Climate change effects on freezing damage in three subarctic bryophytes

A snow manipulation field experiment in a tundra ecosystem in Abisko, Sweden

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Abstract
Climate change is expected to have a large impact on northern ecosystems. Increased temperatures and altered precipitation and snow cover patterns will have a great impact on subarctic tundra. Bryophytes form an important component of tundra ecosystems because of their high abundance and their importance in many ecological processes. The effect of elevation and snow cover on freezing damage in shoots of three subarctic bryophytes: Ptilidium ciliare, Hylocomium splendens and Sphagnum fuscum, was studied in a snow manipulation field experiment at different elevations in Abisko, Sweden, during early spring. The treatments included snow addition, snow removal and control. In addition, bryophyte healthiness at the plot scale was determined by image analysis using colour selection, and soil temperature and moisture data were collected. Freezing damage differed significantly among bryophyte species with P. ciliare having the lowest freezing damage. There was a decrease in freezing damage over time due to the increase in temperature as spring progressed. Counter expectation, freezing damage was higher at low elevation although the mean daily minimum temperature was lower at higher elevation, which might be due to adaptation effects. Snow treatment had only a minor effect on freezing damage, but it did have an effect on proportion of undamaged tissue at the plot scale which increased with increasing snow cover at high elevation, but decreased with increasing snow cover at low elevation. Soil moisture content was also affected by snow treatment. The number of freeze-thaw cycles was less for S. fuscum and H. splendens compared to bare soil plots, which indicates insulating capacities of these bryophytes. Freezing damage could not be explained by the measured climate variables alone; therefore, it is likely the result of a complex set of factors, possibly including solar radiation and disturbance by herbivores.

Key words: freezing damage; bryophytes; tundra; climate change; snow manipulation experiment
Introduction

Climate change is expected to be more severe and have greater impact on high latitudes and high altitudes in terrestrial habitats in the northern hemisphere (IPCC 2001). The mean annual temperature in Arctic regions has increased with 1-3 °C from 1954 to 2003, which is almost twice the rate compared to the rest of the world. An additional arctic warming of 4-7 °C is projected for the next 100 years, with winter temperatures rising more rapidly than summer temperatures (ACIA 2005). An overall increase in winter precipitation is projected for the future, but it is unclear whether this will lead to an increased amount of snow or a decreased amount of snow, as rising temperatures will also enhance snowmelt and reduce the fraction of precipitation that falls as snow (Räisänen 2008).

An increase in temperature causes ecosystem zones to shift: temperate forest move into boreal forest, boreal forest move into tundra and tundra move into polar desert (ACIA 2005; Anisimov et al. 2001). Similarly in mountains, cold-adapted species will move upwards to escape increasing temperatures (Pauli et al. 1996) and avoid competition when the climate gets milder at lower elevations (Callaway et al. 2002). Because plants in tundra and high-altitudinal areas are adapted to cold environments, e.g. by slow growth, high longevity and low competitive ability, their capacity to adapt to a changing climate is low. Arctic and alpine ecosystems are therefore highly threatened and especially where tundra occupies a narrow coastal area such as Fennoscandia, climate change might result in biome extinction (Jägerbrand et al. 2011).

Snow cover is an important feature in (sub) arctic habitats as it lasts for 8-10 months a year. The amount, duration and structure of snow cover affect the climatic conditions, ecological and hydrological processes, biodiversity, permafrost and land use (ACIA 2005; Callaghan et al. 2011). Snow cover duration is mainly determined by elevation and air temperature. At a local scale, snow depth and properties are determined by wind erosion and deposition which result in a variety of exposed areas, snow drifts and dunes (Callaghan et al. 2011). A shortened snow cover season increases the potential growing season; however, as snow has a great insulating capacity which protects the underlying vegetation from freezing temperatures, earlier snowmelt could also lead to cell damage as a result of frost (Kreyling, Haei, et al. 2012). Furthermore, snow cover has an impact on soil-moisture gradients during the growing season, for instance a thick snow layer will result in more snowmelt water. On the other hand, early snowmelt could lead to soil moisture deficits later in summer (Anisimov et al. 2001).

Bryophytes contribute to a large proportion of the biodiversity in northern ecosystems. Although bryophytes and vascular plants decrease with increasing latitude, the decrease in vascular plants is greater and therefore the relative abundance of bryophytes is higher (Jägerbrand et al. 2011, Slack 2011). Bryophytes inhabit a diversity of environments and play an important role in many ecological processes such as primary production, carbon and nutrient cycling, biodiversity, biogeochemistry, competition, facilitation, and feedback responses to climate change (Turetsky 2003; Soudzilovskaia et al. 2010; Jägerbrand et al. 2011; Turetsky et al. 2012; Lindo et al. 2013). More specifically, bryophytes are known to insulate the soil, resulting in a negative feedback on soil temperature which in turn affects thaw depth, season length and nutrient availability (Tenhunen et al. 1992). Bryophytes (especially Sphagna) produce recalcitrant organic matter, therefore they contribute substantially to the carbon sink of arctic ecosystems such as tundra, boreal forest and peatlands (Turetsky 2003; Lindo & Gonzalez 2010). Since bryophytes are such essential components in arctic ecosystems, it is important to study their different responses to climate change.
One of the possible outcomes of climate change as discussed here is an early snowmelt, leading to a shorter snow-cover duration and possibly more freezing damage in spring. Bryophytes are known to withstand freezing temperatures much better than vascular plants. The lower temperature limit for photosynthesis under natural atmospheric CO₂ concentrations and light saturation is thought to be around -8 °C for arctic and subarctic mosses (Glime 2007). Several studies have shown that some bryophytes can survive temperatures of -20 °C (Kennedy 1993; Lenné et al. 2010) to -25 °C (Rütten & Santarius 1992b). However, it can be much colder for longer periods of time in the Arctic; therefore, protection by snow is essential during winter and early spring. Little is known about winter activity of bryophytes, but they possibly start photosynthesis as soon as small amounts of light penetrate the snow (Glime 2007). Young moss leaves are more susceptible to freezing than mature tissue, which makes bryophytes more vulnerable during early spring when the growing season just started and temperatures are still dropping below 0 °C (Rütten & Santarius 1992a).

The formation of crystals inside the bryophyte tissue as the result of freezing is detrimental because it can damage the cell membrane resulting in solutes leaking from the cell. Ice crystals are hygroscopic, which means that they attract water molecules from the cell to the intercellular space where the crystals may form, which causes dehydration of the cell (Glime 2007). Tolerance to desiccation is therefore one way of surviving freezing temperatures, also referred to as freeze avoidance (Kennedy 1993; Lenné et al. 2010). Frost tolerance, in the cells of certain bryophytes, has been found to increase with increasing sugar concentration (Rütten & Santarius 1992b), which seems to be induced by the stress hormone abscisic acid (Nagao et al. 2005). These studies show there is some knowledge about the physiological mechanisms behind freeze avoidance and frost tolerance in bryophytes, but little is known about the impact of climate change on freezing damage in bryophytes in natural conditions. There are, however, a few studies on the impact of climate change on freezing resistance in other plant types, such as alpine treeline plants (Martin et al. 2010) and different populations of *Fagus sylvatica* (Kreyling et al. 2014).

This study aimed to test the effect of elevation, as a substitute for temperature and with a low elevation acting as future climate scenario, and altered snow cover on freezing damage in three common subarctic bryophyte species in a snow manipulation field experiment during early spring. The bryophyte species were *Hylocomium splendens*, *Ptilidium ciliare* and *Sphagnum fuscum*, which were transplanted from a high elevation site. Freezing damage was quantified from individual shoots and healthiness was determined at the plot scale. The hypotheses were that 1) freezing damage would be less at lower elevation because of higher temperatures; 2) freezing damage would be high in plots with little snow because of low insulation, and the effect of snow addition would result in similar levels of freezing damage at both high and low elevation; 3) freezing damage would decrease over time because of increasing temperatures as spring progressed and recovery from freezing damage; 4) the bryophyte species would differ in freezing damage with *P. ciliare* experiencing the lowest damage because of adaptation to high elevations and exposed sites (Signe Lett, personal communication), *H. splendens* experiencing the highest damage because of its high abundance on lower elevations, and *S. fuscum* experiencing intermediate damage because the capillary water-holding capacity of *Sphagnum* mosses (Rydin & Jeglum 2013), assuming that this buffer capacity would reduce the effect of freeze-thaw cycles. Furthermore, *H. splendens* and *S. fuscum* were expected to benefit more from increased snow cover compared to *P. ciliare*, because they were expected to suffer more from freezing temperatures.
Methods

Site description
The field experiment was situated at two sites of different elevations in a subarctic tundra system close to the Stordalen mire in Abisko (Figure 1), which is 200 km north of the polar circle in Sweden. The low site (68°20′50.84″N, 18°56′35.40″O) was situated below the treeline at approximately 350 m above sea level. The high site (68°19′38.16″N, 19°5′6.87″O) was situated above the treeline at approximately 700 m above sea level. Both sites were established in a treeless area with scarce vegetation dominated by Empetrum spp., Betula nana, bryophytes and lichens. The mean temperature in February is -11 °C, the mean temperature in July is 11 °C, and the mean annual precipitation is 310 mm (www.smhi.se).

Experimental design
In summer of 2013, five blocks of experimental plots were established at both sites at approximately the same elevation within each site to avoid microclimate effects. Each block included a snow-addition, a snow-removal, and a control plot. Each plot (approximately 1 m²) consisted of four cores (ø 24 cm), each containing one of the bryophyte species Hylocomium splendens, Ptilidium ciliare, Sphagnum fuscum, or bare soil. Bryophyte cores and soil were collected from 700 m elevation and transplanted to both the high and the low site. On each snow-addition plot a small enclosing squared snow fence (1.2 by 1.2 m) with an open top was placed, which allows a snow layer to build up during the winter and captures additional snow that is blown by the wind. An insulating fabric was placed over the bryophytes and soil cores to provide insulation before snowfall. The snow fences were in place from the end of September to mid-May (2014 and 2015) until the snow within the snow fences was completely melted away. The snow removal treatments were applied for approximately four weeks in late winter and early spring of
2014 and 2015 by removing the snow from the plots with a shovel up to a few centimetres to avoid damage to the moss cores. In 2015, snow removal started on April 14 at the high site and April 15 at the low site and repeated every week until all the snow had melted from the plots.

Sampling
The first sampling took place on May 10 and 11 at the low site and the high site, respectively, and the second sampling took place on 27 and 29 May for the low site and the high site, respectively. To measure freezing damage on individual bryophyte shoots, five healthy-looking shoots of approximately 2.5 cm were collected of each bryophyte species in each plot. Healthy-looking shoots were selected to make sure that bryophytes were active during the present growing season and were not remains of the previous year. The samples were stored overnight in the fridge at 4 °C, and the measuring of freezing damage was started the next day. To measure healthiness at the plot scale, standardized pictures were taken (before collecting the shoots) during the second sampling only.

REL method
The relative electrolyte leakage (REL) method according to Kreyling et al. (2012) was used to quantify freezing damage of the samples taken from the snow-manipulation field experiment. Initial conductivity was measured at room temperature after 24 hours of soaking in 16 ml of 0.1% (v/v) Triton X-100. Final conductivity was measured 24 hours after the samples had been in a water bath of 97 °C for one hour with the purpose to lyse all the cells. Along with each batch of 45 samples (one sampling moment of one site), three blank samples were taken of which the mean initial and mean final conductivity was calculated. The REL was then calculated using the formula:

\[
REL = \frac{\text{initial conductivity} - \text{initial blank}}{\text{final conductivity} - \text{final blank}}
\]  

(1)

Image analysis
To evaluate the damage of freezing on the plot scale, standardized pictures were taken using a digital single-lens reflex camera. A shading tent was used to minimize differences due to direct sunlight. Adobe Photoshop was used to crop the pictures and eliminate the area outside the moss cores, which then turns grey (Figure 2). Next, a colour of healthy-looking tissue was selected by eye from several randomly chosen pictures for each species. This colour was applied to all pictures (both elevations) of the same species using the Colour Range function in Photoshop, keeping the same fuzziness. Each picture was corrected for unwanted selected pixels such as dead leaves of other plants. The fraction of undamaged tissue was then calculated using the following formula (see also Figure 2):

\[
\text{Fraction of undamaged tissue} = \frac{n.o. \ of \ pixels \ of \ selected \ colour}{\text{total no. of pixels} - \text{n.o. of pixels outside the moss core}}
\]  

(2)

Climate data
Data loggers (Echo EM50, Decagon Devices Inc.) were present in four of the five blocks at each site. The loggers measured soil moisture (VWC, v/v) and temperature (°C) every hour at approximately 5-cm depth. Soil moisture and temperature data were collected from the field sites at June 15 and July 7. The data were combined in one dataset and clipped for the experimental period: one month from April 26 (two weeks before the first sampling) to May 26 (one day before the second sampling started). For each combination of site, treatment, and species, the number of
freeze-thaw cycles was calculated, as well as the mean daily minimum temperature. For the VWC (volumetric water content) the daily mean was calculated.

Data analysis and statistics
Repeated measures factorial analysis of variance (ANOVA) combined with linear mixed effects models were used to test for main and interaction effects of the four factors (elevation, snow treatment, bryophyte species, and sampling date) on REL. Elevation, snow treatment, and species were set as fixed factors, block was set as a random factor, and sampling date was set as a repeated measures factor. Stepwise regression (bidirectional elimination) by AIC was used to find the best-suited model. Post-hoc analysis was done using Tukey’s HSD to test for significant differences among more than two groups. The same model was applied for the healthiness (image analysis) and climate data as response variables, except that the repeated measures factor was left out. The statistical analyses on healthiness were performed for each species separately, because the colour selection was species specific. The healthiness data were proportional, therefore they were arcsine transformed before statistical analysis. If necessary, climate data were log- or square root-transformed to meet the assumptions of homogeneity of variances and normality. In addition to the bryophyte species, climate data of the soil cores were included as a fourth level within the bryophyte factor to see if the bryophytes had insulating capacities.

All statistical analyses were conducted with the free statistical software R, version 3.2.1 (R Core Team 2015). The additional packages nlme (Pinheiro et al. 2015), multcomp (Hothorn et al. 2008) lsmeans (Lenth & Hervé 2015) and sciplot (Morales 2012) were used.

Figure 2. Example of a picture of a *Hylocomium splendens* plot used for the image analysis.
Results

Freezing damage

Of the three bryophyte species, *Ptilidium ciliare* had the lowest freezing damage and *Sphagnum fuscum* had the highest freezing damage. Overall, the three bryophyte species all differed significantly from each other in terms of freezing damage (Table 1; pairwise comparisons: p<1e-10 for all comparisons; Figure 3C). Remarkably, the high site had on average lower freezing damage than the low site (Figure 3A). Snow addition resulted in significantly lower freezing damage than control; however, the differences between treatments are small (Figure 3B). Time also had an effect on freezing damage; the second sampling had significantly lower freezing damage than the first sampling (Figure 3D).

A two-way interaction between site and bryophyte species showed that only *S. fuscum* and *H. splendens* had higher freezing damage at the low compared to the high site whereas there was no difference in damage for *P. ciliare* between the two sites (Figure 4A). However, *P. ciliare* responded over time with decreasing freezing damage (pairwise comparisons: p<0.001, whereas the two other bryophyte species were unchanged over time. The interaction between time and bryophyte species was significant (Table 1; Figure 4C), and a significant interaction between site and time showed that freezing damage reduced with time at the high site but not at the low site (Figure 4B).

The three-way interaction including site, bryophyte species, and time specifically showed that the freezing damage at the first sampling point was more equal across sites for all three species, whereas the second sampling showed greater differences between the sites for *H. splendens* and *S. fuscum* (Figure 6; pairwise comparisons: p<0.001 for both). An additional three-way interaction showed that freezing damage of *P. ciliare* and *S. fuscum* was not affected by snow treatment, whereas *H. splendens* had lower freezing damage with snow addition compared to control at the low site (Figure 5; pairwise comparisons: p=0.021).

Table 1. Results of the repeated measures ANOVA and mixed effects model for the Relative Electrolyte Leakage as a measure of freezing damage. Numerator degrees of freedom, denominator degrees of freedom, F-values and p-values are given for the best-fitting model, using stepwise model selection by AIC (the interactions site : snow treatment : time and site : snow treatment : bryophyte : time were excluded). Significant effects are in bold.
Figure 3. Mean ± 1 SE for the Relative Electrolyte Leakage (REL) as a measure of freezing damage for the main effects: elevation (A), snow treatment (B), bryophytes species (C) and sampling time (D). The graphs show only one factor and are averages over the other factors. Statistically significant differences are indicated with a, b, c.

Figure 4. Mean ± 1 SE for the Relative Electrolyte Leakage (REL) as a measure of freezing damage for the site:time interaction, averaged across bryophytes and snow treatments (A); the species:site interaction, averaged across bryophytes and snow treatments; (B) and the species:time interaction, averaged over sites and snow treatments (C). The first sampling was on May 10-11, the second sampling was on May 27-29. Significant effects (p<0.05) within groups of bars are indicated with *.
Figure 6. Mean ± 1 SE for the Relative Electrolyte Leakage (REL) as a measure of freezing damage at the low site at 350 m (left) and the high site at 700 m (right) for different snow treatments and species, averaged for both sampling dates.
Undamaged tissue at the plot scale

*H. splendens* had a higher fraction of undamaged tissue at the high site, *S. fuscum* had more undamaged tissue at the low site, and *P. ciliare* did not differ between sites (Table 2; Figure 7). There was a highly significant interaction between site and snow treatment (Table 2; Figure 7). Undamaged tissue decreased in the order of snow addition to control to snow removal at the high site. On the contrary, undamaged tissue increased from snow addition to control to snow removal at the low site.

Table 2. Results of the factorial mixed effects ANOVA for the fraction of undamaged tissue as a measure of freezing damage at the plot scale. Numerator degrees of freedom, denominator degrees of freedom, F-values and p-values are given. Significant effects (p<0.05) are in bold.

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Climate data

*Soil temperature*

The mean daily minimum temperature was lower at the high site compared to the low site (ANOVA: F=36.02, p<0.001) and lower in snow-removal plots compared to snow-addition and control plots (pairwise comparisons: p<0.001 for both comparisons; Figure 8). The number of freeze-thaw cycles was significantly lower for *H. splendens* and *S. fuscum* compared to the soil plots (Pairwise comparisons: p=0.006 and p=0.003, respectively; Figure 10B). Furthermore, snow-removal plots had a significantly higher number of freeze-thaw cycles compared to snow-addition plots and control plots at the low site (Pairwise comparisons by Tukey: p=0.019 and p=0.008 respectively; Figure 10A).

*Soil moisture content*

Volumetric water content was lower in snow-removal compared to snow-addition plots (pairwise comparisons: p<0.001; Figure 9).

There was no correlation between any of the climate data discussed above and relative electrolyte leakage (freezing damage). See Appendix: Correlation climate variables and REL.
Figure 7. Mean ± 1 SE for the fraction of undamaged tissue (image analysis; selected pixels of a healthy colour) for different snow treatments and sites per species. Note that the y-axes are different to visualise the interaction between site and snow treatment.

Figure 8. Mean ± 1 SE of the daily minimum temperature for April 26 to May 26 2015 per site for treatments and bryophytes, bare soil included.
Figure 9. Mean ± 1 SE of the daily mean volumetric water content (VWC) for April 26 to May 26 2015 per site for treatments and bryophytes, bare soil included.

Figure 10. Mean ± 1 SE of the number of freeze-thaw cycles for April 26 to May 26 2015 for the treatment:site interaction (left figure; add=snow addition, con= control, rem=snow removal) and bryophytes and soil (Hs=Hylocomium splendens, Pc=Ptilidium ciliare, Sf=Sphagnum fuscum; right figure).
Discussion

The aim of this study was to determine the effect of elevation and altered snow cover on freezing damage in *H. splendens*, *P. ciliare* and *S. fuscum* during early spring in the sub-arctic. Bryophytes differed substantially in freezing damage and there was also a clear main effect of elevation, with lower elevation unexpectedly having the highest freezing damage. Increased snow cover had significantly lower freezing damage and as spring progressed, freezing damage decreased, as expected. At the plot scale, undamaged tissue increased with increasing snow cover at high elevation, but decreased with increasing snow cover at the low elevation. Remarkably, freezing damage was not correlated with climate variables, but minimum temperature, freeze-thaw cycles and soil moisture were affected by elevation and snow cover.

Bryophytes

The difference in freezing damage between species was quite consistent for different sites and treatments: despite the assumed (micro-) climatic effects of site and snow treatments, the bryophytes showed similar freezing damage when compared to each other. The bryophyte cores had less freeze-thaw cycles than the bare soil cores, which indicates that bryophytes have insulating capacities (Tenhunen et al. 1992). Although the bryophyte species were expected to differ in their insulating capacities, no difference was found in this study. It has been shown that the temperature regime within bryophyte mats differs greatly between species, but can be fully explained by mat thickness and soil moisture content; therefore species-specific physical properties seem to be more important than biological processes (Soudzilovskaia et al. 2013). Mat thickness was not measured here, but soil moisture content did not differ between species and therefore did not result in differences in insulation.

As expected, *P. ciliare* had the lowest freezing damage. This species grows at high elevation and on exposed sites (around Abisko) and seems to be adapted to these harsh environments through withstanding frost better than the other two species. Moreover, *P. ciliare* was not affected by elevation or snow cover, which supports the presumption that it is well adapted to low-temperature conditions. Probably *P. ciliare* did not suffer as much as the other species and was able to recover from the freezing damage measured earlier, which explains why it decreased in freezing damage over time whereas the other bryophytes did not.

*S. fuscum* had the highest freezing damage, although it was hypothesised that *H. splendens* would have the highest damage. *S. fuscum* was expected to have intermediate damage because of its capillary water-holding capacity, which could decrease freeze-thaw cycles because more water takes more time to freeze and more ice takes more time to thaw. However, the water-holding capacity hypothesis of *S. fuscum* was not supported by the soil moisture data: it did not have higher soil moisture levels compared to the other bryophytes. Although *S. fuscum* belongs to the *Sphagnum* genus (peat mosses), which are known for their capillary water-holding capacity (Rydin & Jeglum 2013), it is also a hummock species, which means that it can persist on drought-exposed mounds (Hájek et al. 2011).

Snow cover

Although soil minimum temperature, freeze-thaw cycles, and soil moisture were affected by snow cover in different ways, there was only a minor effect of snow cover on freezing damage, since only *H. splendens* had lower freezing damage under increased snow cover at low elevation. This is remarkable, knowing that snow cover is an important feature in protecting subarctic vegetation from freezing. It could, however, be that the differences in temperature and moisture caused by the altered snow cover were not substantial enough to cause differences in freezing damage.
Possibly the effect of decreased snow cover on freezing damage becomes more important over the long term, or when it is happening in winter when freezing temperatures are more extreme compared to spring. It has been shown for *H. splendens* that mid-winter snowmelt reduces photosynthetic rates and growth rates in the following growing season by around 50% (Bjerke et al. 2011). In another study understory species composition in a boreal forest was altered by snow removal: vegetation cover declined by more than 50%. Especially the most abundant bryophytes *Pleurozium shreberi* and *Dicranum scoparium* were affected, as well as the dominant dwarf shrubs (Kreyling, Haei, et al. 2012).

**Elevation**

Despite higher minimum temperatures at low elevation, freezing damage was lower at high elevation for *H. splendens* and *S. fuscum*. Possibly because freeze-thaw cycles are more important for causing freezing damage than daily minimum temperature, which is in line with a study of Antarctic mosses where repeated freeze-thaw cycles caused a greater reduction in photosynthesis than continuous freezing (Kennedy 1993). Indeed, more freeze-thaw cycles occurred at low elevation under less snow cover. However, this was not the case for the control plots, where low elevation had less freeze-thaw cycles, or under increased snow cover, which had approximately the same amount of freeze-thaw cycles at both elevations. Therefore, the freeze-thaw cycles can only partially explain the high freezing damage at low elevation. Probably adaptation plays a role here, since the bryophytes were transplanted from high elevation and were therefore adapted to the conditions occurring there. This adaptation might have caused the relatively low damage at high elevation. It has been shown that growth parameters of different populations of *H. splendens* can be correlated with early-summer temperatures and length of the growing season (Callaghan et al. 1997). This might result in a mismatch when bryophytes adapted to a certain climate are transplanted to a different elevation, as elevation has a strong effect on temperature and growing season.

**Plot-scale fraction of undamaged tissue**

Selection of healthy-looking shoots for measuring shoot-scale freezing damage might have caused a bias in the REL results, neglecting unhealthy patches, which were possibly affected by frost. To compensate this, freezing damage was also quantified at the plot scale using image analysis, which makes a distinction between damaged and undamaged parts of the plot. The image analysis seems a valid measure of overall performance of the bryophyte community, in addition to shoot-scale measurements on freezing damage. As with the shoot-scale freezing damage, bryophytes differed highly in undamaged tissue at the plot scale. However, this difference should be ignored for the plot scale since a different colour was selected for each species separately.

For *H. splendens* the fraction of undamaged tissue was higher at high elevation, which is consistent with the lower freezing damage, though unexpected, at high elevation. However *S. fuscum* had less undamaged tissue at the high site which is the opposite of the measured freezing damage. Therefore the fraction of undamaged tissue selected by colour might not be a good indicator for freezing damage in *S. fuscum*. However, a more interesting pattern is that increased snow cover resulted in less damage at high elevation, possibly because the bryophytes were protected against freezing damage in an unfavourable climate. Decreased snow cover resulted in less damage at low elevation, which could be explained by the early start of growing season in a favourable climate, whereas increased snow cover resulted a later start of the growing season. This corresponds to a study that found that shrub growth was enhanced with early snowmelt in years with few frosts, but decreased with early snowmelt in years with frequent frosts (Gerdol et al.)
It seems that increased snow cover can be beneficial at high elevations, but disadvantageous at lower elevations.

Other causes of damage
The REL method seems to be a valid method to measure cell damage within living bryophyte tissue (Strimbeck et al. 2007; Kreyling, Wiesenberg, et al. 2012; Kreyling et al. 2014). Because of the set-up of the snow-maniulation experiment, the measured damage was thought to be the result of frost; low temperatures and/or freeze-thaw cycles. However, no correlation was found between any of the climate variables and REL, nor fraction of undamaged tissue; which indicate there might be other causes of cell damage. Since 2015 was a peak year in vole and lemming densities it is possible that herbivory played a role. Also traces of moose and reindeer have been seen at the sites. Bryophytes are a common food source in northern ecosystems (Prins 1982; Gloutney et al. 2001) and are sensitive to disturbance by trampling (Van Der Wal & Brooker 2004). There were no visible signs of grazing, however some of the plots seemed to be partly damaged by trampling or grubbing. However it remains unclear whether herbivory, possibly, caused damage at the shoot-scale or at the plot-scale.

Solar radiation forms another threat for bryophytes. High light availability at low temperatures can be highly damaging for plants and especially for bryophytes with leaves of only one cell-layer thick (Glime 2007). Furthermore, UV radiation has increased in the Arctic and Antarctic over the past decades as a result of arctic ozone layer depletion. Due to the nature of ozone depletion, UV is generally increased in spring (ACIA 2005). For example, *H. splendens* has been shown to respond to increased UV-B with a reduction in annual growth (Arróniz-Crespo et al. 2011). Therefore, since temperature and soil moisture could not fully explain the patterns in freezing damage, the damage might be partially caused by high UV radiation. It might even explain difference in damage at different elevations, however there are no data available on this.

Further research
This field study indicates that freezing damage differs among bryophyte species and is affected by elevation, time of the year and slightly by snow cover. Bryophytes are known to have an increasing frost tolerance from summer to winter (Rütten & Santarius 1992b). It would therefore be interesting to study seasonal changes in freezing damage. Possibly, effects of snow cover and elevation might become more important in a long-term study, since this study, performed over a relatively short period, showed that effects of factors such as elevation and bryophyte species differed over time. In my study, the consequence is of the measured freezing damage for bryophyte performance is unknown. However, it is known from previous studies that winter warming events could have a strong impact on ecophysiology and growth of *H. splendens* (Bjerke et al. 2011). Therefore, additional experiments would be needed to measure photosynthesis or respiration to see how bryophytes cope with and recover from freezing damage throughout the growing season. Also, it would be relevant to measure the actual temperature limit at which bryophytes are able to withstand frost (see Martin et al. 2010, Kreyling et al. 2012b).

Concluding remarks
Freezing damage differed substantially among bryophyte species. *P. ciliare* was the best adapted species since it had the lowest freezing damage, was least affected by elevation and snow cover, and showed a decrease in freezing damage over time, which indicates recovery. Unexpectedly, freezing damage was lower at higher elevation and snow cover had only a minor effect on freezing damage, which is probably due to adaptation to high elevation conditions because the bryophytes were transplanted from 700 m to 300 m above sea level. Proportions of healthy or undamaged
tissue, on the other hand, seemed to increase with increasing snow cover at the high site and decreased by increasing snow cover at the low site. This indicates that increasing snow cover could reduce freezing damage at high elevations, as was hypothesized. However, this also leads to the thought that increased snow cover could be disadvantageous in a future climate with higher temperatures because it slows down the start of the growing season. Soil temperature, freeze-thaw cycles, and soil moisture content could not alone explain freezing damage. It is therefore likely that other factors, such as solar radiation and disturbance by herbivores, play a role in level of freezing damage in bryophytes.
Acknowledgements
I would like to thank Signe, first of all for the opportunity of doing a very interesting research project at a very beautiful place, second for being a good supervisor and third for being a great person to work with. I learned a lot from our discussions and your feedback and I had a really nice time working on the project!

Second I would like to thank Ellen and Mariet for being my official supervisors, keeping track of my work, providing useful feedback and interesting discussion points and for making it possible for me to graduate in time.

Third I’m very thankful to the field assistants and students who helped me out with field work when I broke my arm. I was very fortunate to be able to continue with the original plan, thanks to Max, Jan, Lotta, Simone, and Charly. Furthermore, I would like to thank Charly and Sylvain for driving me to the hospital, which is quite a time-consuming task in Abisko. Thanks as well to Laurenz for helping with practical stuff, Keith for helping with R stuff and Gesche for helping with the image analysis.

Last but not least, thanks to all people at CIRC and ANS for an amazing time in Abisko. I cherish nice memories and I hope to come back some time!
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Appendix

Correlation climate variables and REL

**Average daily minimum temperature – REL.**

Spearman's rank correlation rho

data:  FDMOSS_climate_complete$av_minT and FDMOSS_climate_complete$REL
S = 121956.6, p-value = 0.05169
alternative hypothesis: true rho is not equal to 0
sample estimates:
rho
0.1981603

**Freeze-thaw cycles – REL**

Spearman's rank correlation rho

data:  FDMOSS_climate_complete$soilfrth and FDMOSS_climate_complete$REL
S = 179559.2, p-value = 0.07675
alternative hypothesis: true rho is not equal to 0
sample estimates:
rho
-0.1805647
**Average daily mean VWC - REL**

Spearman's rank correlation rho

data:  FDMOSS_climate_complete$av_meanVWC and FDMOSS_climate_complete$REL
S = 140166, p-value = 0.4451
alternative hypothesis: true rho is not equal to 0
sample estimates:
rho
0.07843756