

Effects of a warmer climate on seed germination in the subarctic

Ann Milbau^{1,2,*}, Bente Jessen Graae², Anna Shevtsova¹ and Ivan Nijs¹

¹Research Group of Plant and Vegetation Ecology, Department of Biology, University of Antwerp, Universiteitsplein 1, B-2610 Wilrijk, Belgium and ²Climate Impacts Research Centre, Department of Ecology and Environmental Science, Umeå University, SE-98107 Abisko, Sweden

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- **Background and Aims** In a future warmer subarctic climate, the soil temperatures experienced by dispersed seeds are likely to increase during summer but may decrease during winter due to expected changes in snow depth, duration and quality. Because little is known about the dormancy-breaking and germination requirements of subarctic species, how warming may influence the timing and level of germination in these species was examined.
- **Methods** Under controlled conditions, how colder winter and warmer summer soil temperatures influenced germination was tested in 23 subarctic species. The cold stratification and warm incubation temperatures were derived from real soil temperature measurements in subarctic tundra and the temperatures were gradually changed over time to simulate different months of the year.
- **Key Results** Moderate summer warming (+2.5 °C) substantially accelerated germination in all but four species but did not affect germination percentages. Optimum germination temperatures (20/10°C) further decreased germination time and increased germination percentages in three species. Colder winter soil temperatures delayed the germination in ten species and decreased the germination percentage in four species, whereas the opposite was found in *Silene acaulis*. In most species, the combined effect of a reduced snow cover and summer warming resulted in earlier germination and thus a longer first growing season, which improves the chance of seedling survival. In particular the recruitment of (dwarf) shrubs (*Vaccinium myrtillus*, *V. vitis-idaea*, *Betula nana*), trees (*Alnus incana*, *Betula pubescens*) and grasses (*Calamagrostis lapponica*, *C. purpurea*) is likely to benefit from a warmer subarctic climate.
- **Conclusions** Seedling establishment is expected to improve in a future warmer subarctic climate, mainly by considerably earlier germination. The magnitudes of the responses are species-specific, which should be taken into account when modelling population growth and migration of subarctic species.

Key words: Climate change, cold stratification, dwarf shrubs, germination percentage, incubation temperature, mean germination time, seedling establishment, seeds, *Silene*, subarctic species, *Vaccinium*, warming.

INTRODUCTION

In Arctic and subarctic regions, which are expected to be affected more by anthropogenic climate change than other regions in the world (ACIA, 2004), the effects of climate warming on germination have hardly been studied (but see Wookey *et al.*, 1995; Molau and Shaver, 1997; Graae *et al.*, 2008). However, the timing and level of germination strongly affect a plant's recruitment success and may consequently have implications for species migration. Therefore, research on how a warmer climate affects germination is important for our ability to predict population dynamics and future distributions of subarctic species (Higgins *et al.*, 2003; Neilson *et al.*, 2005; Pearson, 2006; Thuiller *et al.*, 2008).

In northern ecosystems, climate warming is not only expected to result in significant increases in temperature, but also changes in the timing and reduction in the depth and duration of the snow cover are projected to occur (ACIA, 2004; Phoenix and Lee, 2004; Keller *et al.*, 2005; IPCC, 2007). The extent of snow cover over Arctic land areas has declined by about 10% over the past 30 years and model projections suggest that it will decrease an additional 10–20% before

the end of the century (ACIA, 2004). Snow is an excellent insulator (Sturm *et al.*, 2005); therefore a warmer climate is likely to result in colder soils and deeper soil frost during the winter when the soil is not insulated by snow (Groffman *et al.*, 2001; Venäläinen *et al.*, 2001; Stieglitz *et al.*, 2003; Öquist and Laudon, 2008). Moreover, important snow quality changes are projected; for instance, the development of hard packed snow due to changing wind patterns and ice layer formation, due to an increase in thawing and freezing in winter (ACIA, 2004), resulting in less insulation and thus colder soils (Körner, 2003). This may have important implications for recruitment from seed, because in subarctic environments, where there is hardly any current season (pre-winter) germination (Körner, 2003), winter temperatures possibly influence seed germination (Vleeshouwers *et al.*, 1995; Baskin and Baskin, 1998).

In cold climate species, experiencing winter (cold stratification) usually increases germination percentages (Probert, 2000; Körner, 2003; Giménez-Benavides *et al.*, 2005) and often contributes to reducing the temperature needed for later germination (Reynolds, 1984; Densmore, 1997; Shimono and Kudo, 2005). Cold stratification studies of Arctic and alpine species have, however, mainly focused on

* For correspondence. E-mail ann.milbau@emg.umu.se

the duration of the stratification period (e.g. Baskin *et al.*, 2000; Cavieres and Arroyo, 2000; Schütz, 2002) rather than on the stratification temperature. This is usually selected close to 5 °C because this is supposed to be optimal for dormancy-breaking in many species (Bewley and Black, 1994). Some recent studies have used more realistic stratification temperatures close to 0 °C for cold climate species (e.g. Baskin *et al.*, 2000; Schütz, 2002; Shimono and Kudo, 2005; Graae *et al.*, 2008), but this only reflects situations where a thick snow cover provides insulation. The effect of colder winter soil temperatures, reached in the absence of snow, has to our knowledge only been tested in two experiments with stratification under field conditions (Baskin *et al.*, 2002; Graae *et al.*, 2008). However, these included only two species, *Empetrum hermaphroditum* and *Vaccinium uliginosum*, and no effects of stratification temperature were observed.

The germination temperature in Arctic and alpine plants is relatively high in comparison with ambient temperatures (Cavieres and Arroyo, 2000; Körner, 2003), which has been thought to be an adaptation to avoid germination in early spring or autumn when the probability of frost is high (Billings and Mooney, 1968; Cavieres and Arroyo, 2000). Further, germination percentages are often positively correlated with the temperature under which seeds are incubated (Baskin and Baskin, 1998; Graae *et al.*, 2008). However, apart from these general trends, detailed knowledge on the germination requirements of (sub)arctic species is lacking (Baskin and Baskin, 1998). This is mainly because most studies use optimal incubation temperatures for testing germinability, often 20/10 °C or 25/15 °C (for a review, see Baskin and Baskin, 1998), independent of the species' habitat. The use of more realistic temperatures adapted to the environment of the species or the application of gradually changing temperature regimes simulating each month of the growing season is still scarce (but see Baskin *et al.*, 1995; Thompson and Naeem, 1996), although essential to apply germination data from laboratory studies in an ecological context. Moreover, the importance of the timing of germination has often been overlooked, despite its significance for plants from harsh environments where the growing season is highly limited (Schütz, 2002; Deines *et al.*, 2007).

To improve our knowledge of how climate warming may affect germination in subarctic ecosystems, an experiment was set up in which the effects of two realistic cold stratification treatments and three warm incubation treatments were tested. The temperature regimes were derived from real soil temperature measurements in subarctic tundra and were gradually changed over time to simulate different months of the year. As stratification temperatures, winter soil temperatures were used from snow-covered and exposed habitats, the latter to simulate a reduced snow cover in a future warmer climate. The incubation temperatures corresponded to (a) current spring and summer temperatures in subarctic tundra, (b) temperatures that are 2.5 °C higher than that, and (c) optimum germination temperatures (20/10 °C). To unravel general patterns, seeds from 20 subarctic species belonging to different functional types were used. Also three boreal tree species were added to test if their current expansion is limited by unsuccessful germination under present subarctic temperatures.

TABLE 1. Average seed mass and functional type of the species studied

Species	Seed mass (mg)	Functional type
<i>Alnus incana</i> (L.) Moench*	0.547	Tree
<i>Astragalus frigidus</i> (L.) A. Gray	2.920	Forb (legume)
<i>Betula nana</i> L.	0.223	Shrub
<i>Betula pubescens</i> Ehrh.*	0.164	Tree
<i>Betula pubescens</i> ssp. <i>czerepanovii</i> (Orlova) Hämet-Ahti	0.236	Tree
<i>Calamagrostis lapponica</i> (Wahlenb.) Hartm.	0.328	Grass
<i>Calamagrostis purpurea</i> (Trin.) Trin.	0.092	Grass
<i>Carex rostrata</i> Stokes†	1.319	Sedge
<i>Carex saxatilis</i> L.†	0.702	Sedge
<i>Deschampsia flexuosa</i> (L.) Trin.	0.498	Grass
<i>Dryas octopetala</i> L.	0.335	Dwarf shrub
<i>Empetrum hermaphroditum</i> Lange ex Hagerup†	1.230	Dwarf shrub
<i>Epilobium angustifolium</i> L.	0.065	Forb
<i>Festuca ovina</i> L.	0.245	Grass
<i>Pinus sylvestris</i> L.*	4.600	Tree
<i>Salix glauca</i> L.	0.156	Shrub
<i>Silene acaulis</i> (L.) Jacq.	0.402	Forb
<i>Silene dioica</i> (L.) Clairv.	0.831	Forb
<i>Solidago virgaurea</i> Praecox	0.327	Forb
<i>Vaccinium myrtillus</i> L.	0.214	Dwarf shrub
<i>Vaccinium uliginosum</i> L.	0.218	Dwarf shrub
<i>Vaccinium vitis-idaea</i> L.	0.202	Dwarf shrub
<i>Vicia cracca</i> L.	12.580	Forb (legume)

* Species with a boreal distribution

† Species excluded from further analyses because of very poor germination (<5%)

Specific research questions were: (a) How are germination percentage and germination time affected by colder winter soil temperatures, occurring under a reduced snow cover? (b) Does a higher germination temperature (+2.5 °C) affect germination of subarctic species, and if so, how? (c) Are the effects general or species specific? (d) Is the range expansion of boreal tree species currently limited by too-low germination temperatures in subarctic tundra?

MATERIALS AND METHODS

Study species

Seeds of 20 species were collected in the surroundings of the Abisko Scientific Research Station (68°21'N, 18°49'E) in subarctic northern Sweden (Table 1). The species were selected to belong to different functional types and were important components of the plant communities in the study area. Additionally, seeds of *Alnus incana* and *Betula pubescens*, tree species with a boreal distribution, were collected close to Umeå (63°50'N, 20°20'E) and seeds of *Pinus sylvestris* were ordered from a seed company and originated from Karesuando (68°20'N, 21°53'E). All seeds (except from *P. sylvestris*) were collected between 15 August and 15 September 2007 and stored dry at room temperature until sowing (6 November 2007). Seeds of fleshy fruits were extracted from the fruits immediately after collection.

TABLE 2A. Temperatures during the different weeks of the stratification treatments

Week of the experiment	Equivalent time of year	Stratification temperature (°C)*	
		Thin snow cover	Thick snow cover
1–4	October–November	0.5	0.5
5–8	December	–5	0.5
9–12	January–February	–10	0.5
13–16	March to 15 April	–5	0.5
17–20	15 April to 15 May	0.5	0.5

* The ‘thin snow cover’ temperatures are based on soil temperature measurements (3 cm depth) during the indicated time periods in wind-exposed poor heath vegetation in subarctic tundra near Abisko (northern Sweden) and the ‘thick snow cover’ temperatures on measurements in meadow vegetation at the same location.

The seed mass of each species, without any attachments, was determined by weighing four times 30 air-dry seeds (ten seeds for *Pinus sylvestris* and *Vicia cracca* because of their large seed size).

Cold stratification and warm incubation treatments

The seeds were subjected to two contrasting cold stratification treatments for 20 weeks. The stratification temperatures (Table 2A) were based on winter soil temperature measurements (3 cm depth) during 2006 and 2007 in subarctic tundra sites with either a thick insulating snow cover throughout winter (meadow vegetation) or a strongly reduced snow cover (wind exposed poor dwarf shrub heath). The measurements were done approx. 8 km from the Abisko Scientific Research Station at 850 m a.s.l. (B. J. Graae, unpubl. res.). Cold stratification occurred in complete darkness.

After stratification, the seeds were allowed to germinate under three different incubation treatments: optimum germination temperatures (20/10 °C) (Baskin and Baskin, 1998), current spring and summer soil temperatures in subarctic tundra (control), and temperatures that are 2.5 °C warmer than that (+2.5 °C; Table 2B). The temperature increase of 2.5 °C is in accordance with the projected temperature rise in Arctic regions (60–90°N) by the year 2050 relative to 1981–2000 (ACIA, 2004). The control incubation temperatures were derived from soil temperature measurements during the spring and summer of 2006 and 2007 in dwarf

TABLE 2B. Temperatures during the different weeks of the incubation treatments

Week of the experiment	Equivalent time of year	Incubation temperature (°C)		
		Control*	+2.5 °C	Optimum
21–22	15–31 May	4/0.5	6.5/3	20/10
23–26	June	10.5/2	13/4.5	20/10
27–33	July–August	12.5/4.5	15/7	20/10

* The ‘control’ temperatures are based on soil temperature measurements in dwarf shrub heath vegetation in subarctic tundra near Abisko. Temperatures were alternated every 12 h

shrubs heath, the dominant vegetation type at the earlier-mentioned location. To simulate the subarctic summer, seeds were kept in 24 h of daylight during incubation, but light intensities were alternated every 12 h coinciding with the temperature intervals. During ‘night’, light was provided with a photosynthetic photon flux density (PPFD) of 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (400–700 nm), whereas during daytime a PPFD of 45 or 110 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was provided every other day, to simulate overcast and clear days. In the ‘optimum’ treatment, the daytime PPFD was always 110 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

For each combination of stratification and incubation treatment, four replications of 30 seeds (ten seeds for *P. sylvestris* and *V. cracca*) were sown on moist commercial pot soil in 90-mm-diameter Petri dishes on 6 November 2007. After sowing, the dishes were wrapped with parafilm to reduce loss of water and they were subsequently put in incubators for the cold stratification treatments.

Germination recording

During incubation, the seeds were checked weekly for germination (protrusion of the radicle) and the germinated seeds were removed to reduce counting time in subsequent weeks. After 10 weeks, most species had completed germination but one final germination counting was done after 13 weeks of incubation. The final germination percentages were arcsine transformed to improve normality and stabilize variances. Species with <5% germination (*Carex rostrata*, *C. saxatilis* and *Empetrum hermaphroditum*) were excluded from further analyses (Table 1).

In addition to percentage germination, the mean germination time (MGT) for each of the species was determined in order to distinguish fast from slowly germinating species and to determine how germination speed was affected by the treatments. It was calculated as

$$\text{MGT} = \sum_{i=1}^i n_i t_i / N$$

where n_i is the number of seeds that germinated 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 germinated (Deines *et al.*, 2007).

RESULTS

Percentage germination

Significant differences in germination percentages were observed between the functional types (ANOVA, $F_{5,474} = 16.0$, $P < 0.001$), with the poorest germination in the grasses (20%) and the highest germination in the forbs (56%; Fig. 1A). Germination percentage slightly increased with seed mass (linear regression, $F_{1,478} = 30.83$, $P < 0.001$, $R^2 = 0.06$).

Germination percentages were significantly affected by the incubation and stratification treatments. In general (all species combined), the ‘thick snow cover’ stratification

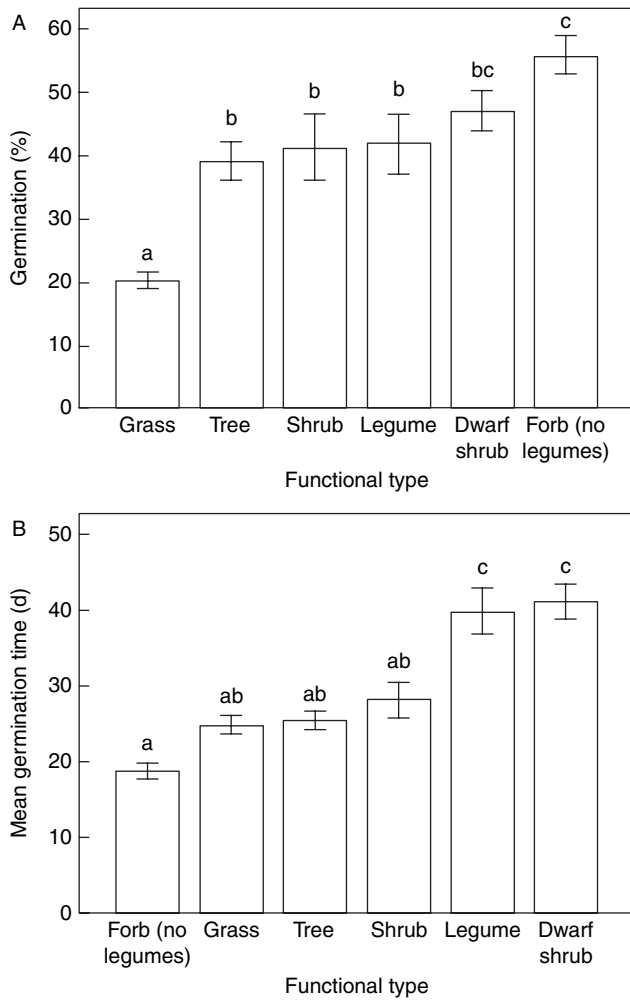


FIG. 1. (A) Germination percentages and (B) mean germination times for the different functional types. Data are averages over all treatments ($n = 24$). Bars denote \pm s.e. Different letters indicate significant differences between functional types at the $P < 0.05$ level (Tukey multiple comparison test).

treatment resulted in more germination (43 %) than the ‘thin snow cover’ treatment (39 %; Fig. 2A and Table 3). In addition, germination percentages were higher in the ‘optimum’ (45 %) incubation treatment than in the ‘+2.5 °C’ (39 %) and ‘control’ (39 %) treatments (no difference between +2.5 °C and control; Fig. 2A). The lack of a significant interaction effect between incubation and stratification (Table 3) suggests that the effects of both are additive, which is supported by the highest germination percentage when ‘thick snow cover’ stratification was followed by ‘optimum’ incubation (Fig. 2A).

Because of strong species \times incubation and species \times stratification interaction effects (Table 3) species-specific analyses were also required. These demonstrated that stratification treatment significantly affected germination percentage in five out of the 20 species, either forbs or dwarf shrubs (Fig. 3). In *E. angustifolium*, *Silene dioica*, *Vaccinium myrtillus* and *V. uliginosum* germination percentages were higher in the ‘thick snow cover’ than the ‘thin snow cover’ treatment. *Silene acaulis*, on the other hand, performed better after

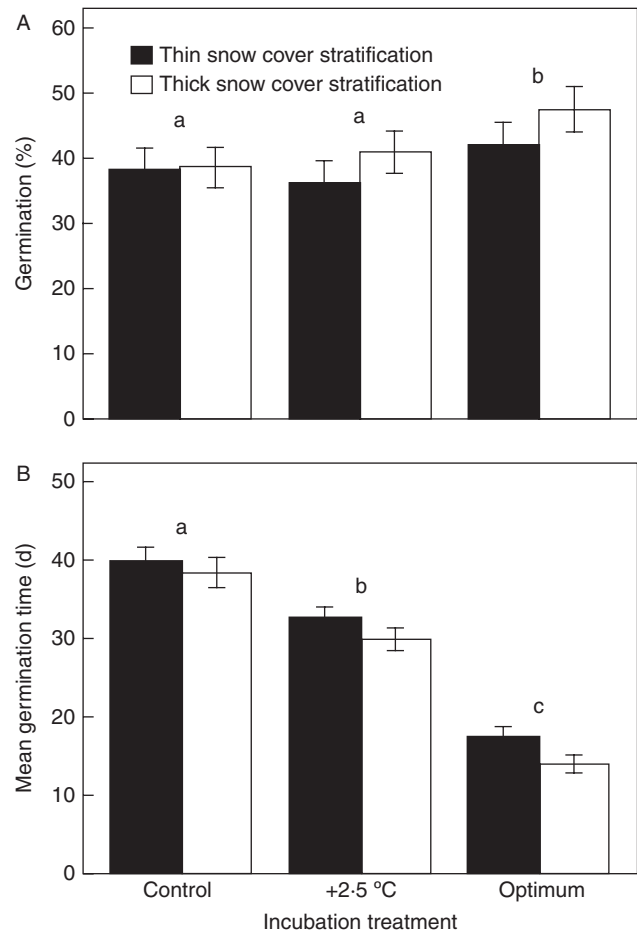


FIG. 2. (A) Germination percentages and (B) mean germination times for the different cold stratification and incubation treatments. See Table 2 for the incubation and stratification temperatures. Data are averages over all species ($n = 80$). Bars denote \pm s.e. Different letters indicate significant differences between incubation treatments at the $P < 0.05$ level (Tukey multiple comparison test).

‘thin snow cover’ stratification (see Table 4 for mean differences in germination percentages).

Significant effects of incubation treatment on germination percentage were found in *S. acaulis* (+15 %), *V. myrtillus* (+67 %), *V. uliginosum* (–15 %) and *V. vitis-idaea* (+14 %) when the ‘optimum’ incubation treatment was compared with the ‘control’ incubation treatment (Fig. 3). Contrary to the other species, germination percentage decreased with increasing incubation temperature in *V. uliginosum*, but only in the ‘thin snow cover’ stratification treatment (significant incubation \times stratification interaction; Fig. 3). In none of the species did a temperature increase of 2.5 °C (‘+2.5 °C’ versus ‘control’ incubation treatment) result in higher germination percentages (Fig. 3 and Table 4).

Mean germination time

The speed of germination differed significantly between the functional types (ANOVA, $F_{5,447} = 29.0$, $P < 0.001$). The dwarf shrubs (41 d) and the legumes (40 d) germinated significantly slower than the other functional types, and the forbs

TABLE 3. Results of two three-way ANOVAs on the effects of incubation treatment, stratification treatment and species identity on germination percentage and on mean germination time

Factor	Germination percentage			Mean germination time		
	d.f.	F-value	P-value	d.f.	F-value	P-value
Incubation (I)	2, 360	12.80	< 0.001	2, 333	407.67	< 0.001
Stratification (S)	1, 360	9.56	0.002	1, 333	11.93	0.001
Species	19, 360	95.19	< 0.001	19, 333	60.96	< 0.001
I × S	2, 360	2.15	0.118	2, 333	0.43	0.653
I × species	38, 360	4.30	< 0.001	38, 333	3.42	< 0.001
S × species	19, 360	4.43	< 0.001	19, 333	1.45	0.103
I × S × species	38, 360	1.18	0.218	38, 333	0.50	0.994

Significant effects ($P < 0.05$) are in bold.

(19 d) were the faster germinating group (Fig. 1B). Large-seeded species germinated a little slower than species with smaller seeds (linear regression, $F_{1,451} = 4.53$, $P = 0.034$, $R^2 = 0.01$).

MGT was significantly affected by the incubation and stratification treatments (Table 3). In general (all species combined), the germination time was shorter after ‘thick snow cover’ (27 d) than after ‘thin snow cover’ (30 d) stratification (Fig. 2B). Further, germination was fastest in the ‘optimum’ incubation treatment (16 d), followed by the ‘+ 2.5 °C’ (31 d) and the ‘control’ (39 d) incubation treatments (Fig. 2B). A lack of interaction between incubation and stratification (Table 3) resulted in the shortest MGT when ‘thick snow cover’ stratification was followed by ‘optimum’ incubation (14 d) and the longest MGT when ‘thin snow cover’ stratification was followed by ‘control’ incubation (40 d).

Analyses on individual species level revealed significant stratification effects on MGT in eleven species, belonging to all functional types except the grasses (Fig. 4). Whereas ‘thin snow cover’ stratification usually delayed germination, the opposite was observed in *S. acaulis*. The strongest effects occurred in *S. dioica* and *V. cracca*, in which germination was on average delayed by 10 d and 12 d, respectively, after ‘thin snow cover’ stratification (Table 4).

In all species except the legumes (*Astragalus frigidus* and *V. cracca*), incubation temperature significantly affected MGT (Fig. 4). Germination was slowest in the ‘control’ treatment and was faster in the ‘+ 2.5 °C’ and the ‘optimum’ incubation treatments, but in *Deschampsia flexuosa* and *Salix glauca* no difference was seen between the ‘control’ and the ‘+ 2.5 °C’ treatment. In *D. octopetala*, *E. angustifolium* and *V. uliginosum*, the strength of the stratification effect depended on the incubation treatment (significant incubation × stratification interaction), whereas for the other species the effects of incubation and stratification were additive (Fig. 4). In ten species, germination occurred at least 1 week earlier in the ‘+ 2.5 °C’ than in the ‘control’ treatment; in *V. myrtillus* and *V. vitis-idaea* MGT was even reduced by 2 weeks (Table 4).

Germination in a future climate

In the majority of species, the positive effect of warming (+2.5 °C) on germination time was diminished if the seeds were first stratified in the ‘thin snow cover’ treatment

(Table 4). Eight species, though, still showed a reduction in MGT by at least 1 week. In *E. angustifolium*, *V. myrtillus* and *V. uliginosum*, the advantage of faster germination was counterbalanced by reduced germination percentages after ‘thin snow cover’ stratification. *Silene acaulis*, on the other hand, showed both an increased germination percentage and faster germination in the scenario of warming combined with a reduced snow cover. *Silene dioica* and *V. cracca* suffered most from the future scenario: *V. cracca* with a strongly delayed germination (+12 d) and *S. dioica* with delayed germination (+3 d) combined with a reduced germination percentage (−15 %).

Germination pattern across time

To examine how germination was influenced by the different temperature steps in each of the incubation treatments, the accumulated germination percentages (averages of the two stratification treatments) were plotted over time for two species (Fig. 5). In *B. nana*, germination started during the first week of incubation and almost immediately reached its maximum percentage in the ‘optimum’ incubation treatment (Fig. 5A). In the ‘control’ and ‘+ 2.5 °C’ treatments, germination started under June temperatures and reached maximum values under summer temperatures. Despite the strong delays in germination under colder incubation temperatures, final germination percentages were similar in all treatments. This pattern was observed in the majority of species (not shown). A different pattern was observed in *V. myrtillus* (Fig. 5B) and *V. vitis-idaea* (not shown), in which germination occurred only after summer temperatures had been reached in the ‘control’ and ‘+ 2.5 °C’ treatments. In the ‘optimum’ treatment, germination started under June temperatures and reached a higher final germination percentage than in the colder treatments, but percentages in the ‘+ 2.5 °C’ and ‘control’ treatments were similar, after an initial delay. Figure 5 clearly shows that germination is strongly temperature regulated, with no germination until a certain temperature threshold has been reached.

DISCUSSION

The results demonstrate strong effects of stratification and incubation temperatures on the timing and level of germination

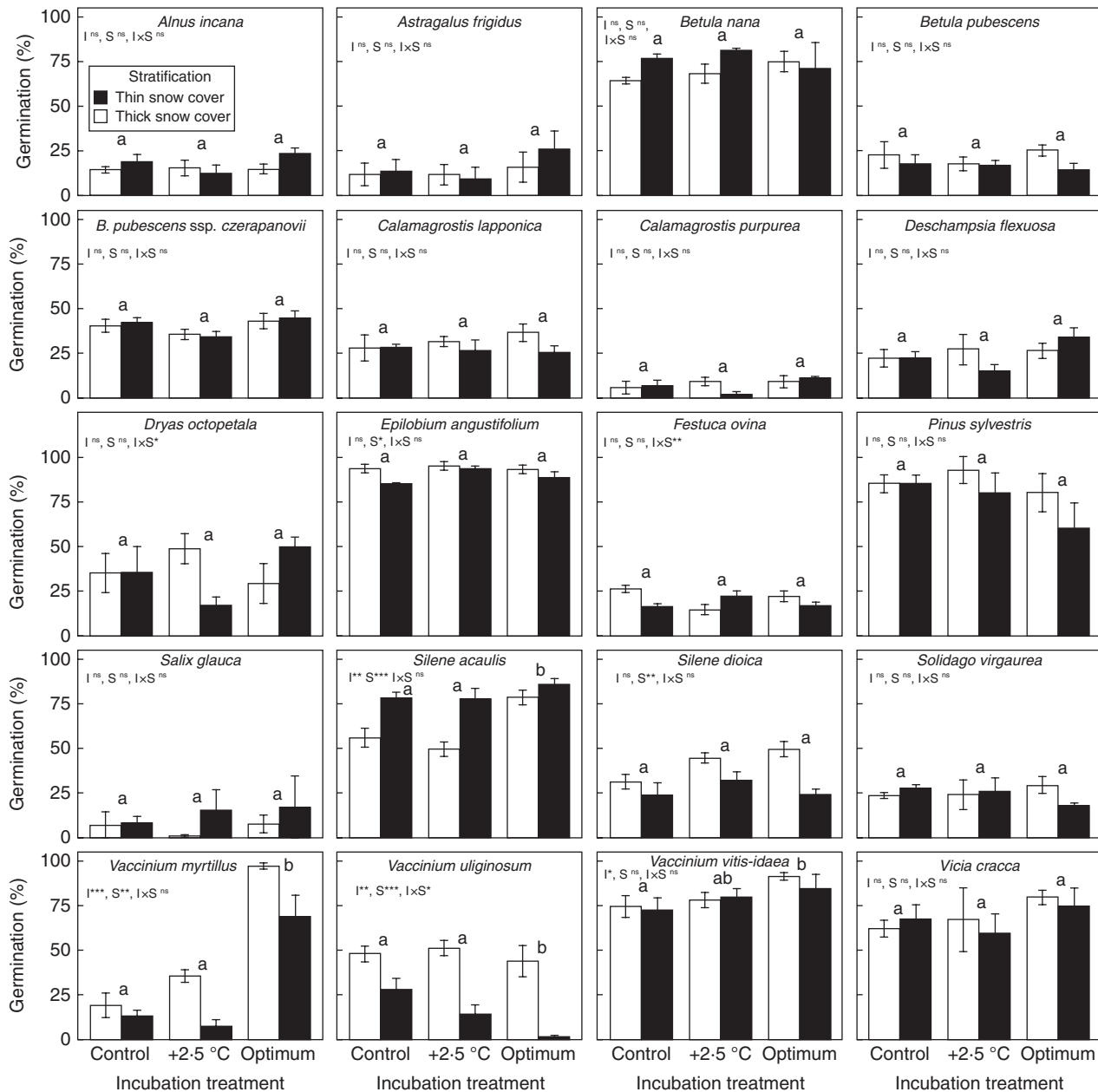


FIG. 3. Germination percentages (means \pm s.e., $n = 4$) of the individual species in the different incubation and cold stratification treatments. See Table 2 for the incubation and stratification temperatures. Significant levels of incubation treatment (I), stratification treatment (S) and I \times S interaction by two-way ANOVA are shown as follows: ^{ns}, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Different letters indicate significant differences between the incubation treatments at the $P < 0.05$ level (Tukey multiple comparison test).

in a large set of subarctic plant species. Although the experiment carried out did not include all abiotic and biotic changes that go along with a warmer climate in a natural environment, we believe it is justified to focus on temperature because this factor has several times been proven to be the most important environmental variable regulating the dormancy state and germination of seeds (e.g. Roberts, 1988; Vleeshouwers *et al.*; 1995; Probert, 2000).

In earlier studies on the influence of snow cover and freezing temperatures on subsequent germination, no significant effects were found. In a study by Graae *et al.* (2008), there was no difference in germination percentage between seeds

of *E. hermaphroditum* and *V. uliginosum* stratified at 0.5 °C in incubators and seeds experiencing colder winter temperatures outdoors at boreal (−0.5 to −2 °C) and Arctic (mean winter temperature of −6.9 °C) sites. Similarly, Baskin *et al.* (2002) could not detect any difference between full- and half-snow-cover treatments on the germination of *E. hermaphroditum*. However, it was found that colder winter soil temperatures delayed the germination in ten species and decreased the germination percentage in four species, whereas the opposite was found in *S. acaulis*. The results are thus the first to demonstrate that the projected reductions in snow depth and duration in (sub)arctic and

TABLE 4. Effects of summer warming (+2.5 °C), a reduced snow cover and the combination of both on germination (Germ) percentage and mean germination time (MGT) for each species

Species	Warming (+2.5 °C)		Reduced snow cover		Warming + reduced snow cover	
	Germ (%)	MGT (d)	Germ (%)	MGT (d)	Germ (%)	MGT (d)
<i>Alnus incana</i>	n.s.	-10	n.s.	n.s.	n.s.	-10
<i>Astragalus frigidus</i>	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
<i>Betula nana</i>	n.s.	-9	n.s.	+2	n.s.	-7
<i>Betula pubescens</i>	n.s.	-9	n.s.	n.s.	n.s.	-9
<i>Betula pubescens</i> ssp. <i>czerepanovii</i>	n.s.	-6	n.s.	+3	n.s.	-3
<i>Calamagrostis lapponica</i>	n.s.	-7	n.s.	n.s.	n.s.	-7
<i>Calamagrostis purpurea</i>	n.s.	-7	n.s.	n.s.	n.s.	-7
<i>Deschampsia flexuosa</i>	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
<i>Dryas octopetala</i>	n.s.	-8	n.s.	+3	n.s.	-5
<i>Epilobium angustifolium</i>	n.s.	-5	-5	+2	-5	-3
<i>Festuca ovina</i>	n.s.	-6	n.s.	n.s.	n.s.	-6
<i>Pinus sylvestris</i>	n.s.	-6	n.s.	+6	n.s.	n.s.
<i>Salix glauca</i>	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
<i>Silene acaulis</i>	n.s.	-6	+19	-2	+19	-8
<i>Silene dioica</i>	n.s.	-7	-15	+10	-15	+3
<i>Solidago virgaurea</i>	n.s.	-5	n.s.	+3	n.s.	-2
<i>Vaccinium myrtillus</i>	n.s.	-18	-21	+5	-21	-13
<i>Vaccinium uliginosum</i>	n.s.	-9	-33	+3	-33	-6
<i>Vaccinium vitis-idaea</i>	n.s.	-14	n.s.	n.s.	n.s.	-14
<i>Vicia cracca</i>	n.s.	n.s.	n.s.	+12	n.s.	+12

For warming, the estimated marginal means of the '+2.5 °C' incubation treatment were compared with the 'control' treatment; a negative value for MGT thus indicates faster germination in case of warming. For reduced snow cover, the marginal means of the 'thin snow cover' stratification treatment were compared with the 'thick snow cover' treatment. 'Warming + reduced snow cover' gives the sum of both treatments.

alpine regions (ACIA, 2004; Keller *et al.*, 2005) may have important implications for seed germination, both by affecting the number of germinating seeds and the timing of germination.

Regarding summer warming, it was observed that even relatively small increases in temperature (+2.5 °C) strongly reduced the germination time in all but four species. The lack of a response in the legumes is possibly due to their physiological dormancy, which is less temperature dependent than physiological dormancy (Baskin and Baskin, 1998; Probert, 2000). Contrary to expectations (Baskin and Baskin 1998; Graae *et al.*, 2008), a positive relationship between incubation temperature and germination percentage was not commonly observed. This occurred only in three species when the optimum treatment was compared with the control treatment; otherwise final germination percentages were similar. Lower incubation temperatures thus mainly resulted in delayed germination, rather than in lower percentages. Because germination studies are usually finished after 4 weeks (for a review, see Baskin and Baskin, 1998), we think that the frequently reported lower germination percentages at lower incubation temperatures may often be an artefact of the experimental procedure. Indeed, if the present experiment had finished after 4 weeks, a similar report on the different germination percentages between the incubation treatments would have been made (see Fig. 5A). Therefore, we stress the importance of continuing germination trials until germination percentages stop increasing.

Most species were able to germinate under June temperatures in the control treatment, suggesting that a temperature of 10.5/2 °C (during several weeks) is generally high enough for germination in subarctic species, although germination

occurred much faster at slightly higher temperatures. As suggested by Körner (2003), a great functional variability in the germination behaviour of the species was found. For instance, the high-altitude species *S. acaulis* and *D. octopetala* started germinating during incubation at 6.5/3 °C, whereas *V. myrtillus* and *V. vitis-idaea* needed 12.5/4.5 °C for several weeks before the onset of germination, suggesting that species from higher elevations may be better adapted to germinate under low-temperature conditions. This contradicts the idea that seeds from higher elevations need higher germination temperatures than those from lower altitudes to avoid germination in early spring when the probability of frost is still high (Billings and Mooney, 1968; Cavieres and Arroyo, 2000). In addition, *S. acaulis* was the only species that benefited from the 'thin snow cover' stratification treatment, both with a higher germination percentage and a shorter germination time. Apparently it is especially well adapted to perform well in the harsh wind-swept habitats where it mostly grows. The protective nature of the dense cushions in which it occurs (Körner, 2003) may enable its seedlings to overcome frost and drought without much damage. The other high-altitude species, *D. octopetala*, did not show the same adaptation, which may explain why this species reproduces predominantly by means of clonal growth (Wookey *et al.*, 1995).

In the majority of species, the combined effect of a reduced snow cover and summer warming resulted in faster germination, but four species were not affected and two species showed slower germination. The effect on germination percentage was positive in one species, negative in four species and not significant in all others. In subarctic ecosystems, the short growing season (approx. 3 months in the tundra near Abisko;

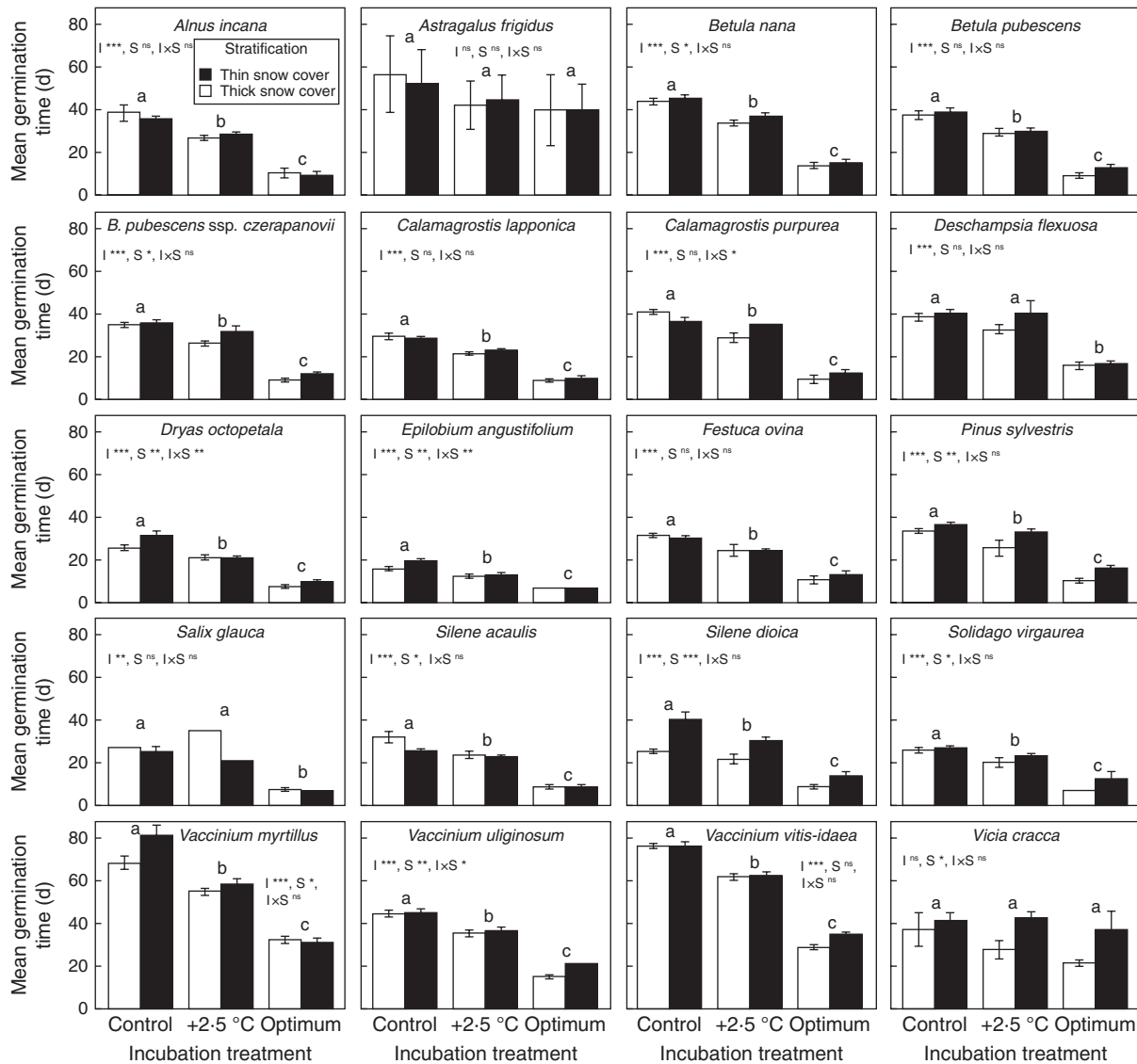


FIG. 4. Mean germination times (MGT) of the individual species in the different incubation and cold stratification treatments (means \pm s.e., $n = 4$). See Table 2 for the incubation and stratification temperatures. Significant levels of incubation treatment (I), stratification treatment (S) and I \times S interaction by two-way ANOVA are shown as follows: ^{ns}, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Different letters indicate significant differences between the incubation treatments at the $P < 0.05$ level (Tukey multiple comparison test).

Molau *et al.*, 2005) is a major barrier for the survival of seedlings because it constrains the period during which seedlings need to attain a critical biomass and acquire resistance to freezing to withstand the harsh and long-lasting winter conditions (Maruta, 1994; Stocklin and Baumler, 1996; Schütz, 2002). Seedling mortalities in cold climates are usually high and 12-month losses often exceed 50% in large-seeded and 99% in small-seeded species (Jolls and Bock, 1983; Körner, 2003). This implies that even relatively small reductions in germination time may have substantial effects for recruitment from seed by improving the chance of seedlings to survive the following winter (Chambers, 1995). Consequently, the present results suggest that a warmer subarctic climate is likely to be beneficial for seedling establishment in most species, even though colder soil temperatures during winter may diminish the positive effect.

The species that may benefit most are trees (*A. incana* and *B. pubescens*), (dwarf) shrubs (*V. myrtillus*, *V. vitis-idaea* and *B. nana*) and grasses (*C. lapponica* and *C. purpurea*), in addition to the forb *S. acaulis*. These species showed considerably earlier germination (>1 week), sometimes combined with an increased germination percentage (especially in *S. acaulis*) when stratified and allowed to germinate under future conditions. *Vaccinium myrtillus*, however, may only improve its recruitment in more protected, snow-rich places (strong negative effect of colder winter temperatures), but its germination percentage is expected to increase substantially if summer temperatures increase >2.5 °C. It has already been documented that dwarf shrubs, grasses and trees will expand in Arctic and subarctic areas (Sturm *et al.*, 2001; Kullman, 2002; Dullinger *et al.*, 2004; Tape *et al.*, 2006) and that they are predicted to perform better in terms of

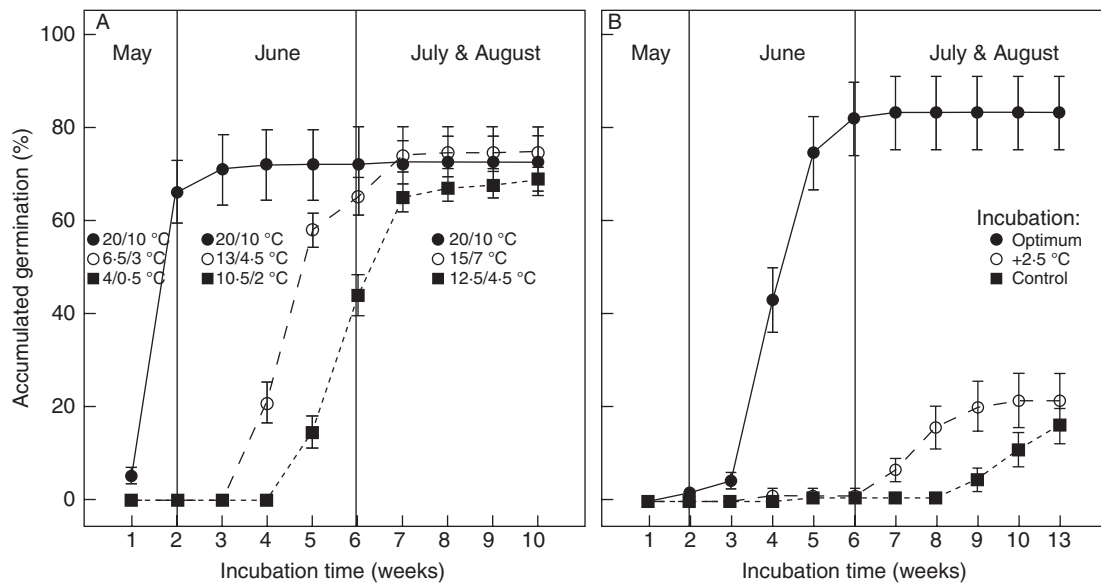


FIG. 5. Accumulated germination percentages (means \pm s.e., $n = 8$) of (A) *Betula nana* and (B) *Vaccinium myrtillus* after different weeks of incubation in the different incubation treatments. Months indicate during which time of year the selected temperatures occur in the Abisko region.

productivity in a warmer climate (Parsons *et al.*, 1995; Arft *et al.*, 1999; van Wijk *et al.*, 2004; Walker *et al.*, 2006). Thus, the functional types that are expected to benefit most from warming in the adult stage may also benefit most in the recruitment phase, possibly resulting in a positive feedback.

The species in which germination was most negatively affected by a warmer climate were three forbs that currently occur in subarctic birch forest (*E. angustifolium*, *S. dioica* and *V. cracca*) and the dwarf shrub *V. uliginosum*. Seeds of these species apparently suffer greatly from below zero temperatures during winter, resulting in less and slower germination. However, they performed well after stratification under mild winter temperatures, so, similarly to *V. myrtillus*, their expansion is expected to be restricted to snow-rich places, for instance close to shrubs which trap and hold snow during winter (Sturm *et al.*, 2005). We can, however, not explain why the combination of ‘thin snow cover’ stratification and ‘optimum’ incubation was extremely unfavourable in *V. uliginosum*.

The observed species-specific responses may have consequences for the patterns of species migration due to warming. In some species, seedling recruitment will improve more than in others in a warmer climate; therefore not all species will be able to shift northward or upslope at the same pace. This may result in plant communities without previous analogues (Davis, 1989; Kullman, 2002) or in shifts in dominance within existing communities (Kelly and Goulden, 2008). In any case, the importance of species-specific reactions, also during the recruitment stage, should be taken into account in models that forecast population dynamics and future species migrations.

Seed germination does not appear to limit northward range expansion in the three boreal tree species of the present experiment. *Alnus incana*, *B. pubescens* and *P. sylvestris* all germinated equally well (similar germination percentages) in the colder stratification and incubation treatments as in the

warmer ones. Moreover, they did not germinate significantly slower under current subarctic conditions than the dominant tree species in the Abisko region, *B. pubescens* ssp. *czerapanovii*. The current distribution of these species is thus most likely limited by other processes than seedling recruitment.

To conclude, it was found that colder winter soil temperatures are likely to result in lower germination percentages and slower germination in about half of the subarctic species. Warmer summers, on the other hand, reduce the germination time and may sometimes increase the number of germinating seeds. The combined effect of both is beneficial in most species and therefore seedling establishment was expected to improve in a future warmer subarctic climate.

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