plants developed under warmer climate will produce seeds with changed germination responses to temperature and/ or cold stratification, but the extent of these changes across species could be driven by seed dormancy traits. Transgenerational plastic adjustments of seed germination and dormancy shown here may result from increased seed viability, reduced primary and secondary dormancy state, or both and may play a crucial role in future plant adaptation to climate change.

Key words: Adaptation, *Cerastium cerastoides*, Climate warming, *Leucanthemopsis alpina*, Parental effects, Plastic responses, Seed dormancy, Seed germination, Seed phenology, Snowbeds, *Veronica alpina*.

INTRODUCTION

Climate has a dominant influence on several life-history traits of species. Among plant reproductive phases, seed germination and seedling establishment are probably the most sensitive to variation in climate conditions (Walck *et al.*, 2011) and represent the major bottleneck to species recruitment (Lloret *et al.*, 2004; Fay and Schultz, 2009; Dalgleish *et al.*, 2010). The strong correlation between climate and plant regeneration from seeds has resulted in the evolution of specific germination requirements across many species (Baskin *et al.*, 2000; Fenner and Thompson, 2005), which play a key role for plant distribution and vegetation dynamics (Silvertown and Charlesworth, 2001; Higgins *et al.*, 2003; Fenner and Thompson, 2005; Neilson *et al.*, 2005; McGill *et al.*, 2006; Pearson, 2006; Thuiller *et al.*, 2008).

In seasonal climates, which are characterized by cyclic variations in temperature and/or rainfall, seed germination is usually synchronized with the changes in environmental conditions, being delayed until the favourable period occurs (Fenner and Thompson, 2005; Baskin and Baskin, 2014). A key mechanism that has enabled the development of such behaviour is seed dormancy, an innate automatism that involves environmental stimuli to avoid seed germination at the time of the year when seedlings emergence would not be successful, since the favourable conditions inducing germination might not persist long enough for seedlings to survive and grow (Vleeshouwers *et al.*, 1995; Geneve, 2003; Finch-Savage and Leubner-Metzger, 2006).

Seed dormancy occurs in all the major angiosperm clades, and the different dormancy types and classes reflect plant adaptation to different climatic and habitat conditions (Finch-Savage and Leubner-Metzger, 2006; Baskin and Baskin, 2014). A commonly accepted hierarchical system of classification distinguishes five classes of seed dormancy: physiological, morphological, morphophysiological, physical, and a combinational dormancy (physical + physiological) (Baskin and Baskin, 2004). Within each class it is possible to distinguish different levels, which provide additional information about dormancy. For example, three levels are distinguished within physiological dormancy: deep, intermediate or non-deep; the latter is the most common kind of seed dormancy. At maturity, seeds with physiological dormancy may be conditionally dormant, and germinate over only a very narrow range of temperatures (Baskin and Baskin, 2014). Indeed, although there are many environmental factors (dormancy-breaking factors), such as moisture, light or chemicals, that contribute to change the dormancy state, temperature is the major driving force for the release or the induction of physiological dormancy (Fenner and Thompson, 2005). Consequently, after perceiving an adequate/accurate thermal stimulus, seeds can forthwith overcome the conditional dormancy and germinate or broaden the range of germination conditions until eventually become non-dormant

(Fenner and Thompson, 2005; Baskin and Baskin, 2014). However, temperature can affect the state of dormancy also when it is experienced by seeds during maturation and, in most of the cases, warmer pre-dispersal temperatures result in seeds with lower dormancy and higher germinability (Fenner, 1991; Gutterman, 2000).

This pivotal role of temperature in driving seed dormancy responses highlights the need to understand how changing environmental conditions will affect seed germination patterns. In this regard, a large body of evidence supports the existence of rapid climate change, the main effect of which is the rise in mean global temperatures (IPCC, 2013). This will be particularly evident in high-elevation biomes, making these ecosystems, inhabited by species highly specialized to low temperatures, among the environments most threatened by the predicted climate change (Diaz and Bradley, 1997; Kullman, 2004; Nogués-Bravo *et al.*, 2007). As a consequence, in the last decades, an increasing number of studies has addressed the effects of climate warming on different life-history traits and functions of plants in arctic and alpine tundra ecosystems (Chapin *et al.*, 1995; Hobbie and Chapin, 1998; Arft *et al.*, 1999; De Valpine and Harte, 2001; Dunne *et al.*, 2003; Hollister *et al.*, 2005; Milbau *et al.*, 2009; Shevtsova *et al.*, 2009; Mondoni *et al.,* 2012; Hoyle *et al.*, 2013; Oberbauer *et al.*, 2013; Mondoni *et al.,* 2015).

Seeds of many alpine species are known to be non-dormant or conditionally dormant at dispersal (Amen, 1966; Schwienbacher *et al.*, 2011). Early studies on germination requirements in high elevation environments showed that freshly collected seeds required relatively high temperature (20 – 30 °C) to germinate (Sayers and Ward, 1966; Billings and Mooney, 1968; Chabot and Billings, 1972; Nishitani and Masuzawa, 1996). This requirement has been considered an adaptation to prevent seed to germinate immediately after dispersal, when early frosts could jeopardize seedlings survival (Billings and Mooney, 1968) and when the extremely short growing season could not be enough to allow seedlings to attain a critical mass for withstand the harsh and long-lasting winter conditions (Maruta, 1994). However, spring germination may occur at lower temperature than those required in the late season, since the cold stratification over winter widens the thermal window for germination towards lower values as a result of the loss of physiological dormancy (Baskin and Baskin, 2014). Hence, seedling emergence in alpine species usually occurs after the snowmelt (Körner, 2003), when seeds meet the environmental conditions (e.g. temperatures, moisture, light) required for germination. In response to this general pattern, seed germination of alpine/arctic species have been shown to vary considerably because of climate warming, for example increasing, reducing or shifting in time (see references above) and such variation was likely due to the different species and approach used (Shevtsova *et al.*, 2009).

Nevertheless, all these studies have considered seeds developed and matured under current climate (i.e. not under warming scenarios). However, warming is not a seasonal-selective event, but acts during all the growing season; it is therefore necessary to consider the effect of the rise in temperature not only on dispersed seeds, but also during all the reproductive cycle phases. Temperatures during seed development and maturation could alter the state of dormancy, both in fresh and stratified seeds. In particular, warmer temperatures during seed maturation, due to altitudinal or inter-annual variations, have resulted in reduced seed dormancy, increasing the germinability of both fresh (Fernández-Pascual *et al.*, 2013; García-Fernández *et al.*, 2015) and cold-stratified seeds (Fernández-Pascual and Jiménez-Alfaro, 2014), or decreasing the duration of cold stratification to satisfy pre-germination vernalization requirements (Cavieres and Arroyo, 2000). Thus, seed dormancy seems to be affected by temperature variations both in space, i.e. seeds of populations from lower and warmer elevations being less dormant than those from higher and colder elevations, and in time, i.e. maternal plastic responses to local seasonality, with the general rule "the warmer the climate, the shallower the dormancy". However, exceptions to this general pattern have been found, e.g. no differences were observed in the germination of fresh seeds of population of *Phacelia secunda* from different elevations (Cavieres and Arroyo, 2000). Moreover, whether and how a warmer parental environment affects the thermal interval for seed germination before and after the cold stratification (i.e. just after seed dispersal and over winter) is still not understood. To this end, the study of climate warming effects on seed dormancy and germination should decouple the effects of pre- and post-dispersal temperatures and analyse their interplay; to the best of our knowledge, no study has yet focused on this purpose.

Understanding the mechanisms affecting the development of seed dormancy plays a crucial role to forecast the future dynamics of alpine plant populations and communities in a warmer climate, since changing in thermal conditions are expected to affect both seed dormancy and germination requirements, which may preclude, delay, or enhance regeneration from seeds (Walck *et al.*, 2011). This is especially true in topographically fragmented and azonal habitats, such as alpine snowbed communities. Snowbeds, which develop in sites characterized by a very short snow-free period, are regarded as especially sensitive to changes in environmental conditions, and, consequently, are retained among the most threatened vegetation types of the alpine life zone under the current climate change (Björk and Molau, 2007). Indeed, a growing body of studies indicates that the environmental conditions of these habitats, such as the length of the growing period, temperatures, and soil nutrients, constrain plant growth and reproduction (e.g. Galen and Stanton, 1995; Huelber *et al.*, 2006; Schöb *et al.*, 2008; Carbognani *et al.*, 2012; Petraglia *et al.*, 2013; Petraglia *et al.*, 2014b; Bernareggi *et al.*, 2015). Consistently, fast alterations in plant community composition and structure have been recently observed (e.g. Elumeeva *et al.*, 2013; Carbognani *et al.*, 2014*a*; Sandvik and Odland, 2014). The predicted climate change involves that plants colonising snowbeds will experience longer and warmer snow-free periods. Since the phenological development of snowbed plants is strongly controlled by micro-climatic conditions (e.g. Galen and Stanton, 1995; Huelber *et al.*, 2006; Hülber *et al.*, 2010; Petraglia *et al.*, 2014a), it is likely that reproductive phenophases will significantly advance under earlier snowmelt coupled with warmer temperatures.

On these bases, the hypothesis that, in the next decades, seeds of snowbed plants will be dispersed during periods characterized by considerably warmer temperatures cannot be ruled out. Nevertheless, still little is known about the effects (direct and indirect) of climate warming on seed germination in snowbed environments, and even less about the effects on seed dormancy. Here, we present the first attempt to evaluate the influence of a warmer parental plant environment on seed dormancy and seed germination requirements in an alpine snowbed habitat. To this aim, three alpine species inhabiting snowbeds were exposed to an experimental warming and to natural conditions in the field. Then, the germinability of seeds produced was tested in laboratory after six cold stratification periods combined with four incubation temperatures. In particular, we wanted to answer to the following questions:

a) Does a warmer pre-dispersal environment influence the germination responses of both fresh and cold-stratified seeds by 1) widening the temperatures range for germination and 2) increasing and speeding the final germination?

b) Are such responses further characterized by reduced duration of cold stratification requirements to overcome seed dormancy?

MATERIALS AND METHODS

Study area

The study was performed in a snowbed area of about 2 ha located in the high Gavia Valley (Stelvio National Park, 46°20′N 10°30′E, 2700 m a.s.l.), in the Italian Rhaetian Alps.

The study site, occurring on siliceous bedrock, is located within the alpine vegetation belt, where *Carex curvula* grassland is the climax vegetation. A more detailed description of the vegetation types were provided by Carbognani *et al.* (2012). The most frequent plant communities are the one dominated by the dwarf shrub *Salix herbacea* and the one dominated by the moss *Polytrichastrum sexangulare* that occur both as spatially separated and mixed patches.

Pre-dispersal treatment

To simulate a moderate climate warming, we used the open top chambers (OTCs, Marion *et al.*, 1997) proposed by the International Tundra Experiment (ITEX), that are known to increase about 1.5 K the mean daily air temperature; such increase is consistent with the most conservative warming scenario of mountain areas for 2055 (Nogués-Bravo *et al.*, 2007). The chambers were constructed by modifying the ITEX protocol as follows: tetragonal chambers 28 cm height, made of 3 mm thick polymethylmethacrylate sheets (ACRIDITE® ACRISUN, Plastidite, Trieste, Italy), with basal width of 90 – 127 cm and summit width of 50 – 71 cm (Carbognani *et al.*, 2014*b*).

The warming treatment started in 2008. From the end of Jun. to mid Jul. 2008, 20 plots were randomly located within the study at snowmelt dates. The OTCs were placed in 10 randomly selected plots; the other 10 plots were left as natural control. Every year, the OTCs were placed in the field immediately after the snowmelt and removed at the end of growing season. Hereafter, the warming treatment will be referred to "W" and the control (i.e. plants exposed to natural climate) with "C".

Microclimatic features

In each plot, the snowmelt dates were monitored by direct observations; soil (-5 cm depth) temperatures of the snow-free period from year 2008 to year 2014 were recorded hourly using 12 probes in total, 6 for W plots and 6 for C plots (Onset, Cape Cod, MA, USA and Tecnosoft, Milan, Italy).

Seed collection

Between 11 Sep. and 04 Oct. 2013, seeds of *Cerastium cerastoides* (L.) Britton (Caryophyllaceae), *Leucanthemopsis alpina* (L.) Heywood (Asteraceae) and *Veronica alpina* L. (Scrophulariaceae) were collected at the time of natural dispersal from both W and C plots. The choice of the species was made on the base of their frequency and abundance in the study site. Seeds collected from different plots of the same treatment were pooled together. After collection, seeds were processed (cleaned) and stored at room temperature until the beginning of the experiment, which occurred within one week after the last collection. The high initial viability (>

80 %) of seed samples was checked through germination tests at conditions previously found to be optimal for germination (20 °C constant + addition of 250 mg L^{-1} GA_3, at 12-h daily photoperiod).

Cold stratification and incubation temperatures

Seeds were exposed to six different periods of cold stratification (0, 2, 4, 8, 12, 20 weeks) at 0 °C in complete darkness using a cooled incubator (LMS Ltd, Sevenoaks, UK). After each interval, seeds were incubated for germination at constant temperatures at 5, 10, 15, 20 °C. Constant temperatures were chosen in order to assess thermal influences isolating the effect of determinate temperature levels on seed germination (which would not have been possible under alternating regime). In this regard, preliminary germination tests confirmed that our species did not required the trigger effect of diurnal temperature variation (e.g. Mooney and Billings, 1961; but see Liu *et al.*, 2013), germinating well (i.e. > 90%) under constant regime (data not shown). For each species, pre-dispersal treatment, stratification periods and incubation temperatures, three samples of 20 seeds (*C. alpina* and *L. alpina*) or two samples of 25 seeds (*C. cerastoides*) were sown on agar held in 50-mm diameter Petri dishes and tested for germination. All germination tests were carried out in temperature and light controlled incubators (LMS 250A, LMS Ltd, Sevenoaks, UK) using a 12-h daily photoperiod (photosynthetically active radiation 40 – 50 µmol m^{-2} s^{-1}). Plates were checked weekly for germination and seeds scored as germinated once radicle protrusion and elongation was more than 2 mm. At the completion of each germination test (4 weeks after sowing), ungerminated seeds were cut-tested to confirm whether there were empty seeds. Ungerminated empty seeds were excluded from the following analyses.

Data analysis

Germination of fresh seeds

Influences of species, pre-dispersal treatments and incubation temperatures on germination of fresh seed (i.e. without cold stratification) were evaluated by means of Generalised Linear Model (GLM) with binomial error and logit link-function. In this GLM, final germination (i.e. number of emerged seedlings out of number of seeds sown) was the response variable, whereas species (as 3-level categorical variable), treatment (as 2-level categorical variable, C and W), temperature (as continuous variable, 5, 10, 15, 20 °C), and their two-way interactions were the fixed effects. In addition, species-specific GLMs were performed with final germination as response variable and pre-dispersal treatment, sowing temperature and their interaction as explanatory variables.

Moreover, to study the effects of warmer incubation temperatures on the speed of seedling emergence, the mean time to germination (MTG) was calculated using the formula:

$\mathrm{MTG} = \Sigma\ (n_i \times t_i) / N$

Where n_i is the number of seeds that emerged within consecutive intervals of time, t_i the time between the beginning of the test and the end of a particular interval of measurement, and N the total number of seeds that emerged. The MTG was calculated using the day of sowing as initial time. The influences of species, pre-dispersal treatments, incubation temperatures, and their two-way interactions on MTG were analysed by means of GLM with gamma error structure and inverse link-function. Within each combination of species, pre-dispersal treatment, stratification periods, and incubation temperatures, seed lots that failed to germinate more than 1 % were excluded from the analysis, although still present in the figures for comparative purposes.

Germination after cold stratification

Differences in final seed germination between species, incubation temperatures, and stratification periods were assessed using GLM with quasibinomial error structure (due to overdispersion) and logit link-function. In this model, seed germination (i.e. number of emerged seedlings out of number of seeds sown) was the response variable, whereas species (as 3-level categorical variable), treatment (as 2-level categorical variable, C and W), temperature (as continuous variable, 5, 10, 15, 20 °C), stratification (as continuous variable, 0, 2, 4, 8, 12, 20 weeks at 0 °C) and their two-way interactions were the explanatory variables. In addition, in order to gain a better understanding of the influences of variation in incubation temperatures and stratification periods on seed germination, further GLMs were performed at the species level and model selection was carried out comparing residual deviance and AIC of different models. In particular, the relationships between temperature and stratification with germination were evaluated testing the explanatory power of GLMs fitted with different combinations of explanatory variables (incubation temperatures and stratification periods) as linear, log-transformed, or polynomial terms. Then, for each species, minimal adequate model was obtained excluding not significant terms following Crawley (2013).

In these analyses, the experimental units were the Petri dishes (each including 20 – 25 seeds) to which post-dispersal treatments (incubation temperatures and stratification periods) were independently applied. Nevertheless, to check for potential influence due to non-independence of seeds within a Petri (e.g. substrate, neighbour, and incubator effects), all models were re-run including Petri as random factor, with the same structures and selection procedures previously described (with the exception of error structure, since in case of overdispersion, quasibinomial distribution was not required due to the presence of the observation-level random effect). However, in all the cases, the two different approaches produced the same outcomes and, consequently, only the results of the GLMs were reported.

All analyses were performed using the R software (version 3.1.2, R Core Team, 2014).

RESULTS

Microclimatic features

During the period 2008 – 2014, the snowmelt at the experimental site occurred on average during the 27th week of the year (WOY), between the 25th WOY (in 2011) and the 29th WOY (in 2008). The mean weekly soil temperature during this week has been detected between 7.4 °C (in 2014) and 11.4 °C (in 2010) in C and between 8.4 °C (in 2014) and 12.0 °C (in 2010) in W (Table 1A). During the snow free period, which lasted 12 – 13 weeks, the OTCs (W) produced a mean soil warming of 1.5 K. The increase in temperature caused an advanced (of about 10 days) of the phenological phases and, coherently, of seed dispersal timing in W. Indeed, seed dispersal occurred between WOY 35 – 36 in W and between WOY 36 – 37 in C with a corresponding difference of soil temperature of about 3 K (Table 1A). The snowfall usually occurs at WOY 40 – 41, in the first half of October (Table 1A).

Seeds of the studied species were collected in 2013, between the 35th and 36th WOY in W and between the 36th and the 37th WOY in C; the mean monthly temperature of this growing season, consistent with the multi-year monthly mean, is shown in Table 1B.

Germination of fresh seeds

(a) Germination phenology

On fresh seeds (i.e. without cold stratification), significant differences in the final germination were observed among species, whose seeds showed a higher germination proportion under warmer incubation temperatures but were not influenced by the pre-dispersal treatment (Table 2A). Furthermore, the effects of [both] pre-dispersal treatment and incubation temperatures were not consistent among the species studied (i.e. significant interaction terms).

The increase in incubation temperatures resulted in a significant increase in germination percentage in all the species and treatments tested (Table 3). At the coldest temperature (i.e. 5 °C), germination percentage was very low or, in some cases, null in all the species and treatments (Fig. 1A). In particular, seeds of *C. cerastoides* germinated between 20 and 25 %, the germination of warmed seeds of *L. alpina* remained less than 1 %, while no emergence was found in control seeds of *L. alpina* and in *V. alpina*. At 10 °C, about 60 – 80 % of seeds of *C. cerastoides* and *V. alpina* germinated and, under higher incubation temperatures (i.e. 15 and 20 °C), the final germination percentage of these species remained, on average, at high levels. Conversely, germination of *L. alpina* remained low (<7 %) until the temperature of 15 °C for warmed seeds and 20 °C for control seeds, after which it showed a significant, but moderate (up to 40 – 50 % in W and to 30 % in C), increase. The pre-dispersal warming had no effects on the germination of *C. cerastoides* and *V. alpina* (Table 3), but it determined a higher increase in seed germination of *L. alpina* (Table 3; Fig. 1A). Despite this, the final germination of this latter species remained lower (< 50 %) in comparison with those of the other two species.

(b) Mean time to germinate

Significant differences in the MTG of fresh seeds were found between both species and incubation temperatures tested (with faster germination under warmer temperatures), but no effect of the pre-dispersal warming was found (Table 2B). We found that the increase in incubation temperatures produced a significant reduction of the MTG in all the species, homogeneously between both the pre-dispersal treatments and the species (of about 1 day per 1 K of temperature increase); however, some species-specific patterns were found (Fig. 1B). In particular, at the coldest temperature (i.e. 5 °C) *C. cerastoides* was the only species able to germinate (values > 5 %) and its seeds kept almost three weeks (20 d) for germinating. Similary, when the other species started to germinate, at 10 °C, the MTG was of 20 − 25 d. With the increase in incubation temperatures the MTG was progressively reduced in all the species, until the lowest value reached at 20 °C.

Germination after cold stratification

Our analyses showed significant differences in germination percentage within all the factors (species, pre-dispersal treatment, incubation temperature, and cold stratification period) tested (Table 4). In particular, germination was on average higher in *C. cerastoides* and *V. alpina* compared to *L. alpina*, in W compared to C seeds, and under warmer incubation temperature and longer cold stratification periods (Fig. 2). Furthermore, significant interactions revealed non-consistent responses of species to all the other factors and non-additive effects of pre-dispersal treatment with different incubation temperatures and stratification periods. Indeed, species-specific GLMs (Table 5) showed that W seeds, compared to C seeds, had higher germination with increasing length of stratification period (in *C. cerastoides*, Table 5A), with increasing temperature of incubation (in *L. alpina*, Table 5B), or with increasing both the length of stratification period and the incubation temperature (in *V. alpina*, Table 5C).

The germination of all the three species showed a saturated-pattern in response to increasing incubation temperatures (Table 5), with higher effects of increasing temperature on germination under colder than warmer conditions (i.e. log-transformed temperatures had the best fit to data). However, while in *L. alpina* the effect of the incubation temperatures was consistent under different stratification periods (Table 5B, Fig. 2B), in the other two species seeds incubated at the lowest temperature (i.e. 5 °C) showed different patterns of germination in response to variation in the length of stratification period compared to those of seeds incubated under warmer temperature levels (Table 5A and 5C, Fig. 2A and 2C).

Finally, seed germination of the species studied strongly differed in response to the duration of the cold stratification. In particular, seed germination of *C. cerastoides* (which had “stratification” as polynomial term in the best model) showed an initial increase followed by a decrease of germination percentage with the increasing of the cold stratification period (Table 5A, Fig. 2A). However, the increase of germination after the earliest cold stratification periods was extremely low in seeds produced at the control (C) plots and therefore the general tendency was to lose germinability increasing the duration of stratification in this pre-dispersal treatment (Fig. 2A). Differently, *L. alpina* seed germination seemed to have a saturated response to increase in cold stratification length, with more pronounced increases of germination during earlier durations of cold stratification (i.e. log-transformed stratification period had the best fit to data) (Table 5B, Fig. 2B). In the case of *V. alpina*, unlike the previous species, the best relationship between cold stratification period and seed germination was linear, with (after the effects of the others factors) a constant increase of germination with increasing duration of the cold stratification (Tab 5C, Fig. 2C).

DISCUSSION

Germination of fresh seeds

The present study described how pre- and post- seed dispersal climate warming influenced seed dormancy and germination requirements of three alpine species inhabiting snowbeds. Here we have shown that after dispersal germination was similar between seeds produced in the control (C) and under warming (W) at lower incubation temperatures (i.e. 5 and 10 °C) in all species, but that seeds of *L. alpina* produced in W showed a slight increase in germination under warmer incubation temperatures (i.e. 15 and 20 °C) (Fig. 1A). This alone indicates that germination/dormancy changes on fresh seeds driven by a warmer parental growth environment are limited and may be expected only if seeds experience warm (e.g. > 15 °C) conditions after dispersal. Interestingly, temperatures recorded at the study area at the time of seed dispersal are usually lower (see Table 1) than those able to stimulate an increase of seed germination in warmed seeds of *L. alpina* (15, 20 °C), indicating that no changes of seedling emergence should be expected in a future warmer climate on fresh seeds of this species. However, the possibility that a warmer parental growth environment (than that used here, about + 1.5 K) could have higher effects on germination of fresh seeds cannot be ruled out.

Fresh seeds of all species did not germinate, or germinated very low at temperature (5 °C) similar to those they currently experience after dispersal (Table 1A), but germination increased significantly after the cold stratification (Fig. 2), indicating that seedling emergence of the species studied is likely programmed to occur after the winter season is passed. Seed germination of alpine plants is indeed know to occur in spring, after they have experienced autumn and winter seasons (Körner, 2003). However, the high germination of *C. cerastoides* and *V. alpina* (approx. 80 %) found at temperature (10 °C) similar to those occurring under warming during the seed dispersal time (9.5 °C, Table 1), suggesting that germination before the winter season should increase in these species in a warmer climate, which may have important implications for seedling survival and establishment (see Mondoni *et al.*, 2012; 2015). Interestingly, our data also highlighted that such increase occurred similarly both on seeds produced under W and C, therefore, regardless the climatic scenarios experienced before seed dispersal. Further, here we have shown that an increase of seedlings emergence at the end of the snow-free season is possible only for seeds exposed to approx. 10 °C for at least approx. 10 and 20 days in *C. cerastoides* and *V. alpina* respectively (see MTG, Fig. 1B). However, a recent study showed that also a short-term heat event may affect the timing of germination and lead seeds to germinate before the winter season (Orsenigo *et al.*, 2015), suggesting that the early germination could be possible even when the exposure of fresh seeds to warm temperatures is shorter than those shown here.

Considering the warming rate predicted for the next decades in the European mid-latitude mountains (ranging from about + 0.3 to 0.5 K per decade, Nogués-Bravo *et al.*, 2007) and the consequent antedating of phenological phases in alpine plants (Wipf and Rixen, 2010; Oberbauer *et al.*, 2013), a significant increase of germination at the end of the snow-free season should be expected at least for C. *cerastoides*, that is, among the snowbed species at the study site, the earliest seed dispersal (Carbognani *et al.*, unpublished data).

Germination after cold stratification

Seeds of our species showed all a certain degree of non-deep physiological dormancy (*sensu* Baskin and Baskin 2014), which varied in its depth depending on the species. Indeed, seed germination increased significantly increasing the period of cold stratification in all species, with a wide variation across them (Table 4). In particular, fresh seeds of *C. cerastoides* germinated at all temperatures, between approx. 20 % (at 5 °C) and 80 – 100 % (at > 10 °C) (Fig. 1A), and its warmed treated seeds showed a significant increase to approx. 90% at 5 °C after three months of cold stratification (Fig. 2A). Indeed, the germination of seeds developed under current climate declined increasing the stratification period, indicating either a loss of viability or the development of a secondary dormancy (see below). Seeds of *V. alpina* showed similar responses, but did not germinate at 5 °C in absence of cold stratification. Conversely, seed germination was low in fresh seeds of *L. alpina* at all temperatures (about 30 – 40 %), increasing mostly after long period cold stratification (i.e. Fig. 2B, Table 5B). On these evidences, although these species should be considered as non-deep physiological dormant, the different temperature windows for germination and durations of cold stratification required to break dormancy, indicate that *L. alpina* is the strongest (physiologically) dormant species, followed by *V. alpina* and *C. cerastoides*. Moreover, the increase in stratification period generally resulted in a significant increase in germination percentage (Table 4, Fig. 2), but the species studied responded differently to the interplay of cold stratification and warming treatment. In seeds of *C. cerastoides,* the increase in stratification duration modelled opposite responses in seeds produced under W and C, increasing and reducing the germination percentage, respectively, and similar responses were observed in *V. alpina* (see Sf × Tr, Table 5A, C). Conversely, parental warming did not affect the germination response to cold stratification in *L. alpina* (Table 5B). Moreover, the significant temperature × treatment interaction found in cold stratified seeds in *L. alpina* (Table 5) indicates that incubation temperatures had different responses in seeds produced under W and C in this species. These are interesting and novel observations indicating that changes of germination requirements induced by the pre-dispersal warming environment depended on the seed dormancy state, with deeper dormant species showing major changes in response to incubation temperature on both fresh and cold stratified seeds (i.e. *L. alpina*) and less dormant species in response to stratification (i.e. *C. cerastoides* and *V. alpina*) (Table 3 e 5). Moreover, changes of pre-dispersal temperatures affected germination response to both stratification and incubation temperatures (at least on cold stratified seeds) in *V. alpina*, which showed an intermediate dormancy behavior between *C. cerastoides* and *L. alpina* (see above).

As a result, higher seed maturation temperatures consistently increased the germination in all species, although to a different extent across them (Fig. 2). Coming back to the other research questions, warming had no affect the MTG, but widened the suitable temperature range for germination and reduced the cold stratification time to break dormancy in *L. alpina*. Indeed, at 15 °C germination was extended to approx. 40 % when fresh seeds of this species matured under warming, while it was lower than 10 % on seeds produced in the control. Moreover, seed germination was significantly higher in warmed treated seeds of *L. alpina*, compared to control ones, just after two weeks of cold stratification (Fig. 2B), indicating that under warming some species will produce seeds with shorter chilling requirement to overcome dormancy. This behaviour occurred at all incubation temperatures, except 5 °C, probably because the cold temperatures constrain the germination (Fig. 2B).

The higher germination responses observed on both fresh and cold stratified seeds produced under warming, compared to those developed under current climate, could be due to changes in the dormancy patterns and/or the seed viability of our species. Warmer maturation conditions are known to lead to better quality of seeds (see reviews by Roach and Wulff, 1987; Wulff, 1995; Gutterman, 2000), and it has been suggested that plants grown in a warmer environment produce longer lived seeds (Bernareggi *et al.*, 2015). In this regard, the different behaviour of warmed and not warmed seeds of *C. cerastoides* might suggest that current alpine climate is not optimal for seed development and maturation, reducing the survival capacities during the long-lasting winter conditions. Similar conclusions may explain the lower germination of not warmed vs. warmed seeds of *L. alpina*. However, after 20 weeks of cold stratification seed germination of *V. alpina* dropped to 0 % at 5 °C, but remained almost complete at the other incubation temperatures, indicating that seeds were still viable. This behaviour reflects the condition of fresh seeds (Fig. 1A, 1C), suggesting that the prolonged permanence at 0 °C could have induced a secondary dormancy state in seeds of this species. Indeed, it is possible that non-dormant seeds that do not meet adequate conditions for germination enter secondary dormancy (Brandel, 2005; Leymarie *et al.*, 2008; Baskin and Baskin, 2014) and, therefore, similar mechanisms may explain also the decrease of germination with increasing cold stratification period in *C. cerastoides* and the lower germination of not warmed vs. warmed seeds in *L. alpina*.

Summarizing, this study suggests that climate warming will increase the likelihood of seed germination both just after dispersal and winter season (i.e. after cold stratification) due to the effects on seed phenology (e.g. advanced seed dispersal), and on environmental conditions (e.g. early snowmelt, warmer temperatures during the snow free period). However, the consequences that these changes will have on plant reproduction success still remains partially investigated. For example, while the germination immediately after seed dispersal has always been considered disadvantageous because constrains seedlings to cope with the harsh winter conditions, a recent study in a glacier foreland showed that a high percentage (about 60 and 75 %) of autumn emerged seedlings can survive through the winter (Mondoni *et al.*, 2015). However, overcoming the winter involve elevated energy consumption (Maruta, 1994), which may reduce the growing capacities in spring. Moreover, the higher germination of warmed treated *vs*. not warmed seeds also after long periods of stratification, suggest an increase in reproductive success. A persistent soil seed bank in several alpine species is thought to be an ecological adaptation to the low chance of establishment in these environments (Schwienbacher *et al.*, 2010). Hence, higher germination may not necessarily be beneficial for alpine plants, as summer drought and late-winter/early spring soil freeze–thaw events are expected to increase due to climate warming (Wipf and Rixen, 2010; Barriopedro *et al.*, 2011), as already shown in some mountain regions (Rikiishi *et al.*, 2004; Scherrer *et al.*, 2004; Mote *et al.*, 2005), reducing the likelihood of seedlings to survive. In order to ensure a realistic evaluation of the recruitment success in alpine environment, it is necessary to consider the all effects, direct and indirect, that climate warming produces in any time of the year. In this regard, *in situ* sowing experiments at the Gavia Pass are underway in order to understand whether a longer and warmer growing season could be advantageous for seedling recruitment and establishment in snowbeds.

ACKNOWLEDGEMENTS

We thank the Stelvio National Park for fieldwork authorization and facilities.

LITERATURE CITED

Amen RD. 1966. The Extent and Role of Seed Dormancy in Alpine Plants. *The Quarterly Review of Biology* **41**: 271–281.

Arft AM, Walker MD, Gurevitch J, *et al.* 1999. Responses of tundra plants to experimental warming: meta-analysis of the International Tundra Experiment. *Ecological Monographs* **69**: 491–511.

Barriopedro D, Fischer EM, Luterbacher J, Trigo RM, García-Herrera R. 2011. The hot summer of 2010: redrawing the temperature record map of Europe. *Science* **332**: 220–224.

Baskin CC, Baskin JM. 2014. *Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination*, 2nd edn. London: Academic Press.

Baskin JM, Baskin CC. 2004. A classification system for seed dormancy. *Seed Science Research* **14**: 1–16.

Baskin JM, Baskin CC, Li X. 2000. Taxonomy, ecology, and evolution of physical dormancy in seeds. *Plant Species Biology* **15**: 139–152.

Bernareggi G, Carbognani M, Petraglia A, Mondoni A. 2015. Climate warming could increase seed longevity of alpine snowbed plants. *Alpine Botany* **125**: 69–78.

Billings WD, Mooney HA. 1968. The ecology of arctic and alpine plants. *Biological Reviews* **43**: 481–529.

Björk RG, Molau U. 2007. Ecology of alpine snowbeds and the impact of global change. *Arctic, Antarctic and Alpine Research* **39**: 34–43.

Brandel M. 2005. The effect of stratification temperatures on the level of dormancy in primary and secondary dormant seeds of two *Carex* species. *Plant Ecol*ogy **178**: 163–169.

Carbognani M, Petraglia A, Tomaselli M. 2012. Influence of snowmelt time on species richness, density and production in a late snowbed community. *Acta Oecologica* **43**: 113–120.

Carbognani M, Tomaselli M, Petraglia A. 2014*a*. Current vegetation changes in an alpine late snowbed community in the south-eastern Alps (N-Italy). *Alpine Botany* **124**: 105–113.

Carbognani M, Petraglia A, Tomaselli M. 2014*b*. Warming effects and plant trait control on the early-decomposition in alpine snowbeds. *Plant and Soil* **376**: 277–290.

Cavieres LA, Arroyo MTK. 2000. Seed germination response to cold stratification period and thermal regime in *Phacelia secunda* (Hydrophyllaceae): Altitudinal variation in the mediterranean Andes of central Chile. *Plant Ecology* **149**: 1–8.

Chabot BF, Billings WD. 1972. Origins and Ecology of the Sierran Alpine Flora and Vegetation. *Ecological Monographs* **42**: 163–199.

Chapin FS III, Shaver GR, Giblin AE, Nadelhoffer KJ, Laundre JA. 1995. Responses of arctic tundra to experimental and observed changes in climate. *Ecology* **76**: 694–711.

Crawley MJ. 2013. The R Book. Chichester: John Wiley & Sons.

Dalgleish HJ, Koons DN, Adler PB. 2010. Can life-history traits predict the response of forb populations to changes in climate variability? *Journal of Ecology* **98**: 209–217.

De Valpine P, Harte J. 2001. Plant responses to experimental warming in a montane meadow. *Ecology* **82**: 637–648.

Diaz HF, Bradley RS. 1997. Temperature variations during the last century at high elevation sites. *Climatic Change* **36**: 253–279.

Dunne JA, Harte J, Taylor KJ. 2003. Subalpine meadow flowering phenology responses to climate change: Integrating experimental and gradient methods. *Ecological Monographs* **73**: 69–86.

Elumeeva T, Onipchenko VG, Egorov AV, *et al.* 2013. Long-term vegetation dynamic in the Northwestern Caucasus: which communities are more affected by upward shifts of plant species? *Alpine Botany* **123**: 77–85.

Fay PA, Schultz MJ. 2009. Germination, survival, and growth of grassland forb seedlings: effects of soil moisture variability. *Acta Oecologica* **35**: 679–684.

Fenner M. 1991. The effects of the parent plant environment on seed germinability. *Seed Science Research* **1**: 75–84.

Fenner M, Thompson K. 2005. *The ecology of seeds*. Cambridge: Cambridge University Press.

Fernández-Pascual E, Jiménez-Alfaro B, Caujapé-Castells J, Jaén-Molina R, Díaz TE. 2013. A local dormancy cline is related to the seed maturation environment, population genetic composition and climate. *Annals of Botany*. **112**: 937–945.

Fernández-Pascual E, Jiménez-Alfaro B. 2014. Phenotypic plasticity in seed germination relates differentially to overwintering and flowering temperatures. *Seed Science Research* **24**: 273–280.

Finch-Savage WE, Leubner-Metzger G. 2006. Seed dormancy and the control of germination. *New Phytologist* **171**: 501–523.

Galen C, Stanton ML. 1995. Responses of snowbed plant-species to changes in growing-season length. *Ecology* **76**: 1546–1557.

García-Fernández A, Escudero A, Lara-Romero C, Iriondo JM. 2015. Effects of the duration of cold stratification on early life stages of the Mediterranean alpine plant *Silene ciliata*. *Plant Biology* **17**: 344–350.

Geneve RL. 2003. Impact of Temperature on Seed Dormancy. *HortScience* **38**: 336 – 341.

Gutterman Y. 2000. Maternal effects on seeds during development. In: Fenner M, ed. *Seeds: The Ecology of Regeneration in Plant Communities*, 2nd edn. Wallingford: CABI Publishing, 59–84.

Higgins SI, Clark JS, Nathan R, *et al.* 2003. Forecasting plant migration rates: managing uncertainty for risk assessment. *Journal of Ecology* **91**: 341–347.

Hobbie SE, Chapin FS III. 1998. The response of tundra plant biomass, aboveground production, nitrogen, and CO_2 flux to experimental warming. *Ecology* **79**: 1526–1544.

Hollister RD, Webber PJ, Tweedie CE. 2005. The response of Alaskan arctic tundra to experimental warming: differences between short- and long-term responses. *Global Change Biology* **11**: 525–536.

Hoyle GL, Venn SE, Steadman KJ, *et al.* 2013. Soil warming increases plant species richness but decreases germination from the alpine soil seed bank. *Global Change Biology* **19**: 1549–1561.

Huelber K, Gottfried M, Pauli H, Reiter K, Winkler M, Grabherr G. 2006. Phenological Responses of Snowbed Species to Snow Removal Dates in the Central Alps: Implications for Climate Warming. *Arctic, Antarctic, and Alpine Research* **38**: 99–103.

Hülber K, Winkler M, Grabherr G. 2010. Intraseasonal climate and habitat-specific variability controls the flowering phenology of high alpine plant species. *Functional Ecology* **24**: 245–252.

IPCC (International Panel on Climate Change). 2013. Summary for policymackers. In: Stocker TF, Qin D, Plattner G-K, *et al.*, eds. *Climate change 2013: the physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge: Cambridge University Press, 3–29.

Körner C. 2003. Alpine plant life: functional plant ecology of high mountain ecosystems. 2nd edn. Berlin: Springer–Verlag.

Kullman L. 2004. Long-term geobotanical observations of climate change impacts in the Scandes of West-Central Sweden. *Nordic Journal of Botany* **24**: 445–467.

Leymarie J, Robayo-Romero ME, Gendreau E, Benech-Arnold RL, Corbineau F. 2008. Involvement of ABA in induction of secondary dormancy in barley (*Hordeum vulgare* L.) seeds. *Plant and Cell Physiology* **49**: 1830–1838.

Liu K, Baskin JM, Baskin CC, Bu H, Du G, Ma M. 2013. Effect of Diurnal Fluctuating versus Constant Temperatures on Germination of 445 Species from the Eastern Tibet Plateau. PLoS ONE 8: e69364. **doi**: 10.1371/journal.pone.0069364.

Lloret F, Peñuelas J, Estiarte M. 2004. Experimental evidence of reduced diversity of seedlings due to climate modification in a Mediterranean-type community. *Global Change Biology* **10**: 248–258.

Marion GM, Henry GHR, Freckman DW, *et al.* 1997. Open-top design for manipulating field temperature in high-latitude ecosystems. *Global Change Biology* **3**: 20–32.

Maruta E. 1994. Seedling establishment of *Polygonum cuspidatum* and *Polygonum weyrichii* var. *alpinum* at high-altitudes of Mt Fuji. *Ecological Research* **9**: 205–213.

McGill BJ, Enquist BJ, Weiher E, Westoby M. 2006. Rebuilding community ecology from functional traits. *Trends in Ecology & Evolution* **21**: 178–185.

Milbau A, Graae BJ, Shevtsova A, Nijs I. 2009. Effects of a warmer climate on seed germination in the subarctic. *Annals of Botany* **104**: 287–296.

Mondoni A, Rossi G, Orsenigo S, Probert RJ. 2012. Climate warming could shift the timing of seed germination in alpine plants. *Annals of Botany* **110**: 155–64.

Mondoni A, Pedrini S, Bernareggi G, *et al.* 2015. Climate warming could increase recruitment success in glacier foreland plants. *Annals of Botany* **116**: 907–916.

Mooney HA, Billings WD. 1961. Comparative Physiological Ecology of Arctic and Alpine Populations of *Oxyria digyna*. *Ecological Monographs* **31**: 1–29.

Mote PW, Hamlet AF, Clark MP, Lettenmaier DP. 2005. Declining mountain snowpack in western North America. *Bulletin of the American Meteorological Society* **86**: 39–49.

Neilson RP, Pitelka LF, Solomon AM, *et al.* 2005. Forecasting regional to global plant migration in response to climate change. *Bioscience* **55**: 749–759.

Nishitani S, Masuzawa T. 1996. Germination Characteristics of Two Species of *Polygonum* in Relation to Their Altitudinal Distribution on Mt. Fuji, Japan. *Arctic and Alpine Research* **28**: 104–110.

Nogués-Bravo D, Araújo MB, Errea MP, Martínez-Rica JP. 2007. Exposure of global mountain systems to climate warming during the 21st Century. *Global Environmental Change* **17**: 420–428.

Oberbauer SF, Elmendorf SC, Troxler TG, *et al.* 2103. Phenological response of tundra plants to background climate variation tested using the International Tundra Experiment. *Philosophical Transaction Royal Society B* 368: 20120481. **doi**: 10.1098/rstb.2012.0481.

Orsenigo S, Abeli T, Rossi G, *et al.* 2015. Effects of Autumn and Spring Heat Waves on Seed Germination of High Mountain Plants. PLoS ONE 10: e0133626. **doi**: 10.1371/journal.pone.0133626

Pearson RG. 2006. Climate change and the migration capacity of species. *Trends in*

Ecology & Evolution **21**: 111–113.

Petraglia A, Carbognani M, Tomaselli M. 2013. Effects of nutrient amendments on modular growth, flowering effort and reproduction of snowbed plants. *Plant Ecology & Diversity* **6**: 475–486.

Petraglia A, Tomaselli M, Petit Bon M, Delnevo N, Chiari G, Carbognani M. 2014*a*. Responses of flowering phenology of snowbed plants to an experimentally imposed extreme advanced snowmelt. *Plant Ecol*ogy **215**: 759–768.

Petraglia A, Tomaselli M, Mondoni A, Brancaleoni L, Carbognani M. 2014*b*. Effects of nitrogen and phosphorus supply on growth and flowering phenology of the snowbed forb *Gnaphalium supinum* L. *Flora* **209**: 271–278.

R Core Team. 2014. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.

Rikiishi K, Hashiya E, Imai M. 2004. Linear trends of the length of snow-cover season in the Northern Hemisphere as observed by the satellites in the period 1972–2000. *Annals of Glaciology* **38**: 229–237.

Roach DA, Wulff RD. 1987. Maternal Effects in Plants. *Annual Review of Ecology and Systematics* **18**: 209–235.

Sandvik SM, Odland A. 2014. Changes in alpine snowbed-wetland vegetation over three decades in northern Norway. *Nordic Journal of Botany* **32**: 377–384.

Sayers RL, Ward RT. 1966. Germination Responses in Alpine Species. *Botanical Gazette* **127**: 11–16.

Scherrer SC, Appenzeller C, Laternser M. 2004. Trends in Swiss alpine snow days: the role of local- and large-scale climate variability. *Geophysical Research Letters* **31**:

L13215, doi: 10.1029/2004GL020255.

Schöb C, Kammer PM, Choler P, Veit H. 2008. Small-scale plant species distribution in snowbeds and its sensitivity to climate change. *Plant Ecology* **200**: 91–104.

Schwienbacher E, Marcante S, Erschbamer B. 2010. Alpine species seed longevity in the soil in relation to seed size and shape – A 5-year burial experiment in the Central Alps. *Flora* **205**: 19–25.

Schwienbacher E, Navarro-Cano JA, Neuner G, Erschbamer B. 2011. Seed dormancy in alpine species. *Flora* **206**: 845–856.

Shevtsova A, Graae BJ, Jochum T, *et al.* 2009. Critical periods for impact of climate warming on early seedling establishment in subarctic tundra. *Global Change Biology* **15**: 2662–2680.

Silvertown JW, Charlesworth D 2001. *Introduction to plant population biology*. 4th edn. London: Blackwell.

Thuiller W, Albert C, Araújo MB, *et al.* 2008.. Predicting global change impacts on plant species' distributions: Future challenges. *Perspectives in Plant Ecology, Evolution and Systematics* **9**: 137–152.

Vleeshouwers LM, Bouwmeester HJ, Karssen CM. 1995. Redefining seed dormancy: an attempt to integrate physiology and ecology. *Journal of Ecology* **83**:1031–1037.

Walck JL, Hidayati SN, Dixon KW, Thompson K, Poschlod P. 2011. Climate change and plant regeneration from seed. *Global Change Biology* **17**: 2145–2161.

Wipf S, Rixen C. 2010. A review of snow manipulation experiments in Arctic and alpine tundra ecosystems. *Polar Research* **29**: 95–109.

Wulff RD. 1995. Environmental maternal effects on seed quality and germination. In: Kigel J, Galili G, eds. *Seed development and germination*. New York: Marcel Dekker, 491–506.

Table Legend

Table 1. (A) Weekly soil temperatures (mean ± s.e. in °C) during the snow-free period of the years 2008 – 2014 in control (C) and warmed (W) plots. Different styling indicates the significant events of snowmelt (underlined), seed dispersal (bold) and snowfall (italic). (B) Monthly mean soil temperatures of the year 2013.

Table 2. Results of GLMs on the effects of species (Sp), pre-dispersal treatment (Tr), incubation temperature (T), and their interactions on (A) final germination and (B) mean time to germinate of fresh seeds (i.e. without stratification). The models were performed with binomial error and logit link-function for the final germination and gamma error and inverse link-function for the mean time to germinate. Seed lots that failed to germinate more than 1% were excluded from the analysis of B. Degrees of Freedom (Df), Deviance (Dev.), Residual Degrees of Freedom (Res. Df), Residual Deviance (Dev. Res), *F*- and *P*-value are shown. Significant values are highlighted in bold.

Table 3. Species specific GLMs (with binomial error and logit link-function) results on the influences of pre-dispersal treatment and incubation temperature on fresh seed final germination. Degrees of Freedom (Df), Deviance (Dev.), Residual Degrees of Freedom (Res. Df), Residual Deviance (Dev. Res), *F*- and *P*-value are shown. Significant values are highlighted in bold.

Table 4. GLM results on the effects of species (Sp), pre-dispersal treatment (Tr), incubation temperature (Te), cold stratification period (Sf) and their two-way interactions on seed germination proportion. The model was performed with quasibinomial error and logit link-function. Degrees of Freedom (Df), Deviance (Dev.),

Residual Degrees of Freedom (Res. Df), Residual Deviance (Dev. Res), *F*- and *P*-value are shown. Significant values are highlighted in bold.

Table 5. Species specific GLM results on the responses of seed germination proportion to the different incubation temperature (T), stratification period (Sf), pre-dispersal treatment (Tr) and their interactions. Estimated coefficient, standard error, t-value and *P*-value are shown. Significant values are highlighted in bold.

Table 1

(A)		Soil Temperature (°C)	
Week of the year	Equivalent time of a normal year	C	W
27	02 – 08 July	9.7±0.1	11.0±0.1
28	09 – 15 July	10.2±0.3	11.0±0.2
29	16 – 22 July	8.8±0.2	10.6±0.2
30	23 – 29 July	8.8±0.1	10.3±0.1
31	30 July – 05 August	9.8±0.2	11.3±0.2
32	06 – 12 August	9.4±0.1	10.7±0.1
33	13 – 19 August	8.1±0.3	9.5±0.3
34	20 – 26 August	8.3±0.3	9.8±0.3
35	**27 August – 02 September**	8.1±0.1	**9.5±0.1**
36	**03 – 09 September**	**7.6±0.1**	**9.2±0.2**
37	**10 – 16 September**	**4.9±0.5**	6.1±0.4
38	17 – 23 September	4.7±0.2	6.3±0.2
39	24 – 30 September	5.9±0.2	7.2±0.3
40 – 41	*1 – 14 October*	*Snowfall*	

(B)		2013 Soil Temperature (°C)	
Month	Weeks of the year (2013)	C	W
July	29 – 31	10.8±0.8	12.7±0.7
August	31 – 35	7.7±1.1	9.3±1.1
September	35 – 40	4.9±1.2	6.5±1.1

Table 2

Factor	Df	Dev.	Res. Df	Dev. Res	F-value	*P*-value
(A) *Final germination*						
Species (Sp)	2	276.9	61	711.9	33.30	**<0.001**
Pre-dispersal treatment (Tr)	1	1.2	60	710.7	0.30	0.589
Incubation temperature (T)	1	465.4	59	245.3	111.93	**<0.001**
Sp × Tr	2	26.9	57	218.4	3.24	**0.047**
Sp × T	2	42.4	55	176.0	5.10	**0.009**
Tr × T	1	6.1	54	168.9	1.46	0.232
(B) *Mean time to germinate*						
Species (Sp)	2	0.4	45	8.8	7.47	**0.002**
Pre-dispersal treatment (Tr)	1	0.0	44	8.8	0.15	0.702
Incubation temperature (T)	1	7.6	43	1.2	293.02	**<0.001**
Sp × Tr	2	0.2	41	1.1	3.11	0.056
Sp × T	2	0.1	39	1.0	1.44	0.250
Tr × T	1	0.0	38	1.0	0.02	0.895

Table 3

Factor	Df	Dev.	Res. Df	Dev. Res	F-value	*P*-value
Final germination						
Cerastium cerastoides						
Pre-dispersal treatment (Tr)	1	3.6	14	158.2	0.71	0.417
Incubation temperature (T)	1	93.4	13	64.8	18.52	**0.001**
Tr × T	1	6.5	12	58.3	1.30	0.277
Leucanthemopsis alpina						
Pre-dispersal treatment (Tr)	1	7.6	16	47.9	5.99	**0.028**
Incubation temperature (T)	1	29.2	15	18.7	22.94	**<0.001**
Tr × T	1	0.1	14	18.6	0.07	0.797
Veronica. alpina						
Pre-dispersal treatment (Tr)	1	3.8	16	91.9	1.23	0.286
Incubation temperature (T)	1	51.6	15	40.3	16.85	**0.001**
Tr × T	1	0.1	14	40.2	0.031	0.863

Table 4

Factor	Df	Dev.	Res. Df	Dev. Res	F-value	*P*-value
Sp	2	621.1	381	3822.3	69.17	**<0.001**
Tr	1	223.4	380	3598.9	49.75	**<0.001**
T	1	1522.1	379	2076.8	339.00	**<0.001**
Sf	1	50.8	378	2026.0	11.32	**0.001**
Sp × Tr	2	90.6	376	1935.4	10.09	**<0.001**
Sp × T	2	98.6	374	1836.8	10.98	**<0.001**
Sp × Sf	2	81.2	372	1755.7	9.04	**<0.001**
Tr × T	1	70.3	371	1685.4	15.66	**<0.001**
Tr × Sf	1	54.2	370	1631.1	12.08	**<0.001**
T × Sf	1	1.8	369	1629.3	0.40	0.526

Table 5

	Estimate	Std. Error	t-value	P-value
(A) *C. cerastoides*				
(Intercept)	-5.6850	0.7610	-7.47	**<0.001**
log(T)	2.8030	0.3237	8.66	**<0.001**
Sf	0.0928	0.0274	3.39	**0.001**
Sf^2	-0.0007	0.0002	-3.81	**<0.001**
Tr	-0.1221	0.2595	-0.47	0.639
log(T) × Sf	-0.0409	0.0117	-3.51	**0.001**
log(T) × Sf^2	0.0003	0.0001	3.43	**0.001**
Sf × Tr	0.0280	0.0044	6.43	**<0.001**
(B) *L. alpina*				
(Intercept)	-4.8571	0.5674	-8.56	**<0.001**
log(T)	1.1410	0.2115	5.39	**<0.001**
Tr	-2.0266	0.7429	-2.73	**0.007**
log(Sf)	0.2996	0.0344	8.72	**<0.001**
log(T) × Tr	1.3783	0.2936	4.69	**<0.001**
(C) *V. alpina*				
(Intercept)	-6.0075	1.4577	-4.12	**<0.001**
log(T)	2.9854	0.5783	5.16	**<0.001**
Sf	0.0734	0.0189	3.88	**<0.001**
Tr	-4.0819	1.2556	-3.25	**0.002**
log(T) × Sf	-0.0283	0.0072	-3.94	**<0.001**
log(T) × Tr	1.6460	0.5109	3.22	**0.002**
Sf × Tr	0.0132	0.0060	2.20	**0.030**

Figure Legend

Fig. 1. (A) Final germination percentage (mean ± s.e.) of control (C, black bars) and warmed (W, grey bars) seeds and (B) mean time to germinate (in days, mean ± s.e.) of control (full circles and continuous lines) and warmed (empty circles and dotted lines) seeds of C. *cerastoides*, *L. alpina* and *V. alpina* under the four incubation temperatures. Seed lots that failed to germinate more than 1% were excluded from the analyses of the mean time to germination, although still present in the figures for comparative purposes.

Fig. 2. Seed germination (in percentage) of (A) *C. cerastoides*, (B) *L. alpina*, and (C) *V. alpina*. For each pre-dispersal treatment (C: control, W: warmed) the influences on seed germination of the length of cold stratification periods and the four incubation temperatures are shown.

Figure 1

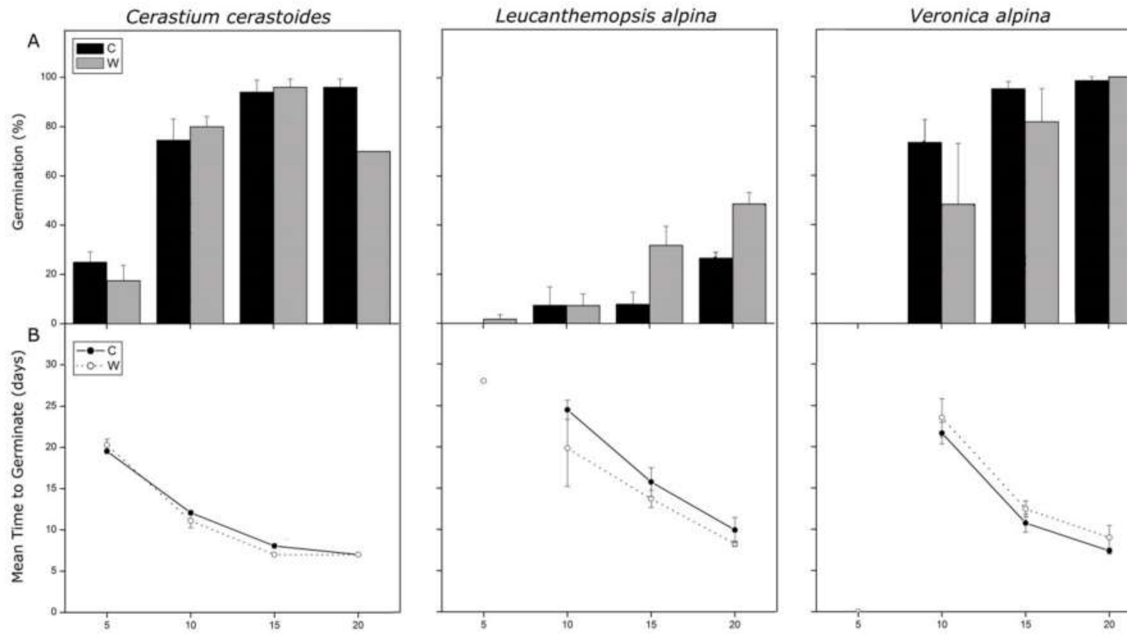
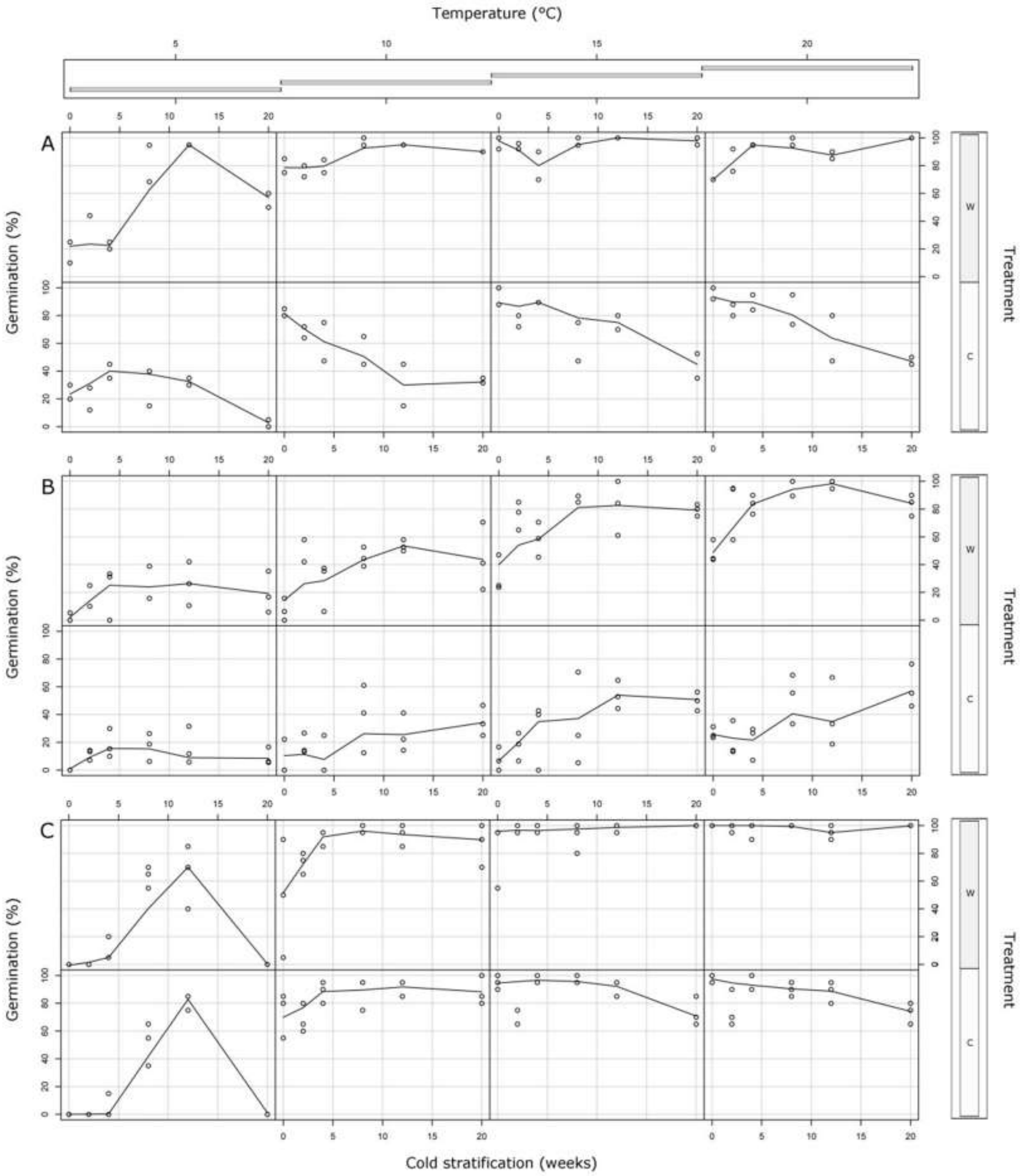


Figure 2


Temperature (°C)
5
10
15
20
A
B
C
Germination (%)
Treatment
W
C
Cold stratification (weeks)
0
5
10
15
20
0
20
40
60
80
100

Article 3

Esther R. Frei, Eva Bianchi, Giulietta Bernareggi, Peter Bebi, Melissa A. Dawes, Carissa D. Brown, Andrew Trant and Christian Rixen.

Experimental evidences for seed and substrate limitation along an elevational climate gradient across treeline in the Swiss Alps

In preparation for submission

Experimental evidence for seed and substrate limitation along an elevation climate gradient across treeline in the Swiss Alps

Running head: Biotic and abiotic interactions on alpine treeline seedling recruitment

Esther R. Frei [1,*], Eva Bianchi[1], Giulietta Bernareggi[1,2], Peter Bebi[1], Melissa A. Dawes[1,3], Carissa D. Brown[4], Andrew Trant[5] and Christian Rixen[1]

[1]WSL Institute for Snow and Avalanche Research SLF, Flüelastrasse 11, 7260 Davos Dorf, Switzerland

[2]Università di Parma, Dipartimento di Bioscienze, Parma, Italy

[3]Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Zurich

[4]Department of Geography, Memorial University, 230 Elizabeth Avenue, St John's, NL, Canada A1B 3X9

[5]School of Environment, Resources and Sustainability, University of Waterloo, 200 University Avenue West, Waterloo, ON, Canada N2L 3G1

Correspondence: Esther Frei, tel. +1 778 834 2485, fax +1 604 822 6150, e-mail: estherfrei@gmx.net

[*] Present address University of British Columbia, 1984 West Mall, Vancouver, BC, Canada V6T 1Z2

Key-words: climate, emergence, establishment, herbivory, microsites, *Larix decidua*, low and high elevation provenances, *Picea abies*, scarification, tree seedling recruitment

Abstract

Alpine treelines are among the most conspicuous vegetation boundaries and potentially responsive to climate warming. Understanding if and how treeline is expanding or will expand with ongoing climate change is a key scientific challenge. Treeline advance depends on natural recruitment, which is influenced by abiotic and biotic factors besides climate. In this study we investigated the effects of different abiotic and biotic factors as well as their interactions on tree seedling recruitment in a treeline ecotone in the Swiss Alps. Using seeding, substrate alternation and herbivore exclosure treatments, seeds of local low and high elevation provenances of two common tree species, European Larch (*Larix decidua* MILL.) and Norway spruce (*Picea abies* (L.) KARST.), were sown into intact vegetation and bare-ground plots in two consecutive years. There was clear evidence for viable seed limitation in the studied treeline ecotone. Seedling emergence at the site below the current treeline was very low because of reduced light availability due to the tree canopy and understorey vegetation. In comparison, seedling emergence was considerable at and above treeline. Our findings suggest that tree seedlings can establish above the current treeline, at least in years with favourable weather conditions and if seeds were transported there. Slightly more seedlings emerged in subplots where the natural vegetation layer was removed, which we interpret as moderate evidence for substrate limitation. Only small differences were observed between the two species as well as between low and high elevation seed provenances. While seedling survival after the first winter was generally high, only a small proportion of seedlings survived the second winter indicating the importance of longer-term research on seedling recruitment. Overall, this study demonstrates that emergence and early establishment of seedlings is limited less by temperature than by multiple interacting abiotic and biotic factors.

Introduction

Global mean temperatures have increased over the past century and future warming is predicted to be most pronounced in high latitudinal and mountain ecosystems (Solomon et al. 2007). Recent warming induced range shifts as species track their optimal climate (Walther et al. 2003; Parmesan and Yohe 2003). Increased shrub abundance in Arctic tundra ecosystems worldwide provides evidence for latitudinal range shifts (Sturm et al. 2005, Myers-Smith et al. 2011; Elmendorf et al. 2012). Upward shifts of shrubs and trees have also been observed in mountain regions (Theurillat and Guisan, 2001; Tinner and Ammann 2005; Kullmann 2002, Moiseev and Shiyatov 2003; Esper and Schweingruber 2004; Devi et al. 2008). Treeline position is widely considered temperature sensitive (Mikola 1952; Körner 2007) and thus potentially responsive to climate warming (Holtmeier and Broll 2005, Körner 2012; Körner and Paulsen 2004). However, treeline advances are not yet a worldwide phenomenon, because they lag behind rates of climate change (Chapin et al., 2005; Lloyd 2005; Loarie et al. 2009). Nevertheless, a global meta-analysis revealed considerable seedling recruitment above treeline consistent with observed rates of recent warming implying the possibility of future treeline advances (Harsch et al. 2009). Although seedling recruitment is not directly linked to treeline shifts (Graumlich et al 2005), upper treeline positions depend on continued natural regeneration (Körner 2012).

In addition to climate, numerous other abiotic factors and biotic interactions are limiting tree seedling recruitment. One pre-requisite for successful seedling recruitment is the availability of viable seed (Turnbull et al. 2000), which commonly declines with elevation (Körner 2012; Holtmeier 2009). Seed limitation at treeline is mainly caused by decreasing quality and quantity of available seed such as extended production time intervals (reviewed in Holtmeier 2009). Seed dispersal may also be a limiting factor with short wind-mediated dispersal distances restricting dispersal beyond treeline (Kullman 2005; Mazepa 2005; Holtmeier 2005; Kuoch 1965). However, other studies contradict the importance of seed limitation at treeline (Körner 2012).

Successful recruitment also strongly depends on the availability of suitable microsites for establishment and survival. A multitude of factors determines the quality of such microsites, such as light, the presence of competitors, climate and herbivory (Leck et al. 2008). Dense vegetation cover may impede recruitment by shading and competition for moisture and nutrients (Hobbie and Chapin III 1998; Schönenberger, 1975; Weih and Karlsson, 1999), but also facilitate recruitment by sheltering seedlings from adverse climate effects (Holtmeier 2009; Oswald 1963; Friedel 1967; Hättenschwiler and Smith 1999). Along environmental stress gradients, biotic interactions are predicted to change from competition to facilitation at high and low stress levels respectively (Callaway 2002). Treeline ecotones are characterized by more open microsites exposing tree seedlings to similar beneficial life conditions as low stature alpine vegetation (Körner et al. 2003). Such open microsites are warmer than seedbeds in montane forests due to surface heating effects that are maintained over the growing season (Scherrer and Körner 2010). Many tree species need open and thus warmer micro-habitats for successful germination and establishment (Bullock 2000; Leck et al. 2008). Seed germination rates of the most common tree species, e.g. *Larix decidua* and *Picea abies*, peak at temperatures above 20 °C (Black and Bliss 1980; Nather 1958; Tranquillini 1979; Sveinbjornsson et al. 1996). In contrast to these positive thermal effects, open microsites with exposure to high solar radiation may also negatively affect seedling recruitment by increasing low-temperature and water stress (Germino et al. 2002; Bader et al. 2007; Maher et al. 2005). Moreover, the lack of shelter can increase the susceptibility of seedlings to herbivores. In fact, herbivory, together with drought, are the most common causes for seedling mortality in these environments (Moles and Westoby 2004; Anschlag et al. 2008).

Few studies focus on individual abiotic and biotic factors affecting seedling recruitment in treeline ecotones (e.g. reviewed in Holtmeier 2009; Zurbriggen et al. 2013), and even fewer studies tested interactions among multiple factors in treeline ecotones (but see Brown et al. 2013; Brown and Vellend 2014; Tingstad et al. 2015). In this study we investigated the effects of climatic and biotic factors and their interactions on seedling recruitment of two common tree species, European Larch (*Larix decidua* MILL.) and Norway spruce (*Picea abies* (L.) KARST.) at three different elevations, i.e. below, at and above the current treeline in the Swiss Alps. The experiment included seeding of local low and high elevation provenances into intact vegetation and bare-ground plots in two consecutive years and with and without herbivore exclusion. Specifically, we addressed the following research questions:

(v) Is seedling recruitment along an elevation climate gradient across treeline limited by the availability of viable seeds?

(vi) Is seedling recruitment influenced by biotic interactions (i.e. open substrate as seed bed, herbivory)?

(vii) If so, do biotic interactions vary along an elevation climate gradient across treeline and over time?

(viii) Are there species- and provenance specific differences in recruitment responses to interacting abiotic and biotic factors?

Materials and methods

Study area and study species

This study was conducted in an alpine treeline ecotone located at the north-east facing slope (inclination 30 – 40°) of the Dischma valley in Davos, Eastern Swiss Alps, Switzerland in vicinity of the Stillberg treeline research site (Bebi et al. 2009). Three experimental sites were established across an alpine treeline ecotone. The lowest site (referred to as forest site) is located at 1930 m a.s.l. below treeline in a subalpine Larch-Spruce forest (Larici-Picetum), the mid-elevation site (treeline site) at 2100 m a.s.l. at the current natural treeline within dense dwarf-shrub vegetation and the uppermost site (alpine site) at 2390 m a.s.l. approximately 300 m above the natural treeline in an alpine meadow (Table S1).

The two conifer species *Larix decidua* MILL. and *Picea abies* (L.) KARST. are characteristic tree species in the Alps. They occur naturally in close vicinity to the study area but with different elevation ranges and thus represent a range of different temperature sensitivities: *L. decidua* is a treeline forming species (natural elevation range 600–2’100 m a.s.l.) with a geographic distribution mostly restricted to the European Alps (Matras 2008), whereas *P. abies* is common within the whole alpine and boreal zones of Eurasia (Skroppa 2003). It is known for its broad ecological niche and has a slightly lower upper elevational range edge (500–1’800 m a.s.l.; Brändli 1998).

Seeds used in this experiment were procured from the Swiss Federal Institute for Forest, Snow and Landscape Research WSL (Birmensdorf, Switzerland), and stored at 5 °C prior to sowing. For each of the two species seeds from one low (approximately in the centre of the elevation range) and one high elevation (close to the current treeline) provenance were used. Seed sampling locations were within a distance of 30 km from the study area (Table S2).

Experimental design

In summer 2013, the three experimental sites were set up following the standard protocol of the global GTREE-initiative (Brown et al. 2013). A scarification treatment was applied using a hand-cultivator to remove surface plant litter, as well as mosses and lichens, but leaving vascular plants rooted in the soil. Seeds of the two studied species were equally spread on half of the subplots in the first year (2013). The other half of the subplots was seeded at the beginning of the following growing season in spring 2014. Immediately after seeding, half of the subplots were covered with durable and stable metallic cages (45 cm x 28 cm) to exclude herbivores ("split subplot"). Each metal cage covered two adjacent subplots. These cages are expected to exclude mice, ungulates, mountain hares, and birds, whereas burrowing animals, such as voles, and small animals like slugs are probably not prevented from entering (M. Schütz, personal communication 2013). Fences were installed at all three sites to protect experimental plots from grazing and trampling by cattle and horses.

In this full factorial experimental design, seeding and substrate treatments were applied at plot level. Treatment combinations were randomly assigned to 20 plots per site, which were 2.24 m long and 0.45 m wide each. This resulted in 5 replications of each treatment combination at each of the three experimental sites. Each of these plots was subdivided into 16 sub-plots (22.5 cm x 28 cm each) to which all possible combinations of the four other experimental factors (species, provenance, herbivory exclosure, and year of seeding) were randomly assigned. Overall, this resulted in 960 subplots.

Seedling emergence and survival were assessed by counting the seedlings in each subplot at the beginning and end of the growing season in 2013, 2014 and 2015. All seedlings were marked to avoid double counts. The height of the seedlings was measured using a hand ruler at the end of growing seasons 2014 and 2015. In subplots with more than ten seedlings, ten arbitrarily chosen seedlings were measured.

At each site, two to five temperature loggers (iButton; Maxim Integrated Products, Sunnyvale, CA, USA) recorded soil temperature at a depth of 5 cm (Table S3). Daily minimum, mean and maximum climate data (air temperature, precipitation, snow depth, wind) was provided by a climate station at 2100 m a.s.l. (Ref) that is located in a distance of approx. 50 m from the mid-elevation site.

Statistical Analyses

Linear and generalized linear mixed-effects models (LMMs and GLMMs; Faraway 2006; Pinheiro & Bates 2000) were used to analyse the response of seedling recruitment (emergence, winter survival and growth) to the investigated abiotic and biotic factors as well as their interactions. Due to the (almost complete) lack of natural germination, unseeded plots were omitted from these analyses, and the forest site was excluded from survival and growth trait models because of the very low seedling recruitment. GLMMs for seedling emergence and first winter survival contained site, substrate, species, provenance, year of seeding, and herbivore exclosure treatment as fixed effects. Because of the small data set for second winter survival, it was not possible to test the influence of substrate treatment, herbivore exclosure and interactions including these factors. All three GLMMs used binomial distributions. Seedling height was modelled using an LMM that contained the fixed effects site, species, provenance, and year of seeding. This data set was too small to analyse interactions between model factors. All models accounted for spatial correlation among subplots by including plot and two subplot-structures as random effects. Significances of model factors were determined by likelihood ratio tests and non-significant fixed effects were eliminated from the final models. All models were fit using standard procedures for model diagnostics (Zuur et al. 2009) with the lme4-library (Bates et al. 2012) in R 3.1.3 (R Core Team 2015).

Results

SEEDLING EMERGENCE

Natural recruitment was observed only at the forest site and during the first baseline census in June 2013, where 15 *L. decidua* seedlings and seven *P. abies* seedlings were found. In the seeded plots, a total of 768 *L. decidua* seedlings and 959 *P. abies* seedlings were counted at the end of the first growing season in all 60 plots. Emergence was significantly higher for *L. decidua* (6.3±0.7 % of viable seeds per subplot; mean ± standard error; Fig. 1) than for *P. abies* (2.9±0.4 %; species $P < 0.001$). Only 12 seedlings (0.1±0.04 % of viable seeds) emerged at the forest site. Seedling emergence was significantly higher at the treeline and alpine sites, where 734 (5.8±0.8 %) and 981 (8.0±0.8 %) seeds emerged, respectively (site $P = 0.013$). Total emergence was similar in both years 2013 and 2014 (year $p = 0.87$). However, the distribution of emergence among sites varied between the two seeding years (year x site $P < 0.001$; Fig. 1a). It was more than three times higher at the treeline site than at the alpine site in 2013 (9.5 % vs. 3.0 %). Conversely, in 2014, emergence at the alpine site was more than six times higher as compared to the treeline site (13.1 % vs. 2.0 %). Emergence of the two *P. abies* provenances differed only slightly (3.3 % for low vs. 2.5 % for high provenance). In *L. decidua*, however, low provenance seeds (10.6 %) emerged about five times more frequently than high provenance seeds (2.1 %; species x provenance $P < 0.001$). Substrate scarification enhanced emergence at the treeline site but not at the alpine site (site x scarified $P = 0.01$) in both species and years (Tab.1; Fig. 1b). The protection against herbivores resulted in a general increase in emergence from 4.0 % to 5.2 % (cage $P < 0.001$). This effect was slightly stronger in 2013 than 2014 (year x cage P =

0.03) and the increase was more pronounced for low than for high provenance seedlings ($P = 0.008$).

EARLY STAGE SEEDLING ESTABLISHMENT

The fraction of seedlings that survived the first winter was significantly lower at the alpine site (8.5±2.0 %) than at the treeline site (51.5±4.4 %, site $P < 0.001$; Fig. 2, Table 2). Survival was generally higher on scarified plots than on plots with an intact vegetation cover (scarified $P < 0.001$), but the difference was more pronounced at the treeline than at the alpine site (site x scarified $P = 0.053$; Fig. 2b). All other interactions were not significant ($P > 0.05$). Low provenance seedlings of both species had slightly higher survival rates after the first winter as compared to high provenance seedlings (provenance $P = 0.037$). Differences between the two species were only marginal (species $P = 0.067$) and herbivore exclosure did not significantly influence winter survival. Absolute numbers of surviving seedlings after the first and second winter can be found in Table S4.

Similar to first winter survival, survival after the second winter was lower at the alpine site (25.9±10.3 % of the seedlings observed at the end of the previous growing season, Table 3) than at the treeline site (79.2±6.0 %; site $P = 0.023$; Fig. 3). Low provenance *L. decidua* seedlings had a higher survival rate during the second winter than their high provenance counterparts, whereas in *P. abies* survival was higher for high than for low provenance seedlings (species x provenance $P = 0.007$). Species and provenance individually, as well as their interactions with site were not significant.

After the second growing season, seedlings had grown to an average height of 3.3±0.1 cm. They were significantly taller at the treeline (3.8±0.1 cm) than at the alpine site (2.6±0.1 cm; site $P < 0.001$; Fig. 4, Table 4). However, there were no differences in seedling height with respect to year of seeding, species and provenance ($P > 0.1$ for all three factors).

CLIMATE

The average mean air temperature for summer (i.e. June, July and August) over the period 1981 – 2010 measured at the climate station at 2100 m a.s.l. was 9.4 °C (Table S3). Summer 2013 with a mean temperature of 10.6 °C was clearly above average, whereas the following summer 2014 with a mean temperature of 8.7 °C was slightly below average. Summer temperature in 2015 was the second highest ever measured (11.8 °C). Measurements at the nearby MeteoSwiss station Davos (1594 m a.s.l.) show that the summers of 2013 (265 mm) and 2015 (261 mm) both had significant precipitation deficits with only 64 % of the normal precipitation sum (410 mm in the 1981 – 2010 period). Conversely, precipitation in summer 2014 (492 mm) was 20 % above average (MeteoSwiss, 2015).

Discussion

Limitation by the availability of viable seeds

This study provides experimental evidence for seed source limitation in an alpine treeline ecotone with almost no natural recruitment. While seed limitation has been reported for different forest ecosystems (e.g. Turnbull et al. 2000; Eriksson & Ehrlén, 1992; Tilman, 1994, 1997), it seems to be particularly common for treeline ecotones (Kuoch 1965; Holtmeier 1999, 2009). This can be partly explained by decreasing seed production with increasing elevation of treeline species such as *P. abies* and *L. decidua*, resulting in good seed crop years only about once a decade (Tschermak, 1950). In addition, seed ripening may be decelerated and the amount of empty, non-viable seeds may be increased due to the shorter growing period at treeline (Körner 2012). As a consequence, natural regeneration from seeds is very critical in the treeline ecotone (Holtmeier, 1993, 1995a, 1999).

The extremely low recruitment at the forest site is expected to be caused by the dense cover of competing understorey vegetation that prevents seedling emergence by strongly reduced light availability at the soil surface. Imbeck and Ott (1987) studied *P. abies* regeneration in a similar subalpine spruce forest rich in tall forbs (*Piceto-Adenostyletum*) near our study site in the Swiss Alps and reported less than 5% of natural recruitment on sites with dense vegetation cover. Our findings also agree with the reduced survival in one-year-old transplanted *P. mariana* seedlings in shaded plots (Cranston and Hermanutz 2013) and the negative correlation between survival and canopy cover in *L. decidua* and *Picea* spp. (Manson et al. (2004). Another important aspect is the asynchronous phenological timing of the understorey forbs and seedling emergence (Imbeck and Ott, 1987). In spring, the understorey vegetation at our forest site is still very short-grown enabling seeds to emerge, as shown by the few naturally emerged seedlings at the start of the experiment in June 2013 that did not survive the first summer. A similar situation applies for the treeline site with the dense tall dwarf-shrub layer limiting seedling recruitment despite single seed bearing trees nearby (Grau et al., 2013; Cranston and Hermanutz, 2013) whereas seed bearing trees are quite far from the uppermost site that is located approx. 300 m above the natural treeline. However, recent studies showed that seedlings of *L. decidua* and *P. abies* have higher altitudinal distribution limits than adult trees of the same species (Lenoir et al. 2009). According to Körner (2012), this difference in range limits between seedlings and adult trees might be due to the enhanced temperatures close to the ground from which seedlings profit whereas adult trees are exposed to lower air temperatures. However, tree seedling occurrence above treeline has to be considered carefully and is not directly linked to climate warming (Körner, 2012). Nevertheless, it shows the potential for an upward range expansion of trees in the Alps in the context of future climate warming (Vitasse et al. 2012).

Biotic and abiotic interactions as recruitment limitations

Suitable substrate as seedbed is decisive for seedling establishment by allowing the germination and successive thrive of those seeds that reach the soil surface. The higher emergence on scarified plots at the treeline site, in comparison with the alpine site, is likely influenced by the dense dwarf-shrub and thick moss layer covering the soil surface and indicating substrate limitation for seedling emergence in this site (Table 1; Fig. 1). In fact, dense vegetation cover may prevent seeds from reaching a suitable seed bed by competition for moisture and nutrients, as well as by shading effects (Schönenberger, 1975; Weih and Karlsson, 1999). Moreover, Hunziker and Brang

(2005) showed that a deep and dense moss mat impeded germination of *P. abies* seeds by preventing seedlings from reaching the soil surface (but see Motta et al., 1994; Munier et al., 2010). Conversely, seedbed at our alpine site was not limiting for germination probably because of the less dense vegetation cover with a relatively high proportion of rock and bare ground. Indeed, reduced competition and exposed mineral soil provide a particularly favourable seedbed for seed recruitment (Munier et al., 2010; Tingstad et al., 2015).

Similar to emergence, seedling survival after the first winter was higher on scarified plots than on intact vegetation in particular at the treeline site (Table 2; Fig. 2b). In contrast, Wheeler et al. (2011) observed lower survival in one-year-old *Picea mariana* seedlings on bare ground than on moss-covered substrate. The authors relate their findings to the protective effect of the moss covered substrate against adverse climate conditions, whereas competing effects of dense moss cover and shading dwarf-shrubs seem to outweigh positive sheltering effects in our study system. Hence, the presence of bare soil generally enhanced seedling recruitment, indicating substrate limitation and suggesting how substrate disturbance may contribute to reduce the competing effect of vegetation.

Post-dispersal predation is known to be an important constraint on seedling recruitment and several experiments about post-dispersal seed and seedling predation in *Picea* and *Abies* species showed that seeds might be eaten by different groups of animals, i.e. invertebrates, rodents, birds, small and large mammals (Côté et al., 2003; 2005; Castro et al., 1999; Itô and Hino, 2004; Dulamsuren et al., 2013). The protection against herbivores in our study resulted also in an increased seedling emergence (Tab. 1) supporting the evidence that herbivores can be an important constraint on seedling recruitment at treeline. However, the overall impact of post-dispersal seed and seedling predation might have been reduced in our study system by the exclusion of invertebrates and digging animals, such as voles, mice and small rodents that seem to have a major influence on seed predation.

Seedling recruitment in different years

The present study provides evidence for the fact that inter-annual climate variability affects seedling emergence (Table 1). Growing season temperatures and precipitation varied considerably among the three study years. The warm and dry summer of 2013 was followed by a cold and wet summer in 2014 and an exceptionally hot summer in 2015 (Fig. S2). In 2013, highest seedling emergence occurred at treeline, whereas in 2014, highest emergence was observed at the alpine site (Table 1; Fig. 1a). This result may be explained by stronger limitations due to summer drought at high elevation, where no tall-growing vegetation such as dwarf-shrubs are protecting against strong solar radiation and prevent drought related early seedling mortality. Indeed, several studies provide evidence for negative effects of high solar radiation on seedling recruitment (Germino et al. 2002; Bader et al. 2007; Maher et al. 2005). However, our result is in contrast to the finding of Castanha et al. (2012), who observed highest germination at an alpine site in an exceptionally warm summer (2009) in a study on seedling recruitment of *Pinus flexilis* and *Picea engelmanni* across a treeline ecotone in the Rocky Mountains (Colorado, USA). The authors relate their findings to the fact that higher temperatures extended the alpine growing season due to earlier snow melt, while they induced emergence constraining drought at the lower forest site (Castanha et al., 2012).

On plots with intact vegetation, seedling emergence from seeds sown in 2014 was higher than emergence from seeds sown in 2013 (Table 1). This possibly reflects the cold and wet conditions of summer 2014 and suggests that the main constraint was not water availability, but water surplus leading to rotting of seeds. The effect of the surrounding vegetation may have been positive in summer 2014, due to higher interception, absorption and evaporation (Hibbert 2007) in contrast to the low water supply in summer 2013, where reduced competition for water on scarified plots may have facilitated seedling emergence. The influence of inter-annual climate variability can thus hardly be generalized, and it is important to consider the timing and duration of drought periods, but also the timing of spring snowmelt and potential water surplus effects.

In our experiment, seedlings that survived the first winter generally survived till the end of the second vegetation period (Table S4). This finding coincides with Batllori et al. (2009) who observed 100 % summer survival after the first winter for *Pinus uncinata* in a seedling transplant experiment in the Pyrenees. While this confirms the hypothesis that first winter is one of the major bottle necks for seedling recruitment, as it bears the greatest risk to survival (Körner, 2012), we have to take into account that, depending on climate, mortality waves are also possible in later stages of seedling recruitment. Indeed, our findings of generally high seedling mortality during the second winter that was more pronounced at the alpine than the treeline site (Tables 3,S4; Fig. 3) may be related to more adverse autumn climate conditions at high elevation (Fig. S1). Likewise, earlier research in our study area showed that seedling survival was high during the first three years of observation for *P. abies*, *L. decidua* and other coniferous species, but was strongly reduced after the third winter for the broadleaved species *Alnus viridis* and *Sorbus aucuparia* (Zurbriggen et al. 2013). This shows the need for studying seedling recruitment also in later stages and that these effects may be strongly species-specific and even affected by provenance differences.

Species and provenance influence on seedling recruitment

In our study, low provenance seedlings of *L. decidua* emerged better than high provenance seedlings of the same species (Table 1). This result may be seen as a contradiction to several investigations and practical experiences with low survival rates of lowland provenances in higher altitudes near treeline (Nather 1987, Schönenberger et al. 1990). However, while most of these investigations and experiences with lowland provenances are based on afforested trees and showed that in particular susceptibility to frost damage during a later stage of seedling development was increased for lowland provenances, this was obviously not yet a critical factor for the one- and two-year old seedlings in our study. The better emergence of low provenance seedlings of *L. decidua*, in our experiment might possibly be because those seeds were considerably heavier compared to the seeds of the high elevation provenance and this larger seed sizes may have favoured germination. In fact, under higher environmental stress, larger seed size may favour germination even when such a relationship is absent under less extreme conditions (Castro et al 1999). The role of provenance is thus likely to have diverging effects at different stages of seedling establishment near treeline.

Seedling establishment varies also among species. In our treeline ecotone, we observed higher survival of *L. decidua* than *P. abies* at the alpine site, whereas the inverse pattern occurred at treeline (Table 2). This finding is in line with the current range limits of the two species in the Central Swiss Alps (Brändli et al 1998, Zurbriggen et al 2013). While it is well known that *L. decidua* is better adapted to the cold environment of treeline sites in the continental Alps compared to the evergreen and less frost tolerant species *P. abies* (Tranquillini 1979), it is less clear if the first years of seedling establishment contribute already to this difference. Other studies in our study area provide evidence that later stages in the seedling development (years 5-15) are also critical bottle necks for tree survival in and above the treeline ecotone and that the effect of elevation on seedling survival is relatively small during the first years, but increasing in later years of seedling development (Barbeito et al 2012, Zurbriggen et al. 2013). In order to understand and disentangle the importance of the different treeline limiting factors, it is thus important to study these effects in different stages of seedling development and to take advantage of long-term observations.

Conclusions

Our results provide experimental evidence for seed source limitation and the importance of a multitude of interacting abiotic and biotic factors in determining seedling recruitment in an alpine treeline ecotone in the Swiss Alps. Seedling emergence and establishment are generally not temperature limited but rather depend on the availability of viable seeds and suitable seedbed conditions. More open alpine microsite conditions promote seedling emergence in contrast to dense subalpine dwarf shrub or forest understorey vegetation. Although elevation dependent temperature differences are not limiting, inter-annual climate variation seems to have an important influence on early seedling recruitment in this alpine treeline ecotone. In addition to this, natural regeneration at treeline is potentially delayed because of, for example, longer masting year cycles or irregular predator outbreak cycles, which further emphasizes the need for longer-term studies on treeline recruitment. Continued research and combining our findings with a global research initiative will allow us to disentangle the importance of different treeline limiting factors and their interacting effects worldwide and will contribute to a widely supported prediction of future treeline expansion.

Acknowledgements

We thank M. Heggli, S. Voegele, E.S. Frei, A. Kulonen, Q. Canelles Trabal, G. Lang, S. Burg, Ch. Vergani, A. Ilari, I. Barbeito, U. Schmid, M. Buchmann, S. Wipf, and the Werkstatt team SLF for helping with setting up the experiment and measuring seedling recruitment. We also thank the Alpgenossenschaft Davos for the permission to work on their land.

References

Anschlag K, Broll G, Holtmeier F (2008) Mountain birch seedlings in the treeline ecotone, subarctic Finland: variation in above- and below-ground growth depending on microtopography. Arct Antarct Alp Res 40:609–616.

Bader MY, van Geloof I, Rietkerk M (2007) High solar radiation hinders tree regeneration above the alpine treeline in northern Ecuador. Plant Ecol 191:33–45.

Barbeito I, Dawes MA, Rixen C, Senn J and Bebi P (2012). Factors driving mortality and growth at treeline: a 30-year experiment of 92 000 conifers. Ecology 93, 389-401.

Batllori E, Camarero JJ, Ninot JM and Gutieérrez 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.

Bates, D.M., Maechler, M. and Bolker, B. (2014). lme4: Linear mixed-effects models using Eigen and S4. R package version 1.0-4, http://CRAN.R-project.org/package=lme4.

Bebi, P., Hagedorn, F., Martin, M., Rixen, C., Senn, J. and Wasem, U. (2009). Langfristige Waldgrenzen-Forschung am Stillberg – vor lauter Bäumen den Wald noch sehen. Forum für Wissen, 87-92.

Black RA, Bliss LC (1980) Reproductive ecology of Picea mariana (Mill.) BSP., at tree line near Inuvik, Northwest Territories. Can Ecol Monogr 50:331–354. doi:10.2307/2937255

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

Brown, C.D., Johnstone, J.F., Mamet, S.D. and Trant, A.J. (2013). Global Treeline Range Expansion Experiment Field Protocols. Mountain Views 7(2): 6-7

Brown, C.D. and Vellend, M. (2014). Non-climatic constraints on upper elevational plant range expansion under climate change. MISSING INFO: 281.

Callaway RM et al (2002) Positive interactions among alpine plants increase with stress. Nature 417:844–848.

Castanha, C., Torn, M.S., Germino, M.J., Weibel, B. and Kueppers, L.M. (2012). Conifer seedling recruitment across a gradient from forest to alpine tundra: effects of species, provenance, and site. Plant Ecology and Diversity 6, 307-318.

Castro J. 1999. Seed mass versus seedling performance in Scots pine: maternally dependetn train. New Phytol 144: 153-161

Côte, M., Ferron, J. and Gagnon, R. (2005). Invertebrate predation of postdispersal seeds and juvenile seedlings of black spruce (Picea mariana) in the boreal forest of eastern Canada. Canadian Journal of Forest Research 35, 674-681.

Côté, M., Ferron, J. and Gagnon, R. (2003). Impact of seed and seedling predation by small rodents on early regeneration establishment of black spruce. Canadian Journal of Forest Research 33, 2362-2371.

Cranston, B.H. and Hermanutz, L. (2013). Seed–seedling conflict in conifers as a result of plant–plant interactions at the forest-tundra ecotone. Plant Ecology and Diversity 6, 319-327.

Crawley, MJ (2007). The R book, Chichester: Chichester : Wiley.

Devi N, Hagedorn F, Moiseev P, Bugmann H, Shiyatov S, Mazepa, V and Rigling A (2008). Expanding forests and changing growth forms of Siberian larch at the Polar Urals treeline during the 20th century. Global Change Biology 14, 1581-1591.

Dulamsuren C, Hauck M and Leuschner C (2013). Seedling emergence and establishment of Pinus sylvestris in the Mongolian forest-steppe ecotone. Plant Ecology 214, 139-152.

Elmendorf, S. C., et al. (2012). Plot-scale evidence of tundra vegetation change and links to recent summer warming. Nature Climate Change 2(6): 453-457.

Eriksson, O. & Ehrlén, J. (1992). Seed and microsite limitation in plant populations. Oecologia, 91, 360-364.

Esper, J. and Schweingruber, F.H. (2004). Large-scale treeline changes recorded in Siberia. Geophysical Research Letters 31, 1-5.

Germino, M.J., Smith, W.K. and Resor, A.C. (2002). Conifer seedling distribution and survival in an alpine-treeline ecotone. Plant Ecology, 157-168.

Grau, O., Ninot , J.M., Cornelissen, J.H.C. and Callaghan, T.V. (2013). Similar tree seedling responses to shrubs and to simulated environmental changes at Pyrenean and subarctic treelines. Plant Ecology and Diversity 6, 329-342.

Graumlich LJ, Waggoner LA, Bunn AG (2005) Detecting global change at alpine treeline: coupling paleoecology with contemporary studies. In: Huber UM, Bugmann KM, Reasoner MA(eds) Global change and mountain regions. An overview of current knowledge. Springer, Dordrecht, pp. 501–508

Haettenschwiler S, Smith WK (1999) Seedling occurrence in alpine treeline conifers: a case study from the central Rocky Mountains, USA. Acta Oecol 20:219–224

Harsch, M.A., Hulme, P.E., McGlone, M.S. and Duncan, R.P. (2009). Are treelines advancing? A global meta-analysis of treeline response to climate warming. Ecology Letters 12, 1040-1049.

Hébert, F., Boucher, J.-F., Bernier, P.Y. and Lord, D. (2006). Growth response and water relations of 3-year-old planted black spruceand jack pine seedlings in site prepared lichen woodlands. Forest Ecology and Management 223, 226-236.

Hibbert, AR. 2007. Water yield improvement potential by vegetation management on western rangelands. Journal of the American Water Resources Association 19: 375-381

Holtmeier, F.-K. (1993a): Der Einfluß der generativen und vegetativen Verjüngung auf das Verbreitungsmuster der Bäume und die ökologische Dynamik im Waldgrenzbereich. Beobachtungen und Untersuchungen in Hochgebirgen Nordamerikas und den Alpen. Geoökodynamik XIV: 153–182.

Holtmeier, F.-K. (1993): Timberlines as indicators of climatic changes: Problems and research needs. Paläoklimaforschung 9: 211–222.

Holtmeier, F.-K. (1995a): Waldgrenze und Klimaschwankungen – Ökologische Aspekte eines vieldiskutierten Phänomens. Geoökodynamik XVI: 1–24.

Holtmeier, F.-K. (1995b): European Larch in Middle Europe with special reference to the Central Alps. In: Ecology and management of Larix forests: A look ahead. In: Proc. int. symp., Whitefish, MT, October 5–9, 1992 (Schmidt, W. C. and McDonald, K. J., eds.): 41–49, USDA For. Serv., Intermtn. Res. Stat., Gen. Techn. Rep. GTR-INT-319.

Holtmeier, F.-K. (1999): Timberline dynamics in view of climate change. In: A terrestrial transect for Scandinavia/Northern Europe: Proc. int. Scantran conference (Turunen, M., Hakkinen, J., Heal, O. W., Saelthun, R. and Holten, J., eds.), Eur. Comm. Ecosystems research report 31: 53–55.

Holtmeier, F.K. (2009). Mountain timberlines : ecology, patchiness, and dynamics, Dordrecht: [Dordrecht] : Springer Science+Business Media B.V.

Holtmeier, F.K. and Broll, G. (2005). Sensitivity and response of northern hemisphere altitudinal and polar treelines to environmental change at landscape and local scales. Global Ecology and Biogeography 14, 395-410.

Hunziker, U. and Brang, P. (2005). Microsite patterns of conifer seedling establishment and growth in a mixed stand in the southern Alps. Forest Ecology and Management 210, 67-79.

Imbeck, H. and Ott, E. (1987). Verjüngungsökologische Untersuchungen in einem hochstaudenreichen subalpinen Fichtenwald, mit spezieller Berücksichtigung der Schneeablagerung und der Lawinenbildung.

Itô H and Hino T (2004). Effects of deer, mice and dwarf bamboo on the emergence, survival and growth of Abies homolepis (Piceaceae) seedlings. Ecological Research 19, 217-223.

Johnstone, J., Boby, L., Tissier, E., Mack, M., Verbyla, D. and Walker, X. (2009). Postfire seed rain of black spruce, a semiserotinous conifer, in forests of interior Alaska. Canadian Journal of Forest Research 39, 1575-1588.

Körner, C. (2012). Alpine treelines : functional ecology of the global high elevation tree limits, Basel: Basel : Springer.

Körner, C. and Paulsen, J. (2004). A world-wide study of high altitude treeline temperatures. Journal of Biogeography 31, 713-732.

Körner, C. (1999): Alpine plant life. Functional plant ecology of high mountain ecosystems. Springer, Berlin/Heidelberg

Körner, C. (2003) Limitation and stress - always or never? J VegSci 14:141–143.

Körner, C. (2007): Climatic treelines: Conventions, global patterns, causes. Erdkunde 61:316–324

Kullman, L. (2002). Rapid recent range-margin rise of tree and shrub species in the Swedish Scandes. Journal of Ecology 90, 68-77.

Kullman, L. (2005): Wind-conditioned 20th century decline of birch treeline vegetation in the Swedish Scandes. Arctic 58: 286–294

Kuoch, R. (1965): Der Samenanflug 1963/1964 an der oberen Fichtenwaldgrenze im Sertigtal. Schweizerische Anstalt für das forstliche Versuchswesen, Mitteilungen 41 (3): 63–85.

Leck, M.A., Parker, V.T. and Simpson, R.L. (2008). Seedling Ecology and Evolution, Cambridge: Cambridge : Cambridge University Press.

Lenoir, J., Gégout, J.C., Marquet, P.A., de Ruffray, P. and Brisse, H. (2008). A significant upward shift in plant species optimum elevation during the 20th century. Science 320, 1768-1771.

Lenoir, J., Gégout, J.C., Pierrat, J.-C., Bontemps, J.-D. and Dhôte, J.-F. (2009). Differences between tree species seedling and adult altitudinal distribution in mountain forests during the recent warm period (1986-2006). Ecography 32, 765-777.

Loarie SR, Duffy PB, Hamilton H, Asner GP, Field CB, Ackerly DD. 2009 The velocity of climate change. Nature 462, 1052–1055. (doi:10.1038/nature08649).

Maher, E.L., Germino, M.J. and Hasselquist, N.J. (2005). Interactive effects of tree and herb cover on survivorship, physiology, and microclimate of conifer seedlings at the alpine tree-line ecotone. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere 35, 567-574.

Manson, W.L., Edwards, C. and Hale, S.E. (2004). Survival and early seedling growth of conifers with different shade tolerance in a Sitka spruce spacing trial and relationship to understorey light climate. Silva Fennica 38, 357-370.

Matras, J. and Pâques, L. (2008). EUFORGEN Technical Guidelines for genetic conservation and use for European Larch (Larix decidua).

Mazepa, V. (2005): Stand density in the last millennium at the upper tree-line ecotone in the polar Ural Mountains. Can. J. For. Res. 35: 2082–2091.

MeteoSwiss (2015): Monthly homogenized values, Davos. Online: http://www.meteoschweiz.admin.ch/product/output/climate-data/homogenous-monthly-data-processing/data/homog_mo_DAV.txt (retrieved on 09.01.2016).

Mikola, P. (1952): The effect of recent climatic variations on forest-growth in Finland. Fennia 75: 69–76

Moiseev, P. and Shiyatov, S. (2003). Vegetation dynamics at the treeline ecotone in the Ural Highlands, Russia. Ecological Studies 167, 423-435.

Moles, A. T. and Westoby, M. (2004). What do seedlings die from, and what are the implications for evolution of seed size? Oikos, 106, 193-199.

Motta, R., Brang, P., Frehner, M. and Ott, E. (1994). Copertura muscinale e rinnovazione di abete rosso (Picea abies L.) nella pecceta subalpina di Sedrun (Grigioni, Svizzera). Monti e Boschi 45, 49-56.

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

Myers-Smith, I. H., et al. (2011). Shrub expansion in tundra ecosystems: dynamics, impacts and research priorities. Environmental Research Letters 6(4).

Nather H (1958) Zur Keimung der Zirbensamen. Zentralbl GesForst 75:61–70.

Nather j. (1987). Der Erfolg bei der Aufforstung ist von vielen Faktoren abhängig. Österr. Forstzeigung 3: 8-9.

Parmesan, C. and G. Yohe (2003). A globally coherent fingerprint of climate change impacts across natural systems. Nature 421(6918): 37-42.

Paulsen, J., Weber, U.M. and Korner, C. (2000). Tree growth near treeline: abrupt or gradual reduction with altitude? Arctic Antarctic and Alpine Research 32, 14-20.

Pinheiro, J.C. and Bates, D.M. (2000). Mixed-effects models in S and S-PLUS, New York: Springer.

R Development Core Team (2015). R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/, Version 3.1.3.

Scherrer D, Koerner C (2010) Infra-red thermometry of alpine landscapes challenges climatic warming projections. Global Change Biol 16:2602–2613

Schönenberger W., Frey W., Leuenberger F.(1990). Ökologie und Technik der Aufforstung im Gebirge: Anregungen für die Praxis WSL-Berichte 325: 1-58.

Schönenberger, W. (1975): Standortseinflüsse auf Versuchsaufforstungen an der alpinen Waldgrenze (Stillberg, Davos). Eidgenössische Anstalt für das forstliche Versuchswesen, Mitteilungen 51 (4): 359–428.

Skrøppa, T. (2003). EUFORGEN Technical Guidelines for genetic conservation and use for Norway spruce (Picea abies).

Sveinbjornsson B, Kauhanen H, Nordell O (1996) Treeline ecology of mountain birch in the Tornetroeask area. Ecol Bull 45:65–70

Tilman, D. (1994). Competition and biodiversity in spatially structured habitats. Ecology, 75, 2-16.

Tilman, D. (1997). Community invasibility, recruitment limitation, and grassland biodiversity. Ecology, 78, 81-92.

Tinner, W; Ammann, B (2005) Long-term responses of mountain ecosystems to environmental changes: resilience, adjustments, and vulnerability. In: Global Change and Mountain Regions: an overview of current knowledge (eds. U. Huber, H. Bugmann, M. Reasoner). Springer, Dordrecht: 133-144

Theurillat, J. P. and A. Guisan (2001). Potential impact of climate change on vegetation in the European Alps: a review. Climatic Change 50(1-2): 77-109.

Tingstad L, Olsen SL, Klanderud K and V. Vandvik (2015). Temperature, precipitation and biotic interactions as determinants of tree seedling recruitment across the tree line ecotone Oecologia (2015) 179:599–608

Tranquillini W (1979) Physiological ecology of the alpine timberline. Tree existence at high altitudes with special references to the European Alps. Ecological studies 31, Springer, Berlin Heidelberg New York

Trant, A.J. and Hermanutz, L. (2014). Advancing towards novel tree lines? A multispecies approach to recent tree line dynamics in subarctic alpine Labrador, northern Canada. Journal of Biogeography 41(6), 1115-1125.

Tschermak, L. (1950). Waldbau auf pflanzengeographisch-ökologischer Grundlage. Wien

Turnbull, L.A., Crawley, M.J. and Rees, M. (2000). Are plant populations seed-limited? A review of seed sowing experiments. Oikos 88, 225-238.

Vitasse, Y., Hoch, G., Randin, C.F., Lenz, A., Kollas, C. and Körner, C. (2012). Tree recruitment of European tree species at their current upper elevational limits in the Swiss Alps. Journal of Biogeography 39, 1439-1449.

Walther, G. R., et al. (2002). Ecological responses to recent climate change. Nature 416(6879): 389-395.

Weih, M. and Karlsson, St. (1999). The nitrogen economy of mountain birch seedlings: Implications for winter survival. J. Ecol. 87: 211–219.

Wheeler, J.A., Hermanutz, L. and Marino, P.M. (2011). Feathermoss seedbeds facilitate black spruce seedling recruitment in the forest–tundra ecotone (Labrador, Canada) Oikos, 1263-1271.

Zurbriggen, N., Hättenschwiler, S., Frei, E.S., Hagedorn, F. and Bebi, P. (2013). Performance of germinating tree seedlings below and above treeline in the Swiss Alps. Plant Ecology 214, 385-396.

Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A. and Smith, G.M. (2009). Mixed effects models and extensions in ecology with R, New York, N.Y: New York, N.Y. : Springer.

Tables

Table 1. Effects of seeding year, experimental site, scarification treatment (scarified), species, provenance and herbivore exclosure treatment (cage), as well as their interactions, on seedling emergence.

Factor	**χ^2**	**Df**	**Pr(> χ^2)**	**Significance ($\alpha = 0.05$)**
year	0.025264229	1	0.873710431	
site	6.181764048	1	0.012907353	*
scarified	2.883306059	1	0.08950209	
species	24.54276987	1	7.27E-07	***
provenance	36.7375166	1	1.35E-09	***
cage	15.38009863	1	8.79E-05	***
year:site	93.02452267	1	5.17E-22	***
year:scarified	3.225830347	1	0.072485027	
year:species	1.08038928	1	0.298610488	
year:provenance	0.897816263	1	0.343367927	
year:cage	4.624882191	1	0.031511425	*
site:scarified	6.463003202	1	0.011014334	*
site:species	0.0216611	1	0.882992232	
site:provenance	0.478995865	1	0.488877501	
site:cage	0.011326132	1	0.915245713	
species:scarified	0.454900164	1	0.500017083	
provenance:scarified	0.354472221	1	0.551592367	
scarified:cage	0.043812323	1	0.834203175	
species:provenance	19.58046866	1	9.65E-06	***
species:cage	0.956126895	1	0.328164637	
provenance:cage	7.029602793	1	0.008017313	**

Values and symbols are χ2-values and significances, respectively, from likelihood ratio tests of mixed-effect models. Significance levels: (*)$P < 0.1$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 2. Effects of experimental site, scarification treatment (scarified), species, provenance and herbivore exclosure treatment (cage), as well as their interactions, on first winter survival.

Factor	**χ^2**	**Df**	**Pr(> χ^2)**	**Significance (α = 0.05)**
site	27.05833918	1	1.97E-07	***
scarified	18.54736328	1	1.66E-05	***
species	3.365937057	1	0.066557709	(*)
provenance	4.330422172	1	0.037436959	*
cage	0.473961241	1	0.491170435	
site:scarified	3.739132591	1	0.053152031	(*)
site:species	5.810687129	1	0.015929069	*
site:provenance	0.109503799	1	0.740709762	
site:cage	1.166019207	1	0.280220699	
species:scarified	1.191007313	1	0.275126464	
provenance:scarified	3.697858298	1	0.054482359	(*)
scarified:cage	1.997239877	1	0.157585941	
species:provenance	0.010684409	1	0.917672962	
species:cage	0.536497429	1	0.463887941	
provenance:cage	0.200002353	1	0.654718947	

Values and symbols are $\chi 2$-values and significances, respectively, from likelihood ratio tests of mixed-effect models. Significance levels: (*)$P < 0.1$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 3. Effects of experimental site, species and provenance, as well as their interactions, on second winter survival.

Factor	χ^2	Df	Pr(> χ^2)	Significance ($\alpha = 0.05$)
site	5.15882155	1	0.02312859	*
species	1.00374778	1	0.31640535	
provenance	0.55284162	1	0.45715893	
site:species	0.1418914	1	0.70640789	
site:provenance	0.15861445	1	0.69043538	
species:provenance	7.36905897	1	0.00663556	**

Values and symbols are $\chi2$-values and significances, respectively, from likelihood ratio tests of mixed-effect models. Significance levels: (*)P < 0.1; *P < 0.05; **P < 0.01; ***P < 0.001.

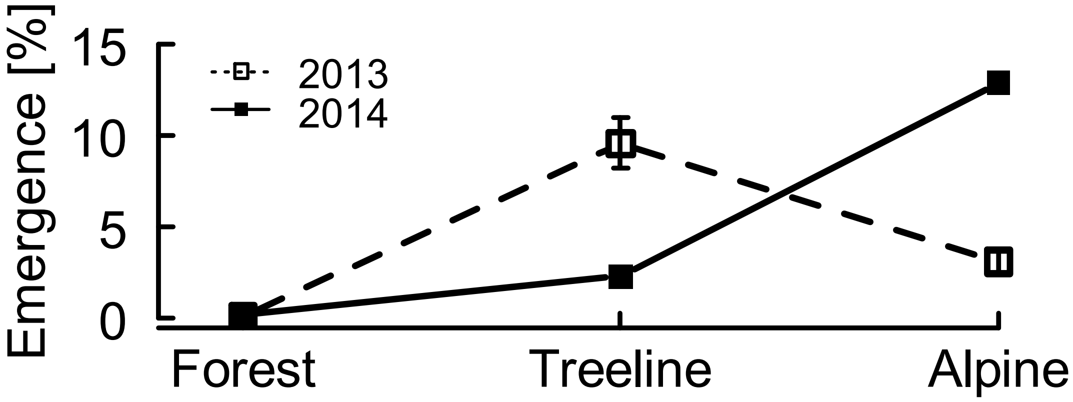
Table 4. Effects of seeding year, experimental site, species and provenance on seedling height.

Factor	χ^2	Df	Pr(> χ^2)	Significance ($\alpha = 0.05$)
year	0.00026151	1	0.98709767	
site	20.9631683	1	4.68E-06	***
species	1.69618866	1	0.19278717	
provenance	2.48948283	1	0.11460938	

Values and symbols are $\chi2$-values and significances, respectively, from likelihood ratio tests of mixed-effect models Significance levels: (*)P < 0.1; *P < 0.05; **P < 0.01; ***P < 0.001.

Figures

a)



b)

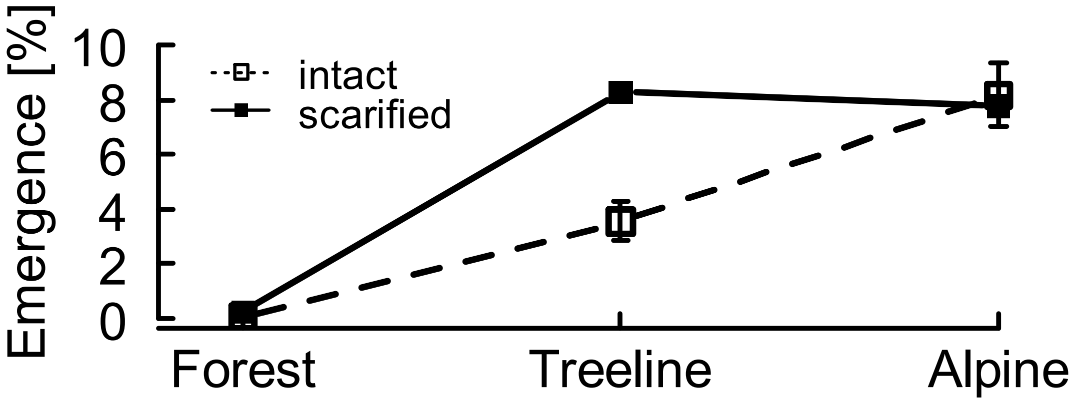


Fig. 1. Seedling emergence as percentage of viable seeds in dependence of experimental site for the two seeding years 2013 and 2014 (a) and for the two substrate treatments (b). Error bars indicate standard errors of trait means.

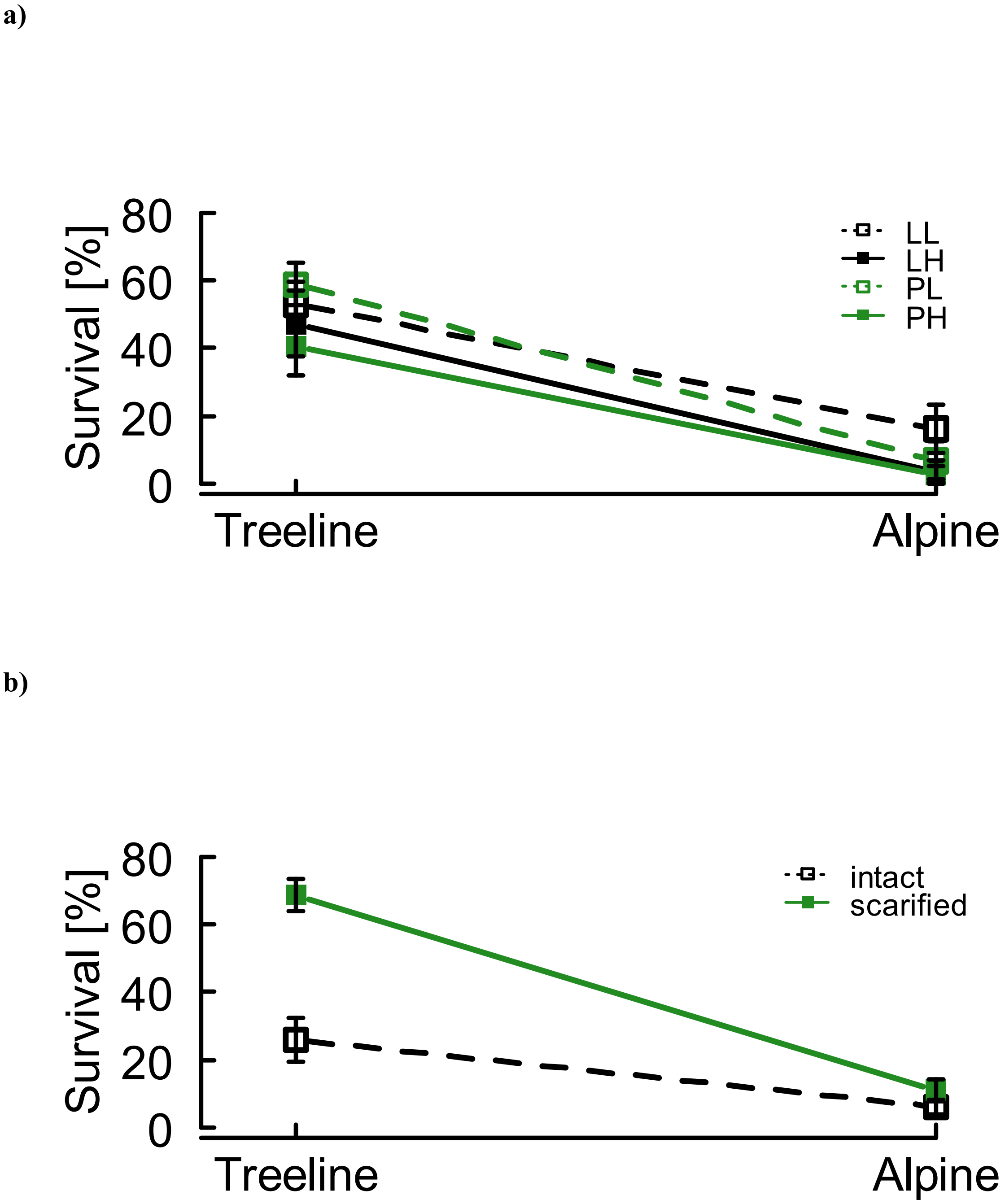


Fig. 2. Seedling survival rates after the first winter in dependence of experimental site for species and provenances (a) and for the two substrate treatments (b). LL: *L. decidua* low elevation provenance, LH: *L. decidua* high elevation provenance, PL: *P. abies* low

elevation provenance, PH: *P. abies* high elevation provenance. Error bars indicate standard errors of trait means.

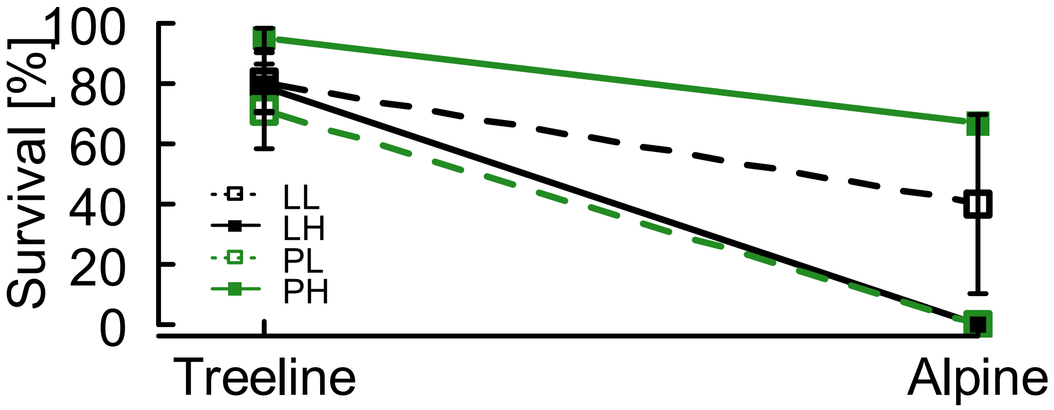


Fig. 3. Seedling survival rate after the second winter in dependence of experimental site for species and provenances. LL: *L. decidua* low elevation provenance, LH: *L. decidua* high elevation provenance, PL: *P. abies* low elevation provenance, PH: *P. abies* high elevation provenance. Error bars indicate standard errors of trait means.

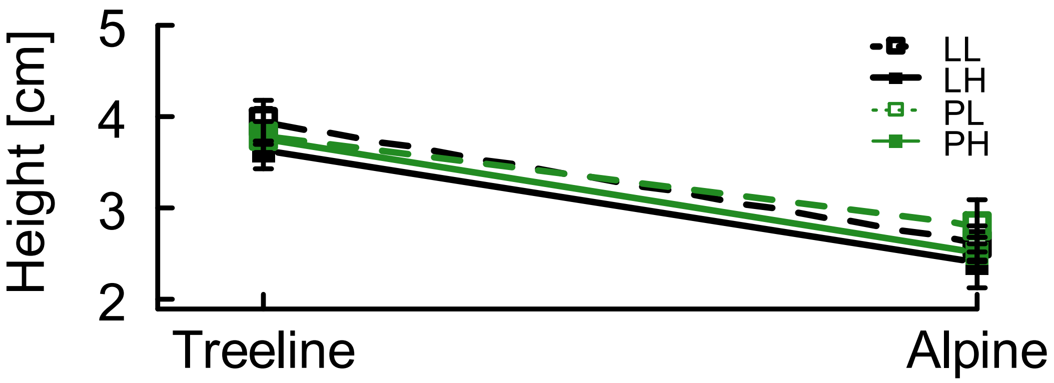


Fig. 4. Seedling height in dependence of experimental site for species and provenances. LL: *L. decidua* low elevation provenance, LH: *L. decidua* high elevation provenance, PL: *P. abies* low elevation provenance, PH: *P. abies* high elevation provenance. Error bars indicate standard errors of trait means.

Supporting information

Table S1. Location of the experimental sites.

Site	Longitude [Swiss Grid]	Latitude [Swiss Grid]	Elevation [m a.s.l.]
forest	2785500	1183500	1930
treeline	2785340	1183220	2100
alpine	2785090	1182570	2390

Table S2. Characteristics of seed provenances.

Species	Provenance	Elevation [m a.s.l.]	Location	Aspect [°]	Collection year	TGW [g]	Viability [%]
L. decidua	low	1350	Wiesen	225	1995	8.5	28
L. decidua	high	1760	Celerina	0	1970	7.3	11
P. abies	low	1000	Küblis	0	1985	6.8	74
P. abies	high	1960	Sertig	90	1983	6.8	61

TGW: thousand grain weight.

Table S3. Soil temperatures (monthly mean ±standard deviation) for the experimental sites from July 2013 to August 2015.

Year	Month	Soil temperature at forest site [°C]	Soil temperature at treeline site [°C]	Soil temperature at alpine site [°C]
2013	July	10.4±1.0	9.9±0.8	14.2±1.9
	August	9.7±1.4	9.2±0.9	11.2±2.2
	September	7.0±1.6	6.8±1.2	6.6±2.5
	October	4.8±1.9	4.8±1.3	3.2±2.2
	November	1.4±1.0	2.1±0.5	0.7±0.1
	December	0.4±0.1	0.8±0.2	0.4±0.1
2014	January	0.4±0.0	0.8±0.0	0.3±0.0
	February	0.4±0.0	0.8±0.0	0.3±0.0
	March	0.4±0.0	0.8±0.0	0.3±0.0
	April	0.5±0.2	0.7±0.0	0.3±0.0
	May	4.8±2.4	3.3±2.0	0.7±0.7
	June	8.7±1.5	8.6±1.4	9.2±3.7
	July	8.8±1.1	9.4±1.1	9.9±2.5
	August	8.6±1.3	9.0±1.3	9.4±1.8
	September	7.3±1.3	7.2±1.3	6.9±1.7
	October	5.2±2.4	4.9±1.6	3.8±2.1
	November	1.9±0.7	1.9±0.3	0.6±0.1
	December	0.3±0.6	0.6±0.4	0.4±0.0
2015	January	0.2±0.1	0.3±0.1	0.3±0.0
	February	0.2±0.0	0.4±0.0	0.2±0.0
	March	0.2±0.0	0.4±0.0	0.2±0.0
	April	0.3±0.0	0.4±0.0	0.3±0.0
	May	4.6±2.2	3.0±2.2	2.7±3.1
	June	9.1±1.5	9.5±1.3	11.1±3.0
	July	11.5±1.2	12.4±1.1	16.3±2.3
	August	10.8±1.4	11.2±1.4	13.3±2.7

Soil temperatures were measured with i-buttons in 5 cm soil depth.

Table S4. Number of seedlings that survived the first (a) and the second winter (b) grouped by species and provenance for site, substrate scarification and seeding year.

(a) Survival 1st winter

	total	Larix	Picea	Larix low	Larix high	Picea low	Picea high
Treeline site	348	164	184	148	16	147	37
Alpine site	60	51	9	50	1	5	4

	total	Larix	Picea	Larix low	Larix high	Picea low	Picea high
scarified	344	173	171	157	16	131	40
intact	64	42	22	41	1	21	1

	total	Larix	Picea	Larix low	Larix high	Picea low	Picea high
seeded 2013	302	132	170	121	11	135	35
seeded 2014	106	83	23	77	6	17	6

(b) Survival 2nd winter

	total	Larix	Picea	Larix low	Larix high	Picea low	Picea high
Treeline site	227	104	123	96	8	94	29
Alpine site	9	7	2	7	0	0	2

	total	Larix	Picea	Larix low	Larix high	Picea low	Picea high
scarified	229	104	125	96	8	94	31
intact	7	7	0	7	0	0	0

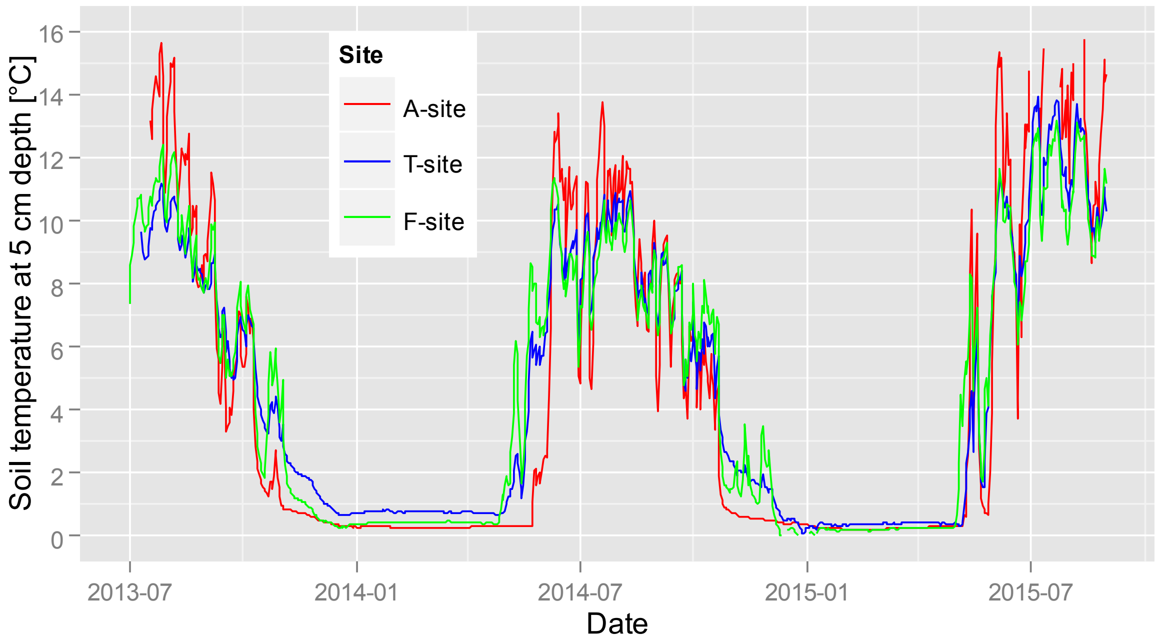


Fig. S1. Soil temperatures for the alpine (A), treeline (T) and forest site (F) from July 2013 to August 2015 measured by i-buttons in 5 cm soil depth.

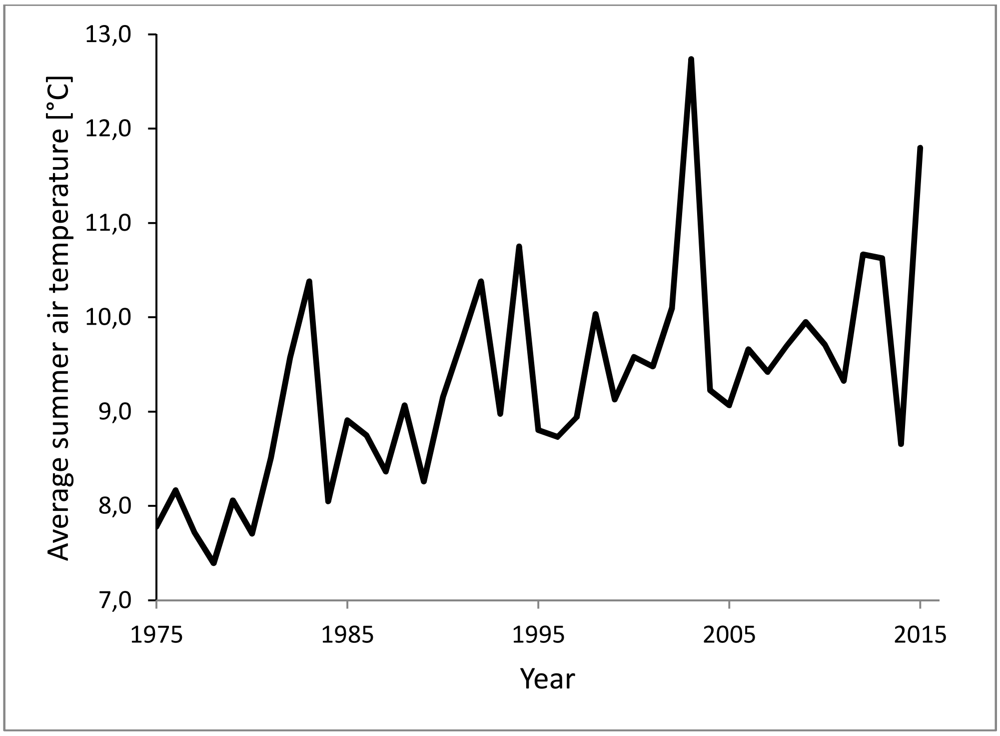


Fig. S2. Annual mean summer air temperatures from 1975 – 2015 from the Stillberg climate station in the research area at 2100m (longitude: 46.773573/ latitude: 9.86716). Mean summer air temperatures were calculated from monthly averages of the months June, July and August.