

# Snow cover manipulations and passive warming affect post-winter seed germination: a case study of three cold-temperate tree species

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**ABSTRACT:** Climate change is leading to increased temperatures globally, which may be especially pronounced in cold-temperate regions. During winter, this may cause changes to thermal insulation provided by snow cover to the ground and lead to altered soil and litter layer temperature regimes, affecting plant regeneration and species' ranges through frost damage. I investigated the effects of changing snow cover and litter temperature regimes on post-winter seed germination of 3 cold-temperate tree species, using snow manipulation and passive warming approaches. Snow manipulation and passive warming led to modest but complex changes in litter layer temperature regimes and caused responses in post-winter seed germination, increasing or remaining constant depending on species and treatment. Despite the modest differences in snow cover and litter temperature among treatments, post-winter seed germination varied up to 3-fold. The results suggest that tree seeds may be susceptible to modest changes in winter conditions as expected in the intermediate term under climate change and may be affecting future forest regeneration and species composition. The mechanisms underlying the observed seed germination response are currently unknown, but possible hypotheses are presented. If confirmed, these mechanisms may be involved in the re-assembly of future species–habitat relationships and control of species' biogeographic ranges.

**KEY WORDS:** Climate change · Eastern white cedar · Forest composition · Green ash · Regeneration · Sugar maple

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## 1. INTRODUCTION

In most cold-temperate regions of the world, the ground is largely snow covered for the majority of the winter period. This snow cover provides important thermal insulation, protecting ground vegetation and litter from very low ambient air temperatures and potentially from freeze–thaw events (Decker et al. 2003). Climate change effects are expected to cause a global rise in air temperatures, which might be especially pronounced during winter and in colder, higher-latitudinal regions, leading to a reduction in extent and duration of the snow cover period (Meehl et al. 2007). Unfortunately, climate change effects in

cold-temperate regions have been less intensely researched (Kreyling 2010) than effects in sub-alpine (Bjork & Molau 2007) and sub-arctic regions (Wahren et al. 2005). The scarcity of studies investigating climate change effects on the winter ecology of cold-temperate regions contributes to our uncertainty about future environmental conditions in these regions (Campbell et al. 2005).

The duration and depth of snow cover in a region depend on temperature and precipitation regimes (i.e. average values as well as temporal variability) during the cold season. However, temperature and precipitation differ in their temporal and spatial variation across scales. Global and regional climate models

largely agree in their predictions for increased winter temperatures in northern latitudes (Christensen et al. 2007, Colombo et al. 2007). However, predictions of precipitation patterns vary between regions and are less certain, with some models predicting increased winter precipitation while other models predict decreases (Christensen et al. 2007, Colombo et al. 2007). It is likely that many high-latitude regions will experience an increase in winter snow accumulation, while other regions might experience no change or a decrease (Brown & Mote 2009). Despite predicted increases in global temperatures, high-latitude winter air temperatures will continue to reach very low values during regional cold spells (Shabbar & Bonsal 2003). If decreased duration and depth of snow cover coincide with such low winter temperatures, the counter-intuitive effect of global warming can be decreasing winter soil temperatures (Brown & DeGaeetano 2011). It is also expected that changes to winter snow and temperature regimes will lead to altered frequencies of freeze–thaw cycles (Henry 2008).

Early life stages of trees, including seeds, are often protected from very low winter air temperatures and freeze–thaw cycles by a thick snow pack, but when snow cover is shallow, these early life stages may experience very low temperatures or freeze–thaw cycles. Adaptations that allow survival at low temperatures include the production of freezing-tolerant membrane constituents (Thomashow 1999). These adaptations, however, require resource investments that may lead to a fitness cost of the acquired cold tolerance (Agrawal et al. 2004). Because of this trade-off, it is likely that investments in cold tolerance are limited and fine-tuned to normal winter temperature regimes. Further, because the current rate of climatic change is fast compared to the typical generation length of trees, selection processes may be too slow for many tree species to be able to adapt to changing temperatures. Therefore, if temperature regimes move outside their normal range, injury or mortality may occur, as demonstrated by Hawkins et al. (2003) in a study on the frost tolerance of conifer seeds.

In a recent snow manipulation study, Drescher & Thomas (2013) found that post-winter germination of seeds of cold-temperate tree species was strongly affected by manipulated snow regimes. However, snow regimes and temperature regimes in that study differed strongly between treatments (average and minimum temperatures differed by 5 and 21°C, respectively). These temperature differences might be more severe than the variation expected due to climate change. Therefore, the question arises whether more modest differences in snow and temperature

regimes, as they are expected under climate change, might also affect post-winter seed germination. Climate model projections for the study region suggest average winter temperature increases of 2 to 4°C by the end of the century (Christensen et al. 2007, Colombo et al. 2007). If it were found that even these smaller temperature differences affect post-winter seed germination, then this would be further evidence suggesting consequences of climate warming for forest regeneration dynamics. Moreover, if seed germination responses are species-specific, then the abundance of certain tree species and thus species composition of future forests may also be affected.

Here I employed a snow manipulation approach in combination with passive warming through open-top chambers (OTCs), to investigate the effects of snow and temperature regimes on post-winter seed germination of 3 tree species native to the study region. Predictions for this region point toward winter temperature increase, but are inconsistent for future snowfall (Christensen et al. 2007, Colombo et al. 2007). Therefore, all snow manipulation treatments (reduced, natural and added snow) included passive warming. The objectives of this study were to determine (1) whether passive warming leads to changed freeze–thaw cycles of the litter layer relative to control conditions, (2) whether manipulated snow leads to changed temperature and frequency of freeze–thaw cycles of the litter layer relative to natural snow, (3) whether modest snow and temperature regime changes affect the post-winter germination of tree seeds and (4) whether this post-winter seed germination response is species-specific. I expected that reduced snow cover would lead to increased exposure of the litter layer to low winter temperatures and to increased frequency of freeze–thaw cycles, which would decrease post-winter seed germination, whereas added snow cover would have opposite effects. Further I expected that passive warming would lead to a rise in average litter layer temperatures, leading to increased freeze–thaw cycles and decreased post-winter seed germination.

## 2. MATERIALS AND METHODS

### 2.1. Snow and temperature regime experiment

I studied the effects of overwintering conditions (i.e. snow cover and temperature) on post-winter seed germination of 3 tree species common to the Great-Lakes-St.-Lawrence forest region of north-eastern North America. I chose 2 deciduous angio-

sperms and 1 conifer. The deciduous species were green ash *Fraxinus pennsylvanica* Marsh. and sugar maple *Acer saccharum* Marsh., and the conifer was eastern white cedar *Thuja occidentalis* L. Seeds for all species were collected from apparently naturally occurring trees within a maximum distance of 50 km surrounding the experimental site. The altitude of the collection sites ranged from 336 to 353 m, and they were located more than 500 km (sugar maple), 900 km (eastern white cedar) and 1000 km (green ash) south of the northern species limit. Climate normals for a regional weather station (Environment Canada 2014) indicate that the tree populations should be adapted to cold winters (Table 1).

The site of the experiment was a maple-beech forest in the Environmental Reserve of the University of Waterloo, Ontario, Canada (43° 28' N, 80° 34' W, altitude: 341 m). The region surrounding the site is characterized by a humid continental climate with very warm summers and cold winters (Table 1). The experiment was carried out in the winter of 2011–2012 using 4 treatments that differed in the type of snow cover manipulation and the presence of OTCs: (1) natural snow without OTC (control), (2) natural snow with OTC (OTC-n), (3) reduced snow with OTC (OTC-r) and (4) added snow with OTC (OTC-a).

The guiding principle of the experimental design was to simulate expected conditions under climatic change. Passive warming was added to all snow treatments except the control. The control treatment (natural snow without OTC) was placed midway between extreme snow treatments (removal and addition) to 'anchor' the snow manipulations with OTCs to natural conditions. This approach was likely to deliver maximum information given that a full factorial design was hindered by logistical limitations.

OTCs are often used in climate change experiments as a simple and reliable method to raise air temperatures. Typical increases of daily mean temperature are about 2°C (Marion et al. 1997). This is

not a strong temperature difference, but lies within the temperature change range expected under climate change for the study region (Christensen et al. 2007, Colombo et al. 2007) and was therefore suitable for this experiment. Open-top hexagon chambers were constructed following the International Tundra Experiment protocol (Marion 1996) and were 0.5 m tall with a distance of 1.5 m between the top edges of opposing sides. Instead of using fibreglass panels (Marion 1996), I used sturdy, transparent polyethylene film with 92% optical transmission (Dura Film® Super 4™, AT Plastics) to cover the chamber sides. 'Natural snow' was snow cover that naturally accumulated and ablated. 'Reduced snow' was snow cover whose natural accumulation was reduced by covering the tops of OTCs with a rigid plastic mesh with approximately 1 cm hole size. The holes were large enough for some snow to fall through the mesh and settle onto the ground below. However, most snow settled on top of the mesh from where it was removed with shovel and broom within 24 h after each significant snowfall event. All residual snow that could not be removed from the top of the mesh was brushed through the mesh and settled on the ground below. 'Added snow' was snow cover whose natural accumulation was increased by transferring to it the snow that was removed from the OTC-r treatment. All snow transferred to the OTC-a treatment was deposited directly surrounding and on top of seed cages located inside the OTCs. Snow in the OTC-r and OTC-a treatments ablated naturally.

Each of the 4 treatments was replicated in 5 different locations, randomly chosen in an approximately 0.25 ha area at the experimental site. The distance between replicate plots of each treatment ranged from approximately 5 to 20 m. Each replicate consisted of 3 seed bags, 1 bag for each species. Seed bags were filled with approximately 30, 21 and 8 seeds for eastern white cedar, green ash and sugar maple, respectively (the number of seeds for sugar

Table 1. Selected climate normals (1981–2010) for the regional weather station (Waterloo Wellington A) closest to the experimental site (Environment Canada 2014)

	Snow regime					Temperature regime					
	No. days with snowfall	Monthly snowfall (cm)	Extreme daily snowfall (cm)	Mean depth (cm)	Extreme depth (cm)	Number of days with max. ≤0°C	Daily mean (°C)	Daily min. (°C)	Daily max. (°C)	Extreme min. (°C)	Extreme max. (°C)
Dec	14.4	37.2	22.4	5	50	14.0	-3.3	-6.8	0.2	-27.2	18.7
Jan	16.1	43.7	16.8	11	58	20.7	-6.5	-10.3	-2.6	-31.9	14.2
Feb	11.9	30.3	17.8	11	74	15.7	-5.5	-9.7	-1.2	-29.2	13.7
Mar	9.0	26.5	21.2	6	77	9.2	-1.0	-5.6	3.6	-25.4	24.4
Apr	3.3	7.3	22.9	0	18	0.6	6.2	0.8	11.5	-16.1	29.2

maple was lower because of a very small seed crop in the region). This resulted in 4 treatments  $\times$  5 replicates  $\times$  3 species  $\times$  approximately 30 (21 or 8) seeds = approximately 1180 seeds in total.

Seed bags were made from light fabric nylon stockings (mesh size  $\sim$ 0.1 mm), which provide little thermal insulation and allow free exchange of gas and moisture between the interior and exterior of the bags. To protect the seed bags from predation, they were deposited in small chicken-wire cages (mesh size  $\sim$ 1 cm). Seed bags inside the cages were covered with a litter layer that was a few leaves thick, to simulate close to natural seed deposition in the litter layer. Next to the seed bags, and also covered with litter, each cage contained a temperature sensor (LogTag<sup>®</sup> TRIX-8, LogTag<sup>®</sup> Recorders) that recorded air temperature at 30 min intervals. Additionally, I measured ambient air temperature at 30 min intervals using the same type of temperature sensor. The air temperature sensor was installed 1.5 m above ground under cover of a large, turned-over white plastic container that provided constant shade to the temperature sensor. Airflow through the container was possible from below and through a series of slots cut into all 4 sides.

Snow depth was measured in all replicates after each significant snowfall event (after snow manipulation), after each significant melting event, and at intermittent times (28 times over a period of approximately 18 wk). Surrounding each seed cage, snow depth was measured in 3 to 4 locations to the nearest millimetre and averaged to represent snow cover of the replicate at that time.

## 2.2. Germination test

Prior to the experiment, all seeds were kept in a refrigerator at 4°C, except when handled (e.g. cleaning of seeds and removal of aborted seeds) or when in transport. Seed bags were deposited on 13 December 2011 and were retrieved from the field on 16 April 2012, after which they were moved to a refrigerator. Germination was tested in the lab to control environmental factors as much as possible and to eliminate the risk of seed predation. In the period of 16 to 18 April, all seed bags were emptied and seeds transferred to moist paper tissues. Desiccation of the paper tissues was limited by depositing them in partially open, clear plastic bags. The plastic bags were deposited on the bottom portion of a greenhouse workbench and shielded from direct sunlight with shade cloth. The seeds were observed

over a period of approximately 4 wk and germinants counted per species. Seeds that showed evidence of an emerging radical were classified as ‘germinated’ and removed from the plastic bags. Seeds that did not show an emerging radical remained in the plastic bags until the end of the observation period when they were classified as ‘non-germinated’. Although some cold-temperate tree species can show prolonged seed dormancy and seeds may germinate up to 3 yr after dispersal, the vast majority of seeds of cold-temperate tree species in the Great-Lakes-St.-Lawrence forest region germinate within 1 yr after dispersal (Burns & Honkala 1990). Even if one were to suspect prolonged dormancy, it is reasonable to assume that germination in Year 1 would also be indicative of germination levels in subsequent years.

## 2.3. Statistical analysis

Germination data were analysed with logistic regression models as described by Agresti (2002). Probability of germination was used as response variable, while treatment and species were used as explanatory variables, both classified as nominal factors. Additionally to these main factors, the interaction term treatment  $\times$  species was included in the model. Also, plot was initially included as random factor in the analyses. Accordingly, the function used for logistic regression analysis was glmer (package ‘lme4’), allowing the fitting of generalized linear mixed models (GLMMs; Zuur et al. 2009):

$$Y_{ij} \sim \text{Bin}(1, p_{ij}) \quad (1)$$

$$\text{logit}(p_{ij}) = \alpha + \beta_1 \times \text{Species}_{ij} + \beta_2 \times \text{Treatment}_{ij} + \beta_3 \times \text{Species}_{ij} \times \text{Treatment}_{ij} + a_i \quad (2)$$

$$a_i \sim N(0, \sigma_a^2) \quad (3)$$

Here,  $Y_{ij}$  is 1 if a seed  $j$  is in plot  $i$  and 0 otherwise. Logit indicates the logistic link for the binary germination response in the GLMM, BIN is binominal response,  $p_{ij}$  is the probability that seed  $j$  in plot  $i$  germinates,  $\alpha$  and  $\beta$  are fitted parameters,  $\text{Species}_{ij}$  indicates the species of seed  $j$ ,  $\text{Treatment}_{ij}$  indicates the treatment experienced by seed  $j$ ,  $a_i$  is the random intercept embodying the random plot effect, which is assumed to be normally distributed with mean 0 and variance  $\sigma_a^2$ .

Quantifying the random factor variance showed that it was close to 0 and therefore of negligible effect on probability of germination (Zuur et al. 2009). Con-



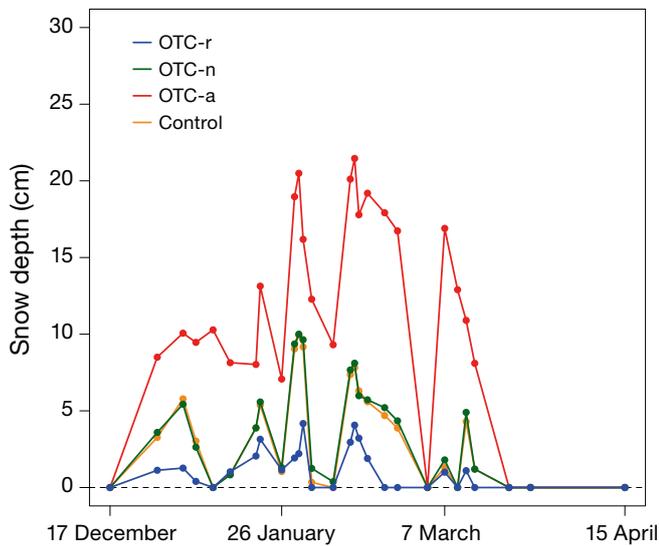


Fig. 1. Seasonal snow depth variations for 4 combinations of snow manipulation and passive warming (OTC-r, OTC-n, OTC-a and control—see Table 2 for explanation of treatment codes) in the seed germination experiment. Presented values are averages of 5 replicates

(Environment Canada 2012b). For this reason, the snow and temperature regimes are divided into a cold period (17 December–7 March) and a warm period (8 March–15 April).

During the cold period, air temperature was on average  $-2.0^{\circ}\text{C}$ , ranging from  $-19.2$  to  $+14.9^{\circ}\text{C}$

(Table 2). Leaf litter temperatures for all treatments were less extreme, showing higher minimum and lower maximum temperatures. Among passive warming treatments (i.e. with OTCs), no pronounced difference was found in average or minimum temperatures. The maximum temperature, however, was about 3 to  $4^{\circ}\text{C}$  lower in the OTC-n treatment than in the OTC-r and OTC-a treatments. Interestingly, the number of freeze–thaw cycles shows a negative relationship with the amount of snow (decreasing frequency from reduced, to natural, to added snow). Compared to the treatment with natural temperature and natural snow (control), the passive warming treatment with natural snow (OTC-n) led to a higher average temperature and less extreme minimum and maximum temperatures, but a comparable number of freeze–thaw cycles (Table 2).

During the warm period, air temperature was on average  $+8.0^{\circ}\text{C}$ , ranging from  $-13.5$  to  $+33.2^{\circ}\text{C}$  (Table 2). Leaf litter temperatures for all treatments were again less extreme than air temperatures, as shown by lower minimum and higher maximum temperatures. Compared to the cold period, deviation of litter temperatures from air were less pronounced. Among passive warming treatments (with OTC), the OTC-r and OTC-n treatments were very similar in temperature and number of freeze–thaw cycles. The OTC-a treatment, however, showed the highest average and maximum temperature and the lowest mini-

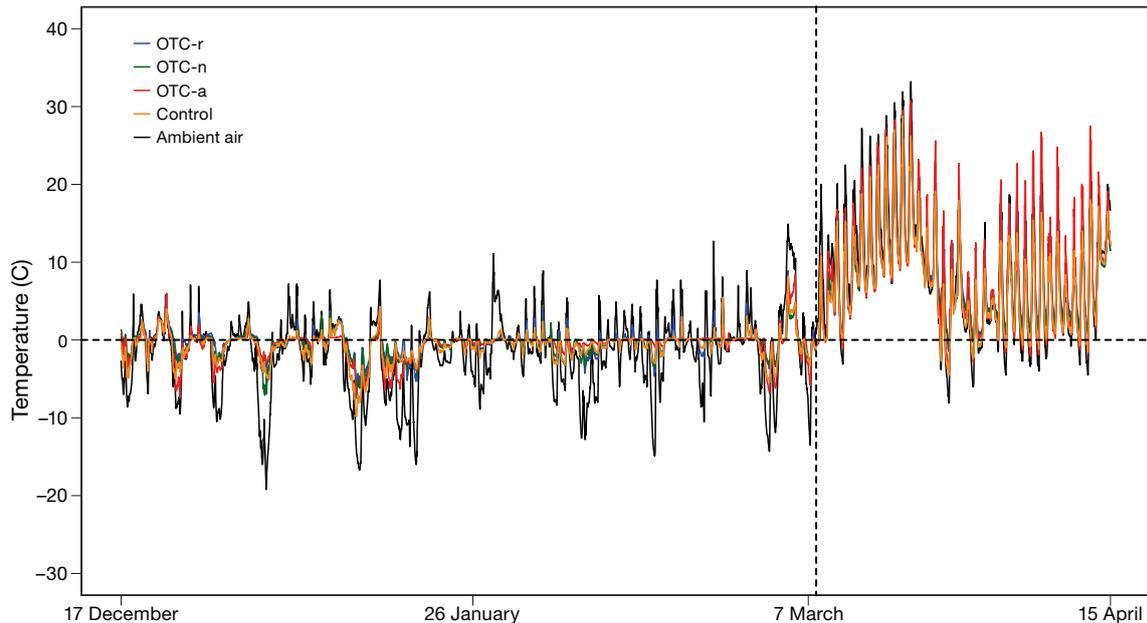


Fig. 2. Diurnal winter temperature variations for ambient air and for the leaf litter layer for 4 combinations of snow manipulation and passive warming (OTC-r, OTC-n, OTC-a and control—see Table 2 for explanation of treatment codes) in the seed germination experiment. Presented values are averages of 5 replicates, except for ambient air, which is only 1 replicate. Vertical broken line: beginning of the warm period

imum temperature; additionally, its number of freeze–thaw cycles was more than 4 times higher compared to the other passive warming treatments (Table 2). The control treatment (natural temperature and snow) led to a lower average temperature, more extreme minimum and maximum temperatures and an increased number of freeze–thaw cycles when compared to the passive warming treatment with natural snow (OTC-n) (Table 2).

### 3.3. Post-winter seed germination

Post-winter seed germination was initially analysed using treatment and species as fixed factors and plot as random factor. The analysis with the full GLMM showed that the estimated random factor variance was 0.0028. This small value indicates that plot had a negligible effect on seed germination and was therefore excluded from further analyses.

Subsequent analyses with GLMs focused on the fixed factors treatment and species. A model with species as sole predictor provided a significantly improved fit over an intercept-only model ( $\chi^2 = 90.37$ ,  $df = 2$ ,  $p < 0.001$ ,  $AIC_{\text{intercept}} = 1563.5$ ,  $AIC_{\text{species}} = 1477.1$ , where AIC is Akaike's information criterion). A model that used both species and treatment as main factors provided a significantly improved fit over the species-only model ( $\chi^2 = 8.67$ ,  $df = 3$ ,  $p = 0.034$ ,  $AIC_{\text{species}} = 1477.1$ ,  $AIC_{\text{species+treatment}} = 1474.5$ ). Finally the full model that included the species  $\times$  treatment interaction term provided a significantly improved fit over the main effect-only model ( $\chi^2 = 14.53$ ,  $df = 6$ ,  $p = 0.024$ ,  $AIC_{\text{species+treatment}} = 1474.5$ ,  $AIC_{\text{species}\times\text{treatment}} = 1471.9$ ).

The significantly improved fit of the model with the species  $\times$  treatment interaction term indicates that the 3 species differed in their response to the treatments (Table 3). Logistic regression analyses of treatment effects on germination at the level of individual species support this finding (Table 4). The results of these analyses indicate that germination of sugar maple and green ash differed between treatment levels, but that germination of eastern white cedar was unaffected. Multiple comparison of treatment levels at the individual species level (Fig. 3) show that for sugar maple, probability of germination was significantly higher for reduced snow treatment ( $z = 2.94$ ,  $p < 0.001$ ) as well as for added snow treatment ( $z = 2.13$ ,  $p = 0.033$ ) than for the control treatment. For green ash, probability of germination was significantly higher for reduced snow treatment ( $z = 2.58$ ,  $p < 0.010$ ) than for the control treatment. Probability of

Table 3. Log likelihood ratio test of predictors in the multi-species seed germination model. Treatment is a combination of snow manipulation with passive warming (control, OTC-r, OTC-n, and OTC-a—see Table 2 for explanation of treatment codes)

Predictor	df	$\chi^2$	p
Species	2	91.41	<0.001
Treatment	3	8.67	0.034
Treatment $\times$ Species	6	14.53	0.024

Table 4. Log likelihood ratio test of treatment effect in the seed germination model at the individual species level. Treatment is a combination of snow manipulation with passive warming (control, OTC-r, OTC-n, and OTC-a—see Table 2 for explanation of treatment codes)

Species	df	$\chi^2$	p
Sugar maple	3	9.97	0.019
Green ash	3	7.81	0.050
Eastern white cedar	3	5.42	0.144

germination was also higher for the added snow treatment than for the control treatment, but this was not statistical significant ( $z = 1.94$ ,  $p = 0.053$ ).

## 4. DISCUSSION

The results suggest that leaf litter temperatures are on average increased and moderated by the passive warming treatment (natural snow with vs. without OTC). Frequencies of freeze–thaw cycles of both treatments were equivalent during the cold period, but lower for the passive warming treatment during the warm period. The snow manipulation results suggest that the litter temperature response depends on the seasonal weather conditions. During the cold period, freeze–thaw cycles in the snow manipulations plots were negatively related to snow cover, while during the warm period, freeze–thaw cycles were positively related to snow cover.

The results of the germination tests suggest that post-winter seed germination was affected by snow manipulations and passive warming and that these effects were species-specific. Overall, seed germination for sugar maple and green ash was higher in the passive warming treatments with either reduced or increased snow cover, while it was generally lower in the treatments with natural snow cover (with and without passive warming). Although a fully factorial

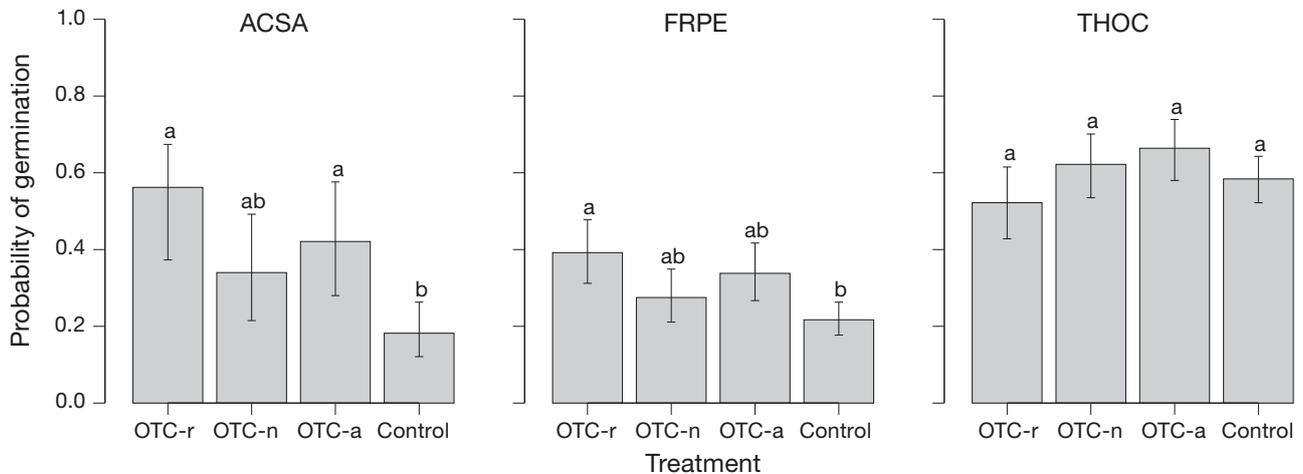


Fig. 3. Probability of post-winter germination for seeds of 3 cold-temperate tree species under 4 combinations of snow manipulation and passive warming (control, OTC-r, OTC-n and OTC-a, see Table 2 for explanation of treatment codes). Shown is the probability of germination plus standard errors. Treatments that do not share the same letter are significantly different ( $p < 0.05$ ). ACSA: sugar maple, FRPE: green ash, THOC: eastern white cedar

design would provide for stronger inference, this observation points toward the possibility that snow cover had a stronger effect on seed germination than passive warming. Despite the relatively small differences in snow cover and litter temperature among treatments, seed germination for sugar maple almost tripled and for green ash doubled under favourable conditions, suggesting possible effects on forest regeneration dynamics and the potential for alterations in future tree species abundance given current climate change projections.

#### 4.1. Snow and temperature regimes

The snow manipulations achieved the desired aim of respectively increasing and reducing snow cover (Table 2). Average snow depth in the snow addition plots continued to build until mid-February, when it reached its peak of 21.5 cm. A similar build-up was not observed in the other treatments, because of more regularly occurring complete snow loss. Throughout the observational period, snow depth in the snow addition plots was approximately 3 and 9 times larger than in the natural snow and snow-reduction plots, respectively. Parallel to this, the frequency of complete loss of snow cover increased from snow addition to natural snow and snow reduction plots. Interestingly, snow cover in the natural snow treatments with and without passive warming (i.e. OTC-n and control) was virtually identical (Fig. 1). Danby & Hik (2007) found that OTCs can have variable effects on snow cover: either increas-

ing, decreasing or not affecting snow depth. The results of the current study do not suggest the existence of any substantial effects of OTCs (i.e. physical structure or passive warming) on snow accumulation given the experimental conditions (i.e. cold-temperate forest interior during a relatively warm and snow-poor winter, Environment Canada 2012a).

The effects of the snow manipulations on leaf litter temperatures were complex and at times different from expectations. However, complex responses have to be embraced when considering realistic climate change experiments (Kreyling & Beier 2013). Even during the cold period, snow in the snow-reduction plots melted and became very shallow when on warm days air temperatures increased above 0°C. On those days, the litter layer in the snow-reduction treatment warmed up, which largely negated any temperature differences between the snow-reduction and snow-addition treatments. Because of the lack of insulation from a thick snow cover, the temperature in the snow-reduction plots often followed the daily fluctuations of the ambient air temperature (Fig. 2), which led to twice as many freeze–thaw cycles as in the snow-addition plots. This freeze–thaw cycle response is in line with other studies. For example, Decker et al. (2003) found that during a warm, snow-poor winter, shallow soil layers in snow-free plots had increased numbers of freeze–thaw cycles relative to control plots with snow.

The warm period was the time when melt-out occurred for all treatments, with melt-out dates ranging from approximately 9 March (snow-reduction

treatment) to 19 March (snow-addition treatment). During this time, the snow-addition plots showed a higher average litter temperature, higher temperature range and larger number of freeze–thaw cycles than the snow-reduction plots. Interestingly, the effects of snow cover on freeze–thaw cycles depend on surrounding conditions; during the cold period, snow addition reduced the number of freeze–thaw cycles, but during the warm period, it increased them, reflecting both the insulating effect of thick snow cover and the temperature-lowering effect of shallow snow cover (Zhang 2005).

The effects of the snow manipulations on litter layer temperatures stand in contrast to the results of other studies. For example, in a snow manipulation study by Simons et al. (2010), soil surface temperatures in plots without insulation reached very low sub-0 temperatures while they remained close to 0°C under natural snow cover. However, the study of Simons et al. (2010) differed from the current study in their snow and temperature regimes. While in their study heavy snowfall occurred frequently and air temperature often fell to very low temperatures, in the current study heavy snowfall occurred seldom and air temperatures were much milder. The unexpected litter layer temperature effects of the snow manipulations in the current study may therefore be the result of the mild and snow-poor winter.

The OTCs were moderately effective in causing the desired passive warming effect (Table 2). During both the cold and the warm period, the average and absolute minimum temperatures were higher with passive warming than without. However, against expectation, the frequency of freeze–thaw cycles without passive warming was higher than with passive warming. Additionally, absolute maximum temperatures were lower with passive warming than without. Complex temperature responses caused by snow manipulations and passive warming have also been found in other studies (Bokhorst et al. 2013). The results of Marion et al. (1997) for a variety of passive warming experiments show that desired increases in air temperature can be limited for certain locations, times of day or seasons. Moreover, the results of Dabros et al. (2010) show that OTCs can sometimes lead to cooling, possibly caused by cold air that is trapped in a chamber and cannot mix with surrounding warmer air. Marion et al. (1997) discussed multiple factors that may affect the temperature response in passive warming devices, including convective heat loss by wind, vegetation shading and efficiency of the chamber heat trap. Given the

forested location of the experimental site and the open design of the chambers, it is possible that in the current study, decreased maximum litter temperatures in passive warming plots were the result of any of these factors.

#### 4.2. Seed germination

The 3 species differed in their seed germination response to the treatments, although the experimental design does not allow for strong inference regarding the individual effects of the 2 factors (snow manipulation vs. passive warming) or their interaction. For sugar maple and green ash, the analyses suggest that a combination of snow reduction and passive warming increased post-winter seed germination relative to the control treatment, while added snow and passive warming led to increased seed germination only for sugar maple. Passive warming alone did not affect seed germination for either of these species. For eastern white cedar, none of the snow manipulation and passive warming treatments had a significant effect on post-winter seed germination. Despite the modest treatment effects, the results show that germination differed—and for sugar maple strongly so—among treatments. Of course, forest regeneration dynamics are affected by processes that occur throughout various life stages of trees including, for example, seedlings and saplings. However, the current results suggest that changes to winter and snow conditions in cold-temperate regions may affect seed germination and may contribute to changes in future forest regeneration dynamics. Additionally, the results indicate an interaction between treatment and species, which suggests species-specific effects of snow cover and litter temperature and consequently the potential for future changes in forest species composition (Henne et al. 2007). Finally, because snow regime changes are expected to occur at regional scales (Venäläinen et al. 2001), changing snow conditions may have the potential to change species' ranges.

The current study focused on investigating whether modest snow and temperature regime changes can affect tree seed germination and did not attempt to reveal any potential mechanisms underlying this response. Therefore, I can only speculate about the potential mechanisms. The increased germination following both reduced and added snow for sugar maple (and to some extent green ash) may suggest that natural snow provided 'Goldilocks' conditions for a process that acted to limit post-winter

germination. Deviations from these conditions (either positive or negative) led to a reduction in the limiting process and therefore increased post-winter germination. This process may be related to biochemical dynamics of germination inhibitors (Webb et al. 1973). However, I speculate that another possible process could be the effect of soil pathogens on overwintering seeds. For example, O'Hanlon-Manners & Kotanen (2004) reported increased seed germination in white birch after fungicide application in the field. Kenerley & Bruck (1983) found a negative relationship between winter soil temperature and propagule density of a pathogenic soil fungus. Cho et al. (2007) found that seed infection with pathogenic fungi showed variable relationships with snow cover (positive, negative or no effect). These results show that the effects of winter conditions on soil pathogens and, by extension, on overwintering seeds, cannot easily be generalized. Instead, they are species-specific and vary with winter conditions. A possible hypothesis based on the current results is that changed snow cover and litter layer temperature regimes in snow-manipulation treatments depresses soil pathogen activity and therefore leads to increased post-winter seed germination.

#### 4.3. Efficacy of experimental treatments

Earlier studies manipulated snow with shovels (Templer et al. 2012) or with snow fences (Natali et al. 2011). However, in my study, falling snow was intercepted with a rigid plastic mesh, leading to a reduction in snow depth in the affected plots, and then transferred to snow addition plots. To my knowledge, this method has never been used before, but it has advantages compared to shovelling and snow fences. First, the danger of disturbing low vegetation or the litter layer, as is the case when removing snow by shovelling, is eliminated when falling snow is intercepted with a mesh. Second, the wind field in forests tends to be weaker and more spatially variable compared to treeless areas (Blanken et al. 2009), leading to higher variability in snow deposition (Trujillo et al. 2009). This makes snow fences less effective in forest interiors, whereas the snow interception method can be used without problem. A potentially complicating factor of using a mesh to intercept snow may be that the mesh effect varied somewhat with snow characteristics. For example, small sized graupel tended to fall through the mesh while ordinary snowflakes settled on the mesh. A more process-oriented analysis of snow manipulation

experiments would have to be sensitive to these differences.

A drawback of any snow treatment that manually adds snow is the unavoidable compaction of redistributed snow. Compacted snow has different insulation and ablation properties than undisturbed snow (Zhang 2005), which might lead to differences in litter layer temperatures. To investigate the effect of snow compaction, it may be possible to intercept snow with a mesh but then add it to the same plot, i.e. not changing the amount of snow. Such a treatment would be an interesting addition to future experiments.

OTCs are simple and effective in inducing passive warming and they are often used in studies investigating the effects of global warming (Arft et al. 1999). However, they are not often used in forests or during winter (exceptions: Dabros et al. 2010, De Frenne et al. 2011). This may be because the passive warming effect of OTCs depends on the trapping of radiant heat that is emitted when sunlight is intercepted and converted into infrared radiation. Shading in forests, the low amount of energy in winter sunlight and the large albedo of snow all may limit the warming effect achieved with passive warming approaches. This may have led to the low amount of passive warming with OTCs in this study (average increase approximately 0.5°C). Using infrared lamps may be more effective at increasing litter layer temperatures in future experiments (Aronson & McNulty 2009).

## 5. CONCLUSIONS

The findings of this study indicate that snow interception and passive warming approaches are useful for modifying snow cover and litter temperature regimes in cold-temperate forests. The resulting modest changes to snow cover and temperature dynamics may be comparable to intermediate-term expectations under climate change, and could affect post-winter germination of tree seeds in species-specific ways. This study adds to the small, but growing, body of research documenting the potential effects of winter climate change on forests in cold-temperate regions. Unfortunately, seeds as a life stage have so far received relatively little attention by studies of the winter effects of climate change on forest regeneration. However, because of the large mortality rates of seeds, this life stage may be especially important for forest regeneration dynamics, species adaptations to climate change and the establishment of future ranges of tree species.

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