

# Short-term climate change manipulation effects do not scale up to long-term legacies: effects of an absent snow cover on boreal forest plants

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## Summary

1. Despite time-lags and nonlinearity in ecological processes, the majority of our knowledge about ecosystem responses to long-term changes in climate originates from relatively short-term experiments.

2. We utilized the longest ongoing snow removal experiment in the world and an additional set of new plots at the same location in northern Sweden to simultaneously measure the effects of long-term (11 winters) and short-term (1 winter) absence of snow cover on boreal forest understorey plants, including the effects on root growth and phenology.

3. Short-term absence of snow reduced vascular plant cover in the understorey by 42%, reduced fine root biomass by 16%, reduced shoot growth by up to 53% and induced tissue damage on two common dwarf shrubs. In the long-term manipulation, more substantial effects on understorey plant cover (92% reduced) and standing fine root biomass (39% reduced) were observed, whereas other response parameters, such as tissue damage, were observed less. Fine root growth was generally reduced, and its initiation delayed by *c.* 3 (short-term) to 6 weeks (long-term manipulation).

4. *Synthesis.* We show that one extreme winter with a reduced snow cover can already induce ecologically significant alterations. We also show that long-term changes were smaller than suggested by an extrapolation of short-term manipulation results (using a constant proportional decline). In addition, some of those negative responses, such as frost damage and shoot growth, were even absolutely stronger in the short-term compared to the long-term manipulation. This suggests adaptation or survival of only those individuals that are able to cope with these extreme winter conditions, and that the short-term manipulation alone would overpredict long-term impacts. These results highlight both the ecological importance of snow cover in this boreal forest, and the value of combining short- and long-term experiments side by side in climate change research.

**Key-words:** minirhizotron, Norway spruce, *Picea abies*, plant–climate interactions, root phenology, snow removal, soil frost, understorey, *Vaccinium*

## Introduction

Ecosystem responses to changes in climate are often complex, nonlinear and can change in the long-term (Brown *et al.* 2001; Leuzinger *et al.* 2011). Despite this complexity, predictions of ecosystem responses to long-term changes in climate are heavily based on the results from short-term experiments (Leuzinger *et al.* 2011; Wolkovich *et al.* 2012). The validity

of such predictions strongly depends on how well short-term experiments can reflect long-term changes, which can be hampered, for example, by a lag period until an effect is present, or a decline in the effect size with time due to genetic adaptation, ontogenetic effects or species interactions (Brown *et al.* 2001; Leuzinger *et al.* 2011; Phoenix *et al.* 2012). Long-term studies of several years to decades are thus a key approach to unravelling complexity in ecosystem responses to climate change, especially when compared to short-term experimental data (typically 1–3 years). Yet, over the course of a long-term

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experiment, background environmental parameters, such as temperature, precipitation patterns or herbivore abundance, can change unrelated to the experimental manipulation (e.g. Elmendorf *et al.* 2012). These factors can potentially complicate a comparison of results from the first year(s) of manipulation to those after many years in case such parameters differ during the year(s) of measurement. Sound separation of short- and long-term effects might therefore benefit from simultaneously (i.e. in the same year) comparing short- and long-term responses. Studying long-term effects of changes in climate is particularly important in ecosystems dominated by long-lived species, such as boreal forests.

Boreal forests are shaped by a distinct seasonality and cold winters with a persistent snow cover that insulates and thus protects understorey vegetation and soils from severe freezing (Haei *et al.* 2013). Boreal snow cover duration and depth are expected to decrease in the near-term, as climate change is most pronounced in high latitudes and during winter (Kirtman *et al.* 2013). The current duration of persistent snow cover of 5–6 months (Laudon & Ottosson Lövvenius 2015) in northern Sweden is predicted to shorten by 73–93 days within the next century due to increasing temperatures and more precipitation as rain instead of snow (Mellander, Lövvenius & Laudon 2007). This reduction in snow cover can lead to the phenomenon of ‘colder soils in a warmer world’ (Groffman *et al.* 2001a), as less insulation during winter increases soil freezing and the frequency of soil freeze–thaw cycles. As such, snow is a critical regulator of understorey vegetation health and composition (Rasmus, Lundell & Saarinen 2011; Kreyling, Haei & Laudon 2012; Saarinen *et al.* 2015) as well as soil processes, and a lack of snow has been shown to alter carbon and nutrient fluxes into the next growing season (Groffman *et al.* 2001a,b, 2006, 2011; Olsson & Falkengren-Grerup 2003; Öquist & Laudon 2008; Haei *et al.* 2013; Campbell, Soggi & Templer 2014). Plant responses, particularly root responses, are suggested drivers of these changes, but remain far less studied than biogeochemical soil alterations (but see Tierney *et al.* 2001; Comerford *et al.* 2013; Campbell, Soggi & Templer 2014).

Fine roots constitute a relatively small proportion of total plant biomass in forest biomes, but play a disproportionately large role in carbon and nutrient cycling (Finzi *et al.* 2015; McCormack *et al.* 2015). In boreal forests, fine roots account for 32% of total plant production and 73% of root production (Yuan & Chen 2010). They turn over more than a magnitude faster than above-ground litter (Ruess *et al.* 2006), are key drivers of soil respiration (Högberg *et al.* 2001) and – together with their associated mycorrhizal fungi – are responsible for 50–70% of stored soil carbon (Clemmensen *et al.* 2013). Compared to above-ground plant parts, roots are usually more protected against frost damage within the soil, and as a result, when exposed to frost, freezing injuries can occur at relatively milder temperatures (Sakai & Larcher 1987; Schaberg *et al.* 2008). In addition to direct cellular frost damage, roots can also be damaged indirectly through ice lens formation and frost heaving of the surrounding soil (Tierney *et al.* 2001; Cleavitt *et al.* 2008; Comerford *et al.* 2013).

Frost-induced damage to the root system of forest plants could influence whole plant health and function, and biogeochemical cycling in the ecosystem. For example, in a northern hardwood forest, snow removal reduced vitality of roots, enhanced overwinter mortality of roots, altered the patterns of root phenology, timing of root growth and changed the total amounts of root production (Tierney *et al.* 2001; Cleavitt *et al.* 2008; Comerford *et al.* 2013).

Boreal forests have an abundant understorey. Consequently, a reduced or absent snow cover not only leads to more severe and deeper soil frost, but also leaves the abundant understorey without insulation. Without this protection, plants of the understorey experience much colder air temperatures, which can lead to frost damage, such as discoloration, bleaching, tissue shrinking and dieback, tearing of tissue or delayed phenology (Sakai & Larcher 1987), and can eventually reduce their abundance (Kreyling, Haei & Laudon 2012). The same processes can also be observed in other ecosystems, such as a variety of arctic ecosystem types (Cooper 2014). All of these potential changes in plant phenology, abundance and health can further feedback on carbon and nutrient cycling, for example, through the changes in nutrient uptake, photosynthesis or growth, and should thus be thoroughly understood.

In this study, we hypothesized that (i) the absence of snow and concomitant stronger (soil-) frost modifies plant biomass, growth and phenology both above- and below-ground in a system where snow is otherwise abundant. We also hypothesized that (ii) conclusions about plant responses to a reduced snow cover differ depending on the duration of the treatments. More specifically, we wanted to find out whether one extreme, snow-free winter already induces ecologically significant changes, or whether effects build up over time, and whether or not results from a short-term manipulation can be extrapolated to long-term effects. To test these hypotheses, we utilized the longest ongoing snow removal experiment we are aware of, situated in the boreal forest of northern Sweden and duplicated the snow removal treatment in new plots during the 11th year of the long-term manipulation, resulting in both 1 and 11 years of snow manipulation. We could thus directly compare the effects of a short- and long-term manipulation under identical measurement conditions.

## Materials and methods

### STUDY SITE

The experiment was situated in the Krycklan Catchment within the Svartberget Field Research Infrastructure (Laudon *et al.* 2013), approximately 50 km north-west of Umeå, northern Sweden (64°14'N, 19°46'E). The site is a 100-year-old Norway spruce forest (*Picea abies* (L.) H. Karst.) with an abundant understorey vegetation dominated by *Carex canescens* L., *Vaccinium myrtillus* L. (bilberry), *Vaccinium vitis-idaea* L. (lingonberry) and a dense bryophyte layer, mainly consisting of *Hylocomium splendens* (Hedw.) B.S.G. and *Pleurozium schreberi* (Brid.) Mitt. Mean annual air temperature was 1.8 °C, mean January temperature –9.5 °C and mean July temperature +14.7 °C for 1981–2010. Mean annual precipitation in the same period was 624 mm, of which approximately 40% fell as snow.

During winter, the snow cover is usually persistent from mid-November to late April and has an annual maximum depth of 76.5 cm (43–113 cm; 1980–2010), although extreme winters with almost no snow and deep soil frost sporadically do occur. For example, the winter of 1995/96 had a snow depth of only c. 20 cm until the end of January, and an average snow depth of only 28 cm over the whole winter, leading to soil frost depth of 54 cm and soil temperatures as low as  $-10\text{ }^{\circ}\text{C}$  at 10 cm depth (Nyberg *et al.* 2001; Mellander, Löfvenius & Laudon 2007; Laudon & Ottosson Löfvenius 2015). Minimum air temperatures usually remain below freezing point from October to April and values reached  $-17.4\text{ }^{\circ}\text{C}$  in December,  $-25.2\text{ }^{\circ}\text{C}$  in January and  $-15.4\text{ }^{\circ}\text{C}$  in February during the winter 2012/13, when the short-term manipulation took place. Air temperatures, precipitation and snow depth, and thus the onset and end of the snow covered period, were recorded at the Svartberget field station, 1.2 km south-west of the study site (Laudon *et al.* 2013), and are available at [www.slu.se/Krycklan](http://www.slu.se/Krycklan).

## EXPERIMENTAL DESIGN

Snow removal in the long-term manipulation started in 2002. In addition to this long-term manipulation, we set up an additional set of plots at the same site during the winter 2012/13, completely analogue to the long-term manipulation, inducing a short-term manipulation of one winter of snow removal which allowed us to compare the long- and short-term effects simultaneously. Three replicate plots per treatment (long-term snow removal, short-term snow removal and control), each  $3 \times 3\text{ m}$ , were randomly distributed along both sides of a very small, first-order stream in a Norway spruce forest. The centres of each plot were three metres away from the stream. Carry-over effects between the plots based on soil water mixing are highly unlikely as previous research at this site has shown that the groundwater flow is unidirectional and homogenous, and as a matrix from uphill areas through the riparian zone to the stream (e.g. Laudon *et al.* 2004; Peralta-Tapia *et al.* 2015). Snow was prevented from falling onto the plots by roofs, consisting of a light blue, clear tarp on wooden constructions at a height of approximately 2.5 m. Additional tarp on the sides of the plots (0–50 cm) prevented snow from drifting in, and as a result, snow was absent from the plots over the whole winter. The roof tarps were put on before snowfall (1st of October 2012) and slit just prior to snow melt (24th of April 2013), such that the same amount of total precipitation and compounds bound in the snow entered the manipulated plots as in the controls, ensuring a similar water balance among the plots. Soil moisture was thus not altered by the treatment (see Öquist & Laudon (2008) for soil moisture measurements in the first years of the experimental manipulation). Light availability in the understorey in summer was not significantly influenced by the wooden constructions (Kreyling, Haei & Laudon 2012).

The use of wooden constructions with rooftops instead of shovelling snow enabled us to perform numerous measurements on undisturbed understorey vegetation, and also ensured that snow-associated nutrients, litter and the same water equivalent entered the plots.

## RESPONSE PARAMETERS

### Soil temperatures with absence of snow

Soil temperatures data from the first 8 years of the experiment (winter 2003/04 to winter 2010/11), recorded every 4 h (CR10, Campbell Scientific, Logan, UT) at 10, 25, 40, 60 and 80 cm depth in each of the long-term and ambient plots, showed that the absence of snow at

this site during winter changed the soil frost patterns. Without snow, soil frost on average reached deeper (down to  $49 \pm 6\text{ cm}$  instead of  $29 \pm 3\text{ cm}$ , mean  $\pm$  standard deviation), lasted longer ( $118 \pm 4$  days at 10 cm depth instead of  $56 \pm 4$  days), delayed thawing by 6 days at the soil surface and 26 days at 10 cm depth and was more severe ( $-5.5 \pm 0.1\text{ }^{\circ}\text{C}$  vs.  $-1.9 \pm 0.1\text{ }^{\circ}\text{C}$  at 10 cm depth,  $-2.9 \pm 0.1\text{ }^{\circ}\text{C}$  vs.  $-0.3 \pm 0.0\text{ }^{\circ}\text{C}$  at 25 cm depth,  $-1.0 \pm 0.0\text{ }^{\circ}\text{C}$  vs.  $0.3 \pm 0.0\text{ }^{\circ}\text{C}$  at 40 cm depth and  $0.2 \pm 0.0\text{ }^{\circ}\text{C}$  vs.  $0.8 \pm 0.0\text{ }^{\circ}\text{C}$  at 60 cm depth, mean  $\pm$  standard error) than under ambient conditions (see also Kreyling, Haei & Laudon 2012, 2013; Haei *et al.* 2013). Both the 'winter' 2012/2013 (period with mean monthly air temperatures below  $0\text{ }^{\circ}\text{C}$ , November – March) and the 'growing season' 2013 (period with mean monthly air temperatures above  $0\text{ }^{\circ}\text{C}$ , April – October) were comparable to the first 8 years of the experiment, with mean monthly air temperatures  $0.6\text{ }^{\circ}\text{C}$  colder in winter and  $0.3\text{ }^{\circ}\text{C}$  warmer in summer.

### Above-ground measurements

We measured above-ground spring phenology, frost damage and shoot elongation on the two most common dwarf shrubs at our site, namely *V. myrtillus* and *V. vitis-idaea*. Dwarf shrubs in general are a main component of the understorey and the two species considered here are among the three most abundant ones in northern Swedish boreal forests (Nilsson & Wardle 2005). Above-ground spring phenology was monitored weekly over a period of 4 weeks, starting at the 18th of May 2013, on the dwarf shrubs *V. myrtillus* and *V. vitis-idaea*. We recorded bud break, unfolding of the first leaf, the formation of additional leaves, opening of first flower and additional flowers using the BBCH scale ('Biologische Bundesanstalt, BUNDessortenamt und Chemische Industrie', German Federal Biological Research Centre for Agriculture and Forestry). This was done on five randomly chosen individuals in each plot, which were marked and revisited every time.

Discoloration of above-ground tissue is one of the most frequent and clearest indications of freezing injury (Sakai & Larcher 1987). Thus, we estimated the proportion of the whole plant that showed visible frost damage, identified as brown discoloration of plant tissue (leaves for *V. vitis-idaea* and stems for *V. myrtillus*), on the same marked individuals in May 2013 and averaged this per plot for statistical analysis.

Shoot elongation of the growing season 2013 was measured in August with a calliper, again on the same individuals and averaged per plot for statistical analysis. Therefore, we measured elongation on several shoots of *V. myrtillus* and averaged for each individual, and measured the total shoot growth of the unbranched *V. vitis-idaea*. Total cover and species-specific cover of the understorey vegetation were measured in August 2013 with a point-intercept method using 100 vertically inserted needles, evenly spaced in the inner  $2 \times 2\text{ m}$  core of each plot (Kreyling, Haei & Laudon 2012). The number of hits was then treated as % cover.

### Below-ground measurements

Fine root (roots  $< 2\text{ mm}$  in diameter) growth and phenology were measured with a minirhizotron system (Bartz Technology Corporation, Carpinteria, CA, USA) consisting of transparent tubes buried in the soil and a camera system. This non-destructive method allows observation of the same fine roots over time (Johnson *et al.* 2001). Three tubes were installed in each plot on the 1st of June 2012, each at a 45 degree angle from the soil surface down to a depth of 50 cm.

We began observing root growth with minirhizotrons at the 20th of April 2013, when control plots still had a snow cover of 30–40 cm, and we sampled every 3 weeks until the 8th of October 2013. Root length was measured at each sampling occasion in the same picture frames using ROOTFLY (version 2.0.2, Clemson University). Both newly appearing roots and elongation of previously present roots between the observations were counted as ‘root growth’, which was pooled together to an average value per plot from all three tubes.

Fine root biomass (< 2 mm diameter) was measured with destructive sampling in August 2013. Three cores of 4 cm diameter were taken in each plot at a depth of both 0–20 cm and 20–40 cm (comprising both humus and mineral layer) and then combined into one sample per plot and depth resulting in a total of 18 samples. Roots were washed clean from soil, discarded as dead if hollow or breaking at touch and then dried at 60° for 72 h and weighed.

## STATISTICAL ANALYSES

We used linear models to test for differences among the treatments in visible frost damage, shoot elongation (for each species separately) and root biomass. If applicable, we included the differences between species, and their interactions in the models. Similarly, we tested for differences among the treatments in the cover of all bryophytes, all vascular plants, the two most common bryophytes (*Hylocomium splendens* and *Pleurozium schreberi*) and the two most common vascular plants (*Carex canescens* and *Vaccinium myrtillus*). For above-ground spring phenology, a linear mixed-effect model with plot identity as random factor was used, to account for repeated measures on the same individuals. Fine root length and growth were analysed with a linear mixed-effect model with random intercept and slope, modelling the interaction between day of year and plot identity as random variables. Tukey’s tests were used to identify homogenous groups. Data were inspected for normality and homogeneity of variances by examining residual vs. fitted plots and qq plots, respectively, of the linear models (Faraway 2005). If conditions were not met, and could not be improved with log or square root transformations, response variables were rank-transformed. All statistical analyses were done in R version 3.1.2 (R Core Team, 2014), with the following packages: MULTCOMP (version 1.3-9), AGRICOLAE (version 1.2-1), NLME (version 3.1-118), LME4 (version 1.1-7), LMTEST (version 2.0-20) and SCIPLOT (version 1.1-0).

## Results

### ABOVE-GROUND

#### Above-ground spring phenology

Bud burst occurred on average 5 days earlier in *V. myrtillus* than in *V. vitis-idaea*, but was not significantly different among the snow manipulation treatments even though it was on average 8 and 4 days later in the short-term and long-term snow treatment, respectively, compared to the control (Fig. 1a, Table 1). As the timing of bud burst was more similar in the short- and long-term treatment than in control for *V. myrtillus*, and more similar in control and long-term treatment than in short-term treatment for *V. vitis-idaea*, there was a significant interaction between snow treatment and species (Table 1). Unfolding of the first leaf also occurred 7 days earlier in *V. myrtillus* than in *V. vitis-idaea*, but there was no

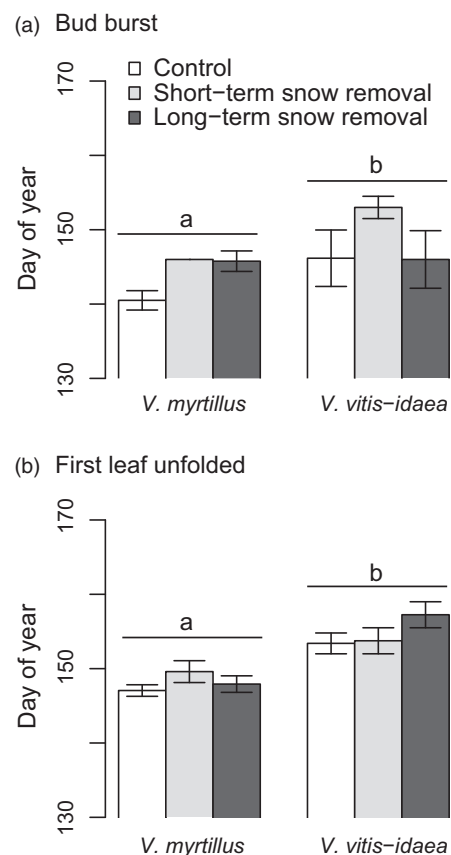
treatment effect and there was no significant interaction between treatment and species (Fig. 1b, Table 1).

#### Visible frost damage

Individuals of both species, *V. myrtillus* and *V. vitis-idaea*, showed visible frost damage as discoloration of above-ground tissues. There were significant differences among the snow manipulation treatments as plants had no frost damage (0%) in the control, little in the long-term treatment (6%) and high frost damage in those exposed to the frost for the first time in the short-term treatment (29%) (Fig. 2, Table 1). All snow manipulation treatments differed from each other (Tukey’s test,  $P < 0.05$ ), and as *V. myrtillus* and *V. vitis-idaea* showed similar patterns of damage, there was no significant interaction between species and treatment (Fig. 2, Table 1).

#### Shoot elongation

The absence of snow and exposure to freezing temperatures significantly reduced shoot elongation of *V. myrtillus*, but not of *V. vitis-idaea* (Fig. 3, Table 1). Therefore, for *V. myrtillus* shoot elongation was only significantly different between the



**Fig. 1.** Day of year at which bud burst (a) and the unfolding of the first leaf (b) occurred in *Vaccinium myrtillus* and *V. vitis-idaea* in the control, short-term snow removal (1 winter) and long-term snow removal (11 winters). Means  $\pm$  SE, data are measured on 5 individuals in each of three plots per treatment.

**Table 1.** Effects of species identity (*Vaccinium myrtillus*, *V. vitis-idaea*), snow treatment (control, short-term snow removal, long-term snow removal) and day of year on above-ground phenology (bud burst and first leaf unfolded), shoot elongation, visible frost damage, root length, root biomass and understorey plant cover

		Response parameter	Source of variation	$F_{df}$	$P$
Above-ground responses	Above-ground phenology	Bud burst	Species (S)	5.9 <sub>1,47</sub>	<b>0.019</b>
			Snow Treatment (T)	1.7 <sub>2,6</sub>	0.257
		First leaf	S × T	4.3 <sub>2,47</sub>	<b>0.019</b>
			Species	47.5 <sub>1,37</sub>	< <b>0.001</b>
	Shoot elongation	<i>V. myrtillus</i>	Snow Treatment (T)	1.2 <sub>2,6</sub>	0.360
			S × T	2.2 <sub>2,37</sub>	0.126
		<i>V. vitis-idaea</i>	Snow Treatment (T)	6.7 <sub>2,6</sub>	<b>0.029</b>
			Snow Treatment (T)	0.9 <sub>2,6</sub>	0.469
		Frost damage	Species	0.01 <sub>1,12</sub>	0.910
			Snow Treatment (T)	47.7 <sub>2,12</sub>	< <b>0.001</b>
	Understorey Plant Cover	Bryophyte cover	S × T	2.2 <sub>2,12</sub>	0.126
			Snow Treatment (T)	0.7 <sub>2,6</sub>	0.533
		Vascular plant cover	Snow Treatment (T)	12.4 <sub>2,6</sub>	<b>0.007</b>
			<i>C. canescens</i>	Snow Treatment (T)	12.5 <sub>2,6</sub>
<i>V. myrtillus</i>		Snow Treatment (T)	9.6 <sub>2,6</sub>	<b>0.013</b>	
<i>H. splendens</i>		Snow Treatment (T)	0.2 <sub>2,6</sub>	0.817	
<i>P. schreberi</i>		Snow Treatment (T)	0.2 <sub>2,6</sub>	0.831	
Below-ground responses		Root length	Day of year (DOY)	37.4 <sub>1,7</sub>	< <b>0.001</b>
	Snow Treatment (T)		3.9 <sub>1,7</sub>	0.089	
	DOY × T		11.5 <sub>1,7</sub>	<b>0.012</b>	
	Root growth	Day of year (DOY)	4.9 <sub>1,10</sub>	<b>0.049</b>	
		Snow Treatment (T)	3.0 <sub>1,16</sub>	0.102	
		DOY × T	0.4 <sub>1,10</sub>	0.542	
	Root biomass	Soil depth (D)	29.5 <sub>1,12</sub>	< <b>0.001</b>	
		Snow Treatment (T)	4.0 <sub>2,12</sub>	<b>0.046</b>	
		D × T	1.7 <sub>2,12</sub>	0.224	

Significant differences are given in boldface.

short-term treatment and the control (Tukey's test). Shoot elongation of *V. myrtillus* was 53% reduced in the short-term treatment compared to the control, and 40% reduced in the long-term treatment compared to the control.

#### Above-ground cover

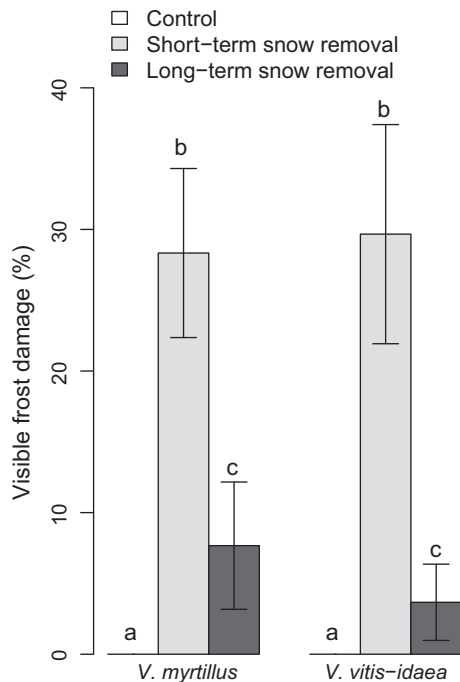
Vascular plant cover strongly declined in response to the absence of snow (Tables 1 and 2). This decline was 42% in the short-term and 92% in the long-term snow treatment compared to the control. Therefore, only the reduction in the long-term treatment was statistically significant (Tukey's test,  $P < 0.05$ , Table 1). The overall decline in vascular species cover was also visible in the two most abundant plant species: the cover of both *Carex canescens* and *V. myrtillus* declined in the absence of snow (Tables 1 and 2). For *C. canescens*, mean cover was reduced in the long-term compared to both short-term treatment and control. Mean abundance of *V. myrtillus* was only significantly reduced in the long-term treatment compared to the control (Tukey's test,  $P < 0.05$ , Table 2). Total bryophyte cover did not differ among treatments. Similarly, the cover of the two most common bryophytes did not differ among the treatments (Tables 1 and 2). Overall, relative abundance of species changed little with the snow manipulation treatments (Table 2). The only major

change was in the abundance of *V. myrtillus*, which shifted from being 2nd to 4th most common in the control vs. long-term treatment. Also, the ratio between bryophyte and vascular plant cover was altered due to the strong reduction in abundance of vascular plants and the relative stability of bryophyte abundance.

#### BELOW-GROUND

##### Fine root growth and phenology

Average root length significantly increased over the growing season, but did not differ significantly among treatments (Table 1). However, the effect of the treatment changed over time (significant interaction between time and treatment, Fig. 4, Table 1). In the long-term treatment, average root length increased 9-fold from the beginning to the end of the season. The control and the short-term treatment, on the other hand, started out with very similar values of root length, c. 6 times higher than in the long-term treatment, but these two treatments then diverged over the season: in the control, root length increased by a factor of 5, whereas root length increased only by a factor of 3.7 in the short-term treatment. The data indicated consistently lower values of root length in the long-term treatment than the other two (Fig. 4), which



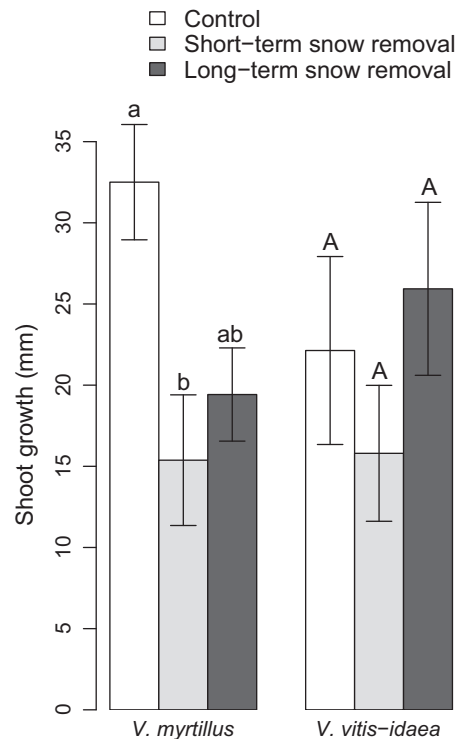
**Fig. 2.** Visible frost damage (percentage of tissue discoloured of each individual) of *Vaccinium myrtillus* and *V. vitis-idaea* in the control, short-term snow removal (1 winter) and long-term snow removal (11 winters). Means  $\pm$  SE, data are averaged from 5 individuals in each of three plots per treatment ( $n = 3$ ); different letters indicate significant differences among treatments (Tukey's test,  $P < 0.05$ ).

occurred at all depths (see Fig. S1 in Supporting Information). Interestingly, fine root length increase in the short-term treatment was more strongly reduced in the deeper soil layers than in the top soil layer (Fig. S1), although soil frost was more severe in the latter.

The timing of root growth (i.e. root phenology) also varied among treatments. Root growth started *c.* 6 weeks later in the long-term treatment and *c.* 3 weeks later in the short-term treatment than under ambient conditions (Fig. 4, Fig. S2). Once root growth had started, it steadily increased in all treatments until the peak of growth in late July to early August (around day of year 216) after which it slowed down again. There was thus a significant effect of day of year on root growth, but no treatment effect (despite the lower increases in root length in the short-term treatment compared to the control that lead to the significant interaction between day of year and treatment for root length), and no interaction between treatment and day of year (Fig. S2, Table 1).

#### Root biomass

Fine root biomass was almost three times higher in the upper soil layer ( $10.17 \text{ mg cm}^{-3} \pm 1.19$  at 0–20 cm depth, mean  $\pm$  SE) than in the deeper soil layer ( $3.55 \text{ mg cm}^{-3} \pm 0.65$  at 20–40 cm depth, Table 1). As was indicated by the minirhizotron data, fine root biomass (soil cores) was reduced by the snow treatments (both sampling depth combined, Fig. 5, Table 1). It was reduced by 39% in



**Fig. 3.** Shoot growth of the investigated growing season in mm of *Vaccinium myrtillus* (average growth of new branches) and *V. vitis-idaea* (the total shoot growth of the only, terminal shoot) in the control, short-term snow removal (1 winter) and long-term snow removal (11 winters), measured in August. Means  $\pm$  SE, data are averaged from 5 individuals in each of three plots per treatment ( $n = 3$ ).

the long-term treatment and by 16% in the short-term treatment compared to the control. The difference, however, was only marginally significant between the long-term treatment and the control ( $P = 0.05$ , Tukey's test). Reductions in fine root biomass with snow removal were similar at both sampling depths (no significant interaction between soil depth and treatment, Table 1).

#### Discussion

Our results showed that a lack of snow negatively impacted boreal forest vegetation in several ways, such as reducing plant abundance in the understorey, inducing frost damage above-ground as well as damaging fine roots, reducing root biomass and altering fine root phenology. Using a short- and long-term manipulation side by side showed that strong effects occur already after a single year of manipulation and that solely measuring short-term responses would have over-predicted long-term impacts. Below, we first discuss plant responses to an absent snow cover and then the inconsistent short- and long-term responses.

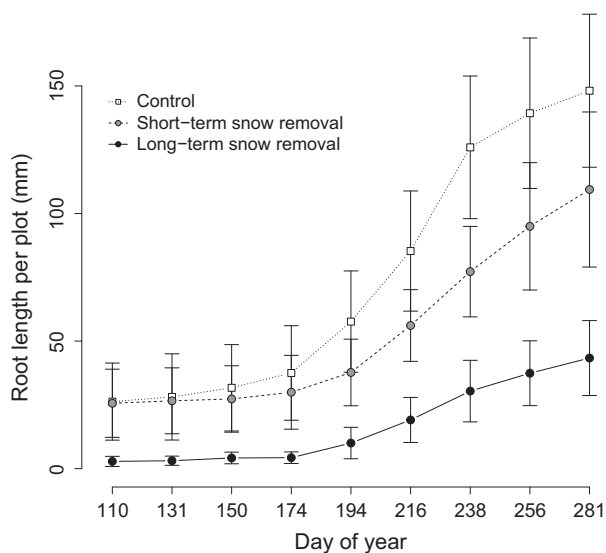
#### PLANT RESPONSES TO AN ABSENT SNOW COVER

Snow removal impeded growth and vitality of boreal forest plants, both above- and below-ground, indicating that the

**Table 2.** Mean above-ground cover (in %) and  $\pm$  SE of all bryophytes, all vascular plants and each species in the understorey per treatment

Species	Growth form	Control	Short-term	Long-term
All bryophyte		84.3 $\pm$ 6.4 n.s.	71.7 $\pm$ 9.3 n.s.	65.3 $\pm$ 16.6 n.s.
<i>Barbilophozia barbata</i>	Bryophyte	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.7 $\pm$ 0.7
<i>Dicranum scoparium</i>	Bryophyte	8.7 $\pm$ 2.8	8.0 $\pm$ 1.7	6.7 $\pm$ 1.8
<i>Hylocomium splendens</i>	Bryophyte	31.3 $\pm$ 12.4 n.s.	29.7 $\pm$ 13.7 n.s.	21.3 $\pm$ 8.4 n.s.
<i>Pleurozium schreberi</i>	Bryophyte	37.3 $\pm$ 12.0 n.s.	33.3 $\pm$ 6.4 n.s.	26.3 $\pm$ 17.4 n.s.
<i>Polytrichum commune</i>	Bryophyte	6.7 $\pm$ 5.7	0.7 $\pm$ 0.7	5.0 $\pm$ 4.5
<i>Ptilium crista-castrensis</i>	Bryophyte	0.3 $\pm$ 0.3	0.0 $\pm$ 0.0	3.0 $\pm$ 3.0
<i>Sphagnum girgensohnii</i>	Bryophyte	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	2.3 $\pm$ 1.5
All vascular		83.3 $\pm$ 12.0 a	48.0 $\pm$ 14.3 ab	6.3 $\pm$ 3.2 b
<i>Carex canescens</i>	Graminoid	46.0 $\pm$ 7.2 a	38.7 $\pm$ 13.4 a	2.0 $\pm$ 1.2 b
<i>Deschampsia flexuosa</i>	Graminoid	0.5 $\pm$ 0.5	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
<i>Luzula pilosa</i>	Graminoid	0.3 $\pm$ 0.3	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
<i>Equisetum sylvaticum</i>	Herbaceous	0.3 $\pm$ 0.3	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
<i>Gymnocarpium dryopteris</i>	Herbaceous	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	1.3 $\pm$ 1.3
<i>Linnaea borealis</i>	Woody	3.7 $\pm$ 3.2	0.0 $\pm$ 0.0	0.3 $\pm$ 0.3
<i>Picea abies</i>	Woody	0.3 $\pm$ 0.3	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
<i>Pinus sylvestris</i>	Woody	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.3 $\pm$ 0.3
<i>Vaccinium myrtillus</i>	Woody	31.0 $\pm$ 11.2 a	5.3 $\pm$ 2.4 ab	0.7 $\pm$ 0.3 b
<i>Vaccinium vitis-idaea</i>	Woody	1.3 $\pm$ 0.9	4.0 $\pm$ 0.6	1.7 $\pm$ 1.2

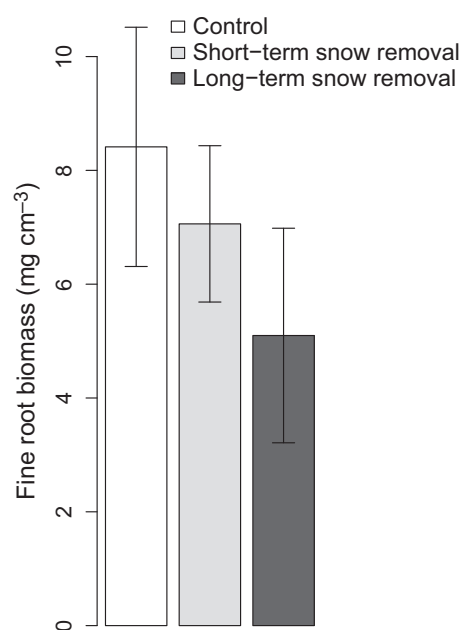
Significantly different groups for all bryophytes combined, all vascular plants combined and the two most common bryophyte and vascular plant species are indicated by different letters (Tukey's test,  $P < 0.05$ ).



**Fig. 4.** Fine root length observed per plot at nine occasions over the growing season, in control (open squares), short-term snow removal (1 winter, grey circles) and long-term snow removal (11 winters, black circles). Means  $\pm$  SE, data are averaged from three minirhizotron tubes installed down to a depth of 50 cm in each of three plots per treatment ( $n = 3$ ).

colder soil and air temperatures during winter outweighed any potential positive effects of a longer snow-free period.

Reduced growth and vitality above- and below-ground resulted in a decline in vascular plant cover in the short- and long-term snow manipulation treatments. As almost all species declined in a similar way in response to the snow manipulation treatments, the relative abundance of species remained remarkably similar. There was no indication of replacement of species with more frost-resistant ones after 11 years of



**Fig. 5.** Fine root biomass (in  $\text{mg cm}^{-3}$ , roots  $< 2$  mm diameter) in 0–40 cm depth in the control, short-term snow removal (1 winter) and long-term snow removal (11 winters). Control and long-term snow removal differ at  $P = 0.05$  (Tukey's test), means  $\pm$  SE,  $n = 3$ .

snow removal, probably a consequence of the limited species pool in boreal forests (Kreyling, Haei & Laudon 2012).

The two most common dwarf shrubs of the understorey, *V. myrtillus* and *V. vitis-idaea*, showed abundant discoloration of leaf and stem tissue and partly reduced growth, especially in the short-term treatment. Both discoloration and growth reduction are common signs of frost damage in woody plants (Sakai & Larcher 1987; Gu *et al.* 2008) and have previously been observed in these species if exposed to an earlier

snowmelt or insufficient snow depth (Raatikainen & Vänninen 1988; Wipf 2009; Gerdol *et al.* 2013; Wheeler *et al.* 2014; Saarinen *et al.* 2015). We generally found *V. myrtillus* to be more sensitive than *V. vitis-idaea*. In accordance, *V. myrtillus* preferably grows in sites with a thick snow cover within the boreal forest and is more affected by winter warming events compared to *V. vitis-idaea* (Bokhorst *et al.* 2011; Rasmus, Lundell & Saarinen 2011). Low levels of snow can also lead to additional stresses besides low temperatures, such as desiccation and high light levels, limiting or even preventing carbon gain through photosynthesis, at least in *V. vitis-idaea* which usually keeps its photosynthetic capacity through winter (Lundell *et al.* 2008; Lundell, Saarinen & Hänninen 2010; Saarinen *et al.* 2015).

As for below-ground responses, standing fine root biomass was reduced by the snow removal treatment. Snow removal has previously been shown to induce compensatory root growth (Tierney *et al.* 2001; Cleavitt *et al.* 2008; Gaul, Hertel & Leuschner 2008) but we observed the opposite: the absence of snow led to reduced fine root growth. Thus, while the control and the short-term snow removal treatment had similar amounts of root length in the beginning of the season, this diverged over the course of the growing season. This reduction in root growth and in standing fine root biomass could be the result of direct cellular root damage as it has been shown in mature trees (Cleavitt *et al.* 2008), or indirect root damage through frost movement of the soil as it can occur in boreal systems (Bergsten *et al.* 2001). It could also be a result of the injury of the understorey vegetation and the strongly reduced plant cover and consequently less photosynthetically fixed carbon available for root growth, altered biomass allocation in the plants or a combination of all of the above. Both a damaged and reduced root system during the growing season can negatively affect the plant water and nutrient uptake and may thus affect the plant health (Schaberg *et al.* 2008; Comerford *et al.* 2013).

In addition to reducing root growth, also root phenology was altered by the treatments despite no significant differences in above-ground phenology. Root growth in the snow removal treatments was initiated later and continued for longer on higher levels than in the control. Soils were also frozen for longer in the snow removal plots; thus, a lag in the start of the root growing season might not be surprising (Repo, Lehto & Finér 2008). However, this is in direct contrast to the previously observed earlier root growth with snow removal and consequently deeper and more severe soil frost than under ambient conditions (Tierney *et al.* 2001; Cleavitt *et al.* 2008). The timing of root production is important and changes therein might amplify temporal asynchronies between plant and microbial processes, especially in spring when soil microbial activity precedes root growth and nutrient uptake (Tierney *et al.* 2001; Groffman *et al.* 2012; Finzi *et al.* 2015).

Almost all knowledge of root responses of forest systems to soil frost stems from potted seedlings, with the exception of a few important studies in mature forest systems, mainly northern hardwood forests ecosystem of the Hubbard Brook

Experimental Forest (Tierney *et al.* 2001; Cleavitt *et al.* 2008; Gaul, Hertel & Leuschner 2008; Comerford *et al.* 2013; Campbell, Succi & Templer 2014). Despite similarities in mean January and July air temperatures between our study area and the northern hardwood forest ecosystem of the Hubbard Brook Experimental Forest, root responses differed distinctly: we found no compensatory growth but reduced growth, and later rather than earlier initiation of root growth. It remains unclear whether those differences arise from slight dissimilarities in the snow manipulations (snow was absent from our sites over the whole winter, not only in the first weeks), from differences in the tree species (the evergreen *Picea abies* compared to a mixture of evergreen and deciduous tree species) or from the presence of a rich understorey vegetation in our study system.

Understorey vegetation is very abundant at our study site (understorey vascular plant cover of 83% in the control) and is a major ecosystem driver in boreal forests of northern Sweden (Nilsson & Wardle 2005). Above-ground net primary productivity of the dwarf shrubs in the understorey is over half of that of trees, they have strong positive effects on litter decomposition and soil microbial activity and thus influence nutrient availability and plant growth (Nilsson & Wardle 2005). Understorey shrubs are a greater nitrogen sink than trees, and understorey performance is thus imperative in carbon and nitrogen cycling within the boreal forest ecosystem (Buchmann, Gebauer & Schulze 1996; Olsson & Falkengren-Grerup 2003; Leppälampi-Kujansuu *et al.* 2014). Snow removal and soil frost induce increased losses of nitrogen and dissolved organic carbon, decrease soil CO<sub>2</sub> efflux and lower soil CO<sub>2</sub> concentrations, and all of these effects can reach into the next growing season (Groffman *et al.* 2001a,b, 2006, 2011; Öquist & Laudon 2008; Haei *et al.* 2013; Campbell, Succi & Templer 2014). Root responses have been suggested as drivers of these changes, just as reduced uptake by roots was responsible for increased nitrogen leaching observed with soil frost (Comerford *et al.* 2013). While our results clearly show negative effects of absence of snow and thus soil frost on the root system, they also highlight that nitrogen leaching might, at least partly, be driven by damage to and reduction in the understorey vegetation (Kreyling, Haei & Laudon 2012).

#### INCONSISTENT SHORT- AND LONG-TERM RESPONSES

A key finding of this study is that the magnitude of plant responses differed between short- and long-term treatments and that the short-term treatment alone would have led to different conclusions about plant responses to a reduced snow cover. As expected, in some of the measured response parameters, such as above-ground cover and root biomass, the effects of the reduced snow cover continued in the same direction and accumulated over time, leading to stronger effects in the long-term than in the short-term treatment. These were factors with potentially far-reaching ecosystem effects, as they can alter carbon sequestration and nutrient cycles. However, even though these response parameters were affected more strongly in the long-term treatment, the



magnitude of the response was still less than what would be predicted as long-term effect from the short-term treatment. As an example, if one would assume a similar effect size and thus a constant relative (proportional) decrease every year and predict its value after 11 years of treatment, this would be a vascular plant cover of 0.19% and a fine root biomass of 1.2 mg cm<sup>-3</sup>. However, measured vascular plant cover after 11 years of treatment was 6% (corresponding to what would have been predicted to be present after 4–5 years) and fine root biomass 5.1 mg cm<sup>-3</sup> (corresponding to what would have been predicted to be present after 2–3 years). Surprisingly, some other above-ground responses of the understorey were not only less severe in the long-term treatment than predicted, but actually stronger in the short-term treatment than in the long-term one in absolute terms. The most obvious of those was visible frost damage which reached almost 30% on the individuals in the short-term treatment and < 6% in the long-term treatment. Likewise, shoot growth and spring phenology showed stronger responses in the short-term treatment, although the differences were not always significant. The latter was probably due to our relatively small sample size of three replicate plots.

A lack of, or a weaker, response in the long-term vs. the short-term treatment points to survival of only those individuals that can cope with these extreme conditions. This discrepancy in extrapolation or even stronger response in the short-term treatment of some possibly plastic parameters shows that the response is not simply steady and proportional but varies over time and predictions made on those assumptions would overpredict long-term effects of an absent snow cover on boreal forest plants. Similar declines in effect sizes of the response variables over time, and also genetic adaptations, even within a relatively short time, have been shown for other long-term experiments when comparing the results to earlier years of the study (Leuzinger *et al.* 2011). However, the effect size of a manipulated environmental variable may depend on another, non-manipulated one and thus vary among years, for instance depending on amounts of precipitation of a particular year. This usually complicates a comparison of short-term and long-term effects in the traditional approach of comparing early to later years of the same experiment, and a particular strength of our study lies in the simultaneous comparison of the short- and long-term effects (i.e. same measurement year).

Our results highlight the key value of long-term studies to reveal lasting responses of natural ecosystems to climate change, especially in systems with long-lived species, where complex drivers trigger nonlinear responses over large temporal and spatial scales (Brown *et al.* 2001; Leuzinger *et al.* 2011; Groffman *et al.* 2012; Lindenmayer *et al.* 2012; Phoenix *et al.* 2012; Parmesan *et al.* 2013).

Our results also show that ecologically significant changes can already occur after one snow-free winter in the boreal forest. The complete absence of snow was a rather drastic treatment that is not necessarily expected to occur to the same extent. However, having one winter with very little snow and severe frost is not unlikely and has, for example, occurred at

the study site in the winter of 1995/96 which had soil frost patterns comparable to our manipulation in magnitude and extent (Nyberg *et al.* 2001; Mellander, Löfvenius & Laudon 2007; Laudon & Ottosson Löfvenius 2015). A decline in vascular plant cover had previously been shown after 8 years of manipulation in this experiment; however, it remained unclear when these changes occurred (Kreyling, Haei & Laudon 2012). We show here that one winter without snow already significantly impacted changes that can feedback onto the carbon and nutrient cycling of the whole ecosystem.

## Conclusions

Using a short- and long-term snow removal experiment in a boreal forest ecosystem, we illustrated that a lack of snow, as hypothesized, impaired plant vitality by injuring the understorey, reducing understorey abundance, damaging fine roots, reducing root biomass and altering fine root phenology. This highlights that winter conditions feedback into the next growing season, as shoots and roots were not only damaged but their growth impaired afterwards. All of these responses do not only modify vegetation structure but can also pose critical feedback onto carbon and nutrient cycling. In addition, root phenological responses in this boreal forest differed distinctly from those in other mature forest systems, highlighting the need for more studies on winter climate change and root ecology in forest ecosystems. Comparing the short- and long-term responses first showed that some strong effects occurred after 1 year of manipulation, which is a realistic scenario. Secondly, solely measuring the short-term responses would have overpredicted the long-term impacts, possibly due to an adaptation of the plant species to the altered environmental conditions leading to a decline in effect size in the long-term. This further underlines the importance of long-term experiments and also highlights the value of comparing the short- and long-term effects side by side for forecasting ecosystem responses to climate change.

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## Data accessibility

Data are deposited in the Dryad Digital Repository <http://dx.doi.org/10.5061/dryad.t3g68> (Blume-Werry *et al.* 2016).

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