Received: 11 August 2023 | Accepted: 25 October 2023

DOI: 10.1111/1365-2745.14236

Journal of Ecology | BRITISH ECOLOGICAL SOCIETY

RESEARCH ARTICLE

Frost damage measured by electrolyte leakage in subarctic bryophytes increases with climate warming

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Funding information
Kempestiftelserna, Grant/Award Number: JCK-1112; Centrum för Miljövetenskaplig Forskning (CMF); Vetenskapsrådet (VR); Wallenberg Academy Fellowship

Handling Editor: Robert G. Björk

Abstract

1. Observed climate change in northern high latitudes is strongest in winter, but still relatively little is known about the effects of winter climate change on tundra ecosystems. Ongoing changes in winter climate and snow cover will change the intensity, duration and frequency of frost events. Bryophytes form a major component of northern ecosystems but their responses to winter climate changes are largely unknown.
2. Here, we studied how changes in overall winter climate and snow regime affect frost damage in three common bryophyte taxa that differ in desiccation tolerance in a subarctic tundra ecosystem. We used a snow manipulation experiment where bryophyte cores were transplanted from just above the tree line to similar elevation (i.e. current cold climate) and lower elevation (i.e. near-future warmer climate scenario) in Abisko, Sweden. Here, we measured frost damage in shoots of *Ptilidium ciliare*, *Hylocomium splendens* and *Sphagnum fuscum* with the relative electrolyte leakage (REL) method, during late winter and spring in two consecutive years. We hypothesized that frost damage would be lower in a milder climate (low site) and higher under reduced snow cover and that taxa from moister habitats with assumed low desiccation tolerance would be more sensitive to lower temperature and thinner snow cover than those from drier and more exposed habitats.
3. Contrary to our expectations, frost damage was highest at low elevation, while the effect of snow treatment differed across sites and taxa. At the high site, frost damage was reduced under snow addition in the taxon with the assumed lowest desiccation tolerance, *S. fuscum*. Surprisingly, frost damage increased with mean temperature in the bryophyte core of the preceding 14 days leading up to REL measurements and decreased with higher frost degree sums, that is, was highest in the milder climate at the low site.
4. *Synthesis* Our results imply that climate warming in late winter and spring increases frost damage in bryophytes. Given the high abundance of bryophytes in tundra ecosystems, higher frost damage could alter the appearance and functioning of

the tundra landscape, although the short and long-term effects on bryophyte fitness remain to be studied.

KEYWORDS
desiccation tolerance, frost sensitivity, mosses, relative electrolyte leakage (REL), snow manipulation experiment, tundra, winter ecology

1 | INTRODUCTION

Northern high-latitude ecosystems experience a faster rate of climate change compared with the global average, with the strongest temperature increase occurring during winter (IPCC, 2021). At the same time, winter precipitation is expected to increase in the Arctic and northern Europe (AMAP, 2019; IPCC, 2021). However, snowfall and snow cover are highly variable in space and time, and concurrent rising temperatures may result in a larger fraction of precipitation falling as rain (Bintanja & Andry, 2017). Warmer winters and more variable temperatures are furthermore associated with an increasing frequency of winter extreme climatic events such as rain-on-snow events (Vikhamar-Schuler et al., 2016). In alpine and arctic tundra, snow cover can last for 8–10 months a year and provide insulation against frost for the low-stature vegetation underneath. Changes in snow cover depth and duration can therefore have strong implications for tundra vegetation, such as severe (frost) damage and mortality (Bokhorst et al., 2009, 2010).

Bryophytes form an important component of tundra ecosystems due to their contribution to biodiversity (Mateo et al., 2016) as well as above-ground biomass, primary production and ground cover (Longton, 1997; Oechel & Sveinbjörnsson, 1978). Furthermore, they regulate many ecological processes such as carbon and nutrient cycling (Turetsky et al., 2012), moisture balance and microclimate (Elumeeva et al., 2011; Soudzilovskaia et al., 2013), and they provide food and habitat for various vertebrates and invertebrates (Gerson, 1982; Prins, 1982; Soininen et al., 2013). Tundra bryophytes have been found to decline with increasing summer temperature, likely as a result of increased competition from vascular plants (Elmendorf et al., 2012). However, the direct effects of changes in overall winter climate and especially snow conditions in tundra ecosystems on bryophytes are not well known (but see e.g. Küttim et al., 2019).

Most bryophytes are poikilohydric, which means they cannot actively control their water status. Bryophytes have therefore evolved to be, at least partially, tolerant to desiccation (Proctor et al., 2007; Proctor & Tuba, 2002). Freezing can induce similar dehydration effects when external or extracellular ice crystals are present: Due to the lower water potential of ice, growing ice crystals will draw water from adjacent cells until the water potential of the cell and of the ice are equal (Lenné et al., 2010; Pearce, 2001). When desiccated, bryophytes become photosynthetically inactive. In this dormant state, bryophytes can survive extreme stresses such as heat and frost, because ice formation within cells cannot take place when they are dehydrated (Lenné et al., 2010). However, frost damage may eventually occur if freezing-induced dehydration exceeds the dehydration tolerance of the cell. This results in damage to the membrane structure and consequently leakage of electrolytes and other solutes (Pearce, 2001). The degree to which bryophytes can recover from dehydration and withstand related stresses differs among species, and this is often related to their habitat preferences. As such, bryophytes of moist and shady places are typically the least tolerant, while those from exposed, arid places are most tolerant of desiccation (Proctor et al., 2007). A higher degree of desiccation tolerance likely results in a higher level of frost tolerance (Glime, 2017; Segreto et al., 2010), and vice versa, frost may induce desiccation tolerance in some species (Hájek & Vicherová, 2014). Therefore, it also seems likely that species differences in frost sensitivity may be predicted from habitat preferences (i.e. moisture conditions), but this has so far not been tested.

Under standardized conditions, several cold-acclimated bryophyte species have been found to survive and regenerate after exposure to temperatures down to −80°C (Perera-Castro et al., 2021; Segreto et al., 2010). Nevertheless, freezing-induced dehydration and thus potential frost damage becomes progressively greater with lower temperatures (Pearce, 2001), and photosynthetic recovery from freezing in an Antarctic moss species has been found to depend on the absolute minimum temperature (Kennedy, 1993). In addition to minimum temperature, repeated freeze–thaw cycles can inflict more damage than constant freezing over the same period (Kennedy, 1993) and lead to photoinhibition and sucrose leakage across bryophyte species (Lovelock et al., 1995; Melick & Seppelt, 1992; Rütten & Santarius, 1992a). Therefore, it remains unsettled how different aspects of winter climate change and the resulting changes in frost events—it being intensity, duration or frequency—cause actual frost damage in bryophytes in nature, and whether changes in snow cover alter the general impact of winter climate.

In this study, we investigate how changes in snow cover regime affect frost damage in tundra bryophyte species with contrasting habitat preferences and across different winter climates. For this, we used a bryophyte transplantation experiment in Abisko, subarctic Sweden, where cores of three common bryophyte species (*Hylocomium splendens*, *Ptilidium ciliare* and *Sphagnum fuscum*) were transplanted from the alpine tree line to two elevations differing in overall temperature, with the higher elevation representing a current cold climate and the lower elevation representing a near-future climate scenario. In addition, the bryophyte cores were subjected to

whole-winter snow addition, autumn and spring snow reduction, or control. We then quantified frost damage from individual bryophyte shoots after snowmelt in spring in two consecutive years after two and three winters of treatment. We expect frost damage to decrease with a more benign climate, that is higher minimum temperatures and more snow cover because of the insulating properties of snow, and that bryophyte taxa differ in frost sensitivity depending on their ecology. More specifically, we hypothesize that (1) frost damage is lower at low elevation; (2) frost damage decreases with snow addition and increases with snow reduction; and (3) bryophyte taxa from moister habitats with assumed low desiccation tolerance (*S. fuscum*) will be more sensitive to lower temperature and thinner snow cover than those from drier and more exposed habitats with assumed intermediate (*H. splendens*) and high desiccation tolerance (*P. ciliare*). Furthermore, to assess whether snow cover and temperature affected bryophyte performance, we measured shoot growth in *H. splendens* and *P. ciliare* within the same field experiment.

2 | METHODS

2.1 | Field sites and experimental design

The study was conducted using two 'field common gardens' situated at different elevations in natural tundra near Abisko, northern Sweden (Lett et al., 2017). One common garden, the 'high site' (68°19′38,16″ N, 19°5′6,87″ E) was situated approximately 50 m above the tree line at 710 m a.s.l. and represents current tundra climate. The other common garden, the 'low site' (68°20′50,84″ N, 18°56′35,40″ E), was situated at 380 m a.s.l. situated in a large opening within the birch forest and simulated a future tundra climate. The low site was 5 km from Abisko, which has subarctic climate with a mean annual temperature of −0.1°C, a mean December–February temperature of −9.4°C, and 335 mm of mean annual precipitation, of which 50% falls as snow (1980–2010, Abisko Scientific Research Station). Both sites were characterized by sparse low-growing heath and barren frost boils. The vegetation at both sites was dominated by low-stature (max 10 cm) vascular plant species such as *Empetrum nigrum ssp. hermaphroditum* (Hagerup) Böcher and *Betula nana* L., *Vaccinium uliginosum* L., *Vaccinium vitis-idaea* L., *Andromeda polifolia* L. and *Carex* spp., as well as various bryophyte and lichen species. At each elevational site, five blocks were established at 20–70 m distance, to ensure adequate independence among blocks given the high small-scale heterogeneity within these communities (Björk et al., 2007; Opedal et al., 2015). Each block consisted of three 1 m^2 plots and was 6 to 10 m apart and included each of three snow treatments (see below). Plots were placed away from tall objects such as rocks, shrubs or trees, which could affect snow thickness. All plots were selected to have horizontal surfaces with low vascular plant cover and limited frost movement which was identified through the absence of frost boils. Each plot included three transplanted intact bryophyte cores; each core covered by one of three bryophyte species as described below. The use of field common gardens as described here logistically allowed for labour-intensive snow manipulations and for repeated sampling under challenging winter and early spring conditions. Given the low and temporary impact of the experiment on the landscape, a fieldwork permit was not required.

2.2 | Bryophyte transplantations

The bryophyte species *Ptilidium ciliare* (L.) Hampe, *Hylocomium splendens* (Hedw.) Schimp. and *Sphagnum fuscum* (Schimp.) H. Klinggr. were selected for the transplantations because they are common both in tundra heath and subarctic birch forest in the area. They represent different growth forms and evolutionary lineages and are found in somewhat different habitat types. *Ptilidium ciliare*, a liverwort, grows in thin tufts on exposed ridges and extends into undergrowth of shrubs; the feather moss *H. splendens* often grows in thick homogenous wefts mixed with dwarf shrubs; and *S. fuscum* is a hummock-forming peat moss that grows in wetter parts of heathlands or the driest parts of mires. The intact, circular, monospecific bryophyte cores (Ø 25 cm, 6 cm deep) were collected from a site similar in climate compared with the high site, that is above the tree line at 670–710 m a.s.l., on 27 August 2013. All cores were cut at 6 cm depth, as gravel and rocks below this depth underneath *P. ciliare* prevented deeper coring. Soil was often present under *P. ciliare* and *H. splendens* cores, as these species typically form thinner colonies than *S. fuscum*. Patches of bryophytes were selected based on minimal abundance of vascular plants. If vascular plants were present in the cores, they were cut off rather than pulled to keep the structure of the bryophyte growth forms intact. In each plot, three holes with similar diameter to the bryophyte cores were dug to 15 cm depth and filled with 9 cm of standardized local soil, consisting of a mix of organic soils originating from both sites and from a *Betula pubescens* stand (next to the low site) and a *Pinus sylvestris* stand (2 km from the low site) at 380 m a.s.l. The bryophyte cores were placed on top of the standardized soil. By transplanting bryophyte cores to a similar climate (the high site) and to a near-future climate scenario (the low site) and subjecting them to different snow regimes (see below), we can assess how bryophytes would perform in a future climate compared with their current climate.

2.3 | Snow manipulation treatments

The snow manipulation consisted of three treatments: snow addition throughout the snow cover season, snow reduction during start and end of the snow cover season, and no snow manipulation, that is control. Snow manipulation treatments were carried out from autumn 2013 to spring 2016, that is during three consecutive winter seasons. Snow addition plots were created by placing 50 cm tall snow fences around each plot, enclosing an area of 1 × 1 m, which caused snow to accumulate inside each fence independent of wind direction to a depth four times higher compared with controls (Lett et al., 2017). Furthermore, the ground and bryophyte transplants in these plots

were covered with four layers of white, moisture-penetrable insulation fleece (gardening fleece; 17 g/cm^2, polypropene Nelson Garden AB, Sweden) in late autumn, to simulate insulation by snow before onset of natural snowfall. Fleeces and snow fences were in place from late autumn (end of October/beginning of November) and were removed in early spring (mid-May) as soon as the snow within the snow fences had melted. In the snow reduction treatment, snow was removed from the plots weekly for 2–4 weeks in late autumn (2013–2015) and 4–8 weeks in early spring (2014–2016) with a shovel or brush to ca. 3 cm snow depth to avoid damage to the bryophytes. Snow was removed in these periods only, that is during the seasonal transitions when climate change is expected to affect snow cover and ecosystem responses most strongly because temperatures fluctuate across the freezing point (Ernakovich et al., 2014).

2.4 | Sampling and frost damage assessment

We assessed the effects of the treatments on frost damage in bryophyte shoots after snow melt in spring, using the modified version of the relative electrolyte leakage (REL) assay as described by Kreyling, Wiesenberg, et al. (2012), based on Strimbeck et al. (2007). The principle behind this method is that frost-induced damage to the cell membrane causes leakage of solutes, which affects the conductivity of a liquid. Frost damage can therefore be quantified by relating measured conductivity before and after forced lysis of all cells of a plant sample. The electrolyte leakage principle has been applied to measure frost sensitivity or resistance in a wide range of vascular plants (e.g. Bucher & Rosbakh, 2021; Gurvich et al., 2002; Pérez-Harguindeguy et al., 2013).

To capture periods when bryophytes may be more vulnerable to freezing, we sampled multiple times after snowmelt (i.e. when all plots had melted out at least partially), and across two consecutive years to increase the generality of our findings. In 2015, sampling took place on May 10 and 27 at the low site and on May 11 and 29 at the high site. In 2016, we sampled on May 2, 11 and 16 and June 1 at the low site and on May 3, 10 and 17 and June 2 at the high site. At each sampling occasion, we collected bryophyte shoots from each species in each plot. Five living (i.e. healthy looking) individual shoots of approximately 2.5 cm length were selected randomly from each core, at a minimum of 5 cm distance from the core edge. After sampling, bryophyte shoots were stored at 4°C overnight until the REL assay was started the following day.

For REL estimation, each sample, consisting of five shoots, was soaked in 16 mL of 0.1% (v/v) Triton X-100 (VWR chemicals) for 24 h at 20°C to allow electrolyte leakage to equilibrate, after which we measured initial conductivity (SevenCompact S230, Mettler-Toledo AG, Switzerland). We then placed the samples in a water bath at 97°C for 1 h, with the purpose to lyse all cells. Final conductivity was measured after another 24 h at 20°C. Along with each batch of 45 samples (one sampling moment of one site), three blank samples, consisting of 16 mL of 0.1% (v/v) Triton X-100, were taken of which the mean initial and mean final conductivity was calculated. REL was then calculated as (initial conductivity − initial blank conductivity)/(final conductivity − final blank conductivity).

2.5 | Bryophyte performance

To assess the impact of snow and elevation on growth as a measure of actual fitness of the bryophytes, we tied cotton threads around two *H. splendens* and three *P. ciliare* shoots. Shoot growth was not measured on *S. fuscum* as shoots were not strong enough to withstand this handling. Distance from thread to tip was measured at the beginning and end of the growing season of 2014, and the difference was calculated to represent shoot length growth. For each plot, the shoot with the highest growth was used to assess growth response. This was done to avoid counting growth of shoots that were potentially affected by the measurement; that is, tying a thread around the moss shoot may disturb or even kill the shoot. In this way, we were more likely to be assessing actual treatment responses.

2.6 | Microclimate in the bryophyte cores

In spring 2015, temperature (°C) and moisture (volumetric water content (VWC), in $m^3 m^{-3}$) in the bryophyte cores were measured hourly (5TM sensors and ECH2O Em50 loggers, Decagon Devices Inc., WA, USA). Sensors were placed vertically in bryophyte cores so that measurements were taken at 0–5 cm depth (integrated) from the bryophyte surface and down. Sensors were placed in four out of five blocks in all bryophyte species and snow treatments at both sites. In spring 2016, bryophyte core temperature was measured as in 2015 (but using Tinytags, Plus2 TGP-4017 external sensors, Gemini Co., UK; and ECH2O Em50 loggers, Decagon Devices Inc., WA, USA) and in *H. splendens* cores only, and only in three out of five blocks, from mid-April until the beginning of June. All data were checked visually for discrepancies; untenable moisture values (i.e. VWC < 0 and VWC > 1) and bryophyte core moisture values measured when the soil was frozen (i.e. concurrent temperature ≤0°C) were removed. We further removed unreliable data from one control plot (showing exceptionally high temperatures right at the start of the spring snow treatments).

After data cleaning, we calculated the following microclimate variables: average daily mean temperature; absolute minimum temperature; no. of diurnal freeze–thaw cycles (if temperature reaches below and above 0°C in one calendar day); number of frost days (daily average temperature <0°C); frost degree sums (the absolute value of the sum of daily minimum temperatures below 0°C; that is, higher values correspond to more frost); and finally mean moisture content. We calculated these variables over the entire experimental period in 2015, that is 16 April to and including 26 May, that is right after the start of the spring snow treatments and just before the final REL sampling in 2015. In addition, for each REL sampling occasion in 2015 and 2016, we calculated all the same microclimate variables over the 14 days leading up to sampling, as well as an additional

variable for each measurement date that is the number of days since the last diurnal freeze–thaw cycle.

2.7 | Frost damage in unmanipulated bryophyte shoots

To assess the REL of healthy, nontransplanted bryophytes in spring, shoots *of H. splendens* and *P. ciliare* were collected on the 28 April 2016 just before snowmelt, from a half-open birch forest close to the low site. For each moss species, shoots were sampled from one site with thin snow cover (7–10 cm) and one site with thick snow cover (53–40 cm). For each snow regime and species, six samples containing five shoots each of about 5 cm length were collected. The samples were consistently subjected to +5°C for 144 h with alternating tubular lighting every 12 h in environmental test chambers (MLR-350, Sanyo Electric Biomedical Co., Ltd., Japan) before REL measurements were taken following the same protocol as described above for bryophytes exposed to experimental treatments in the field common gardens.

2.8 | Data analysis

To analyse the effects of sites and snow manipulations on frost damage in the different bryophyte species, we used a linear mixed effects model with all REL measurements as the response variable and site, snow treatment, species, year and their two-way and three-way interactions as fixed effect variables. To take account of the block structure in the experimental set-up and the repeated measures over time, we added a random effect structure of block, snow treatment nested in block, and bryophyte core (species) nested in snow treatment. In case of significant (interaction) effects, we performed pairwise comparisons using estimated marginal means (EMMs) with specified contrasts and Tukey adjustments. Bryophyte performance, that is shoot growth, was analysed similarly with a linear mixed effects model, but with site, snow treatment and species as fixed effects, and using a random effect structure of snow treatment nested in block.

We analysed the differences in microclimate variables between sites, snow manipulations and species with linear mixed effects models, with site, snow treatment, species and their two-way interactions as fixed factors, and a random effect structure of block, snow treatment nested in block, and bryophyte core (species) nested in snow treatment. Only data from 2015 were used as this data included all species and treatments. Here, we left out the three-way interaction because data cleaning resulted in too few microclimate calculations (observations) for several site-treatment-species combinations, which were therefore deemed unreliable. Differences in the no. of freeze–thaw cycles and no. of frost days were analysed assuming a Poisson distribution with a generalized linear mixed effects model, with the same model structure as for the other microclimate variables. Pairwise comparisons using estimated marginal means (EMMs) with specified contrasts and Tukey adjustments were performed in case of significant (interaction) effects.

TABLE 1 Results of a linear mixed effects model testing for the effects of site, snow treatment, species, year and their two-way and three-way interactions on relative electrolyte leakage (REL) in bryophytes. Significant effects are in bold and denoted with *($p < 0.05$) and ***($p < 0.001$).

	df	*F*	*p*
Site	1	28.58	**<0.001*** **
Snow treatment	2	2.09	0.157
Species	2	324.92	**<0.001*** **
Year	1	17.69	**<0.001*** **
Site × Snow treatment	2	3.72	**0.047***
Site × Species	2	1.07	0.352
Snow treatment × Species	4	0.80	0.532
Site × Year	1	5.79	0.017*
Snow treatment × Year	2	1.54	0.217
Species × Year	2	0.59	0.552
Site × Snow treatment × Species	4	3.46	**0.015***
Site × Snow treatment × Year	2	2.24	0.108
Site × Species × Year	2	3.18	**0.042***

To further explore the relationships between different microclimate variables and REL, we used linear mixed effects models, with REL as response variable and each microclimate variable per 14 days as explanatory variable in separate models, using a random effect structure of bryophyte core (species) nested within treatment, treatment nested within block and block nested within site (using data from 2015 and 2016). Marginal and conditional coefficients of determination (R^2) were calculated for each model to provide a measure of the variance explained by the fixed effect and the entire model (fixed and random effects), respectively (Nakagawa & Schielzeth, 2013).

For all linear mixed effects models, data were checked visually for homoscedasticity and normality to meet the assumptions for parametric testing. The analyses were performed in R version 4.1.1 (R Core Team, 2021), using the packages nlme (Pinheiro et al., 2021), lme4 (Bates et al., 2015), emmeans (Lenth, 2021) and MuMIn (Barton, 2020).

3 | RESULTS

Overall, REL as a measure of frost damage was higher at the low site at 380 m a.s.l. (0.42 ± 0.009; mean ± SE) than at the high site at 710 m a.s.l. (0.38 ± 0.008; Table 1). Frost damage differed strongly between taxa (all pairwise comparisons with $p < 0.0001$), with *P. ciliare* having the lowest overall REL (0.27 ± 0.006), followed by *H. splendens* (0.41 ± 0.007) and *S. fuscum*, which had the highest REL (0.51 ± 0.007; Table 1). Overall, frost damage was higher in 2015 (0.42 ± 0.009) than in 2016 (0.39 ± 0.007; Table 1).

The effect of snow treatment on frost damage depended on site and species (significant interactions for snow treatment × site and

site × snow treatment × species, Table 1). As such, REL in *H. splendens* was significantly lower under snow addition compared with snow reduction at the low site (EMMs, *t* ratio = −3.03, *p* = 0.021; Figure 1), whereas at the high site, REL in *H. splendens* did not differ between snow treatments. At the high site, REL in *S. fuscum* was significantly lower under snow addition compared with control (EMMs, *t* ratio = −3.12, *p* = 0.017) and compared with snow reduction (EMMs, *t* ratio = −2.88, *p* = 0.028; Figure 1), whereas there was no such effect for *S. fuscum* at the low site. Snow treatments did not affect REL in *P. ciliare* at any of the sites. Furthermore, REL was higher at the low site than at the high site for *H. splendens* under snow reduction (EMMs, *t* ratio = −2.88, *p* = 0.028) and for *S. fuscum* under snow addition (EMMs, *t* ratio = 4.39, *p* = 0.002; Figure 1). Frost damage was lower in 2016 than in 2015 at the low site for *H. splendens* (EMMs, *t* ratio = 2.48, *p* = 0.014) and *S. fuscum* (EMMs, *t* ratio = 3.93, *p* < 0.001) and for *P. ciliare* at the high site (EMMs, *t* ratio = 2.63, *p* = 0.009; Figure S1).

Maximum shoot growth during the growing season of 2014 was similar between *P. ciliare* and *H. splendens* (1.7 ± 0.42 and 1.4 ± 0.33 mm, respectively; averaged over sites and treatments). For both species, maximum shoot growth was ca. three times higher at the low site (2.3 ± 0.38 mm) than at the high site (0.8 ± 0.3 mm; Table S1). Snow treatment tended to affect maximum shoot growth, with snow addition resulting in higher growth (2.4 ± 0.54 mm) than control (1.1 ± 0.35 mm) and snow reduction (1.1 ± 0.41 mm), but this result was not statistically significant (Table S1).

Microclimate variables in the bryophyte cores were most strongly affected by site, snow treatment and the interaction between the two, while species identity and the interaction between site and species during the experimental period in spring 2015 had a smaller effect (Table 2; Figures 2 and 3). As expected, the high site had lower mean and minimum temperature and higher frost degree sums (meaning more severe frost) and number of frost days than the low site. Mean moisture was higher at the low site compared with the high site. Interestingly, the number of diurnal freeze–thaw cycles was unaffected by site, but it was significantly higher under snow reduction at the low site (Figure 2) and highest in *P. ciliare* compared with the other species (Figure 3). Also mean temperature was higher under snow reduction compared with control and snow addition at the low site, while minimum temperature and frost degree sums were lower under snow reduction regardless of site (Figure 2).

Counterintuitively, REL increased with increasing mean temperature and lower frost degree sums (meaning less severe frost over a 14-day period) and decreased with increasing moisture content, although their effects were small and only detectable when the random effect structure was considered (Table 3; Figure 4). There was no effect on REL of absolute minimum temperature, diurnal freeze–thaw cycles, number of frost days or number of days since last diurnal freeze–thaw cycle (Table 3).

Frost damage of unmanipulated shoots was about half as low in *P. ciliare* as in *H. splendens*. For both species, shoots growing under thinner snow cover had higher REL (Figure S2).

4 | DISCUSSION

4.1 | Milder climate leads to more frost damage

Our study reveals that frost damage as measured by REL was highest at the low site, representative of a near-future climate that is more benign, in contrast to our first hypothesis. This finding indicates that warming of the general climate may increase rather than decrease frost damage in bryophytes. It further shows that in nature, lower temperatures do not result in more frost damage per se, which is confirmed by the absence of a relationship between minimum temperature and frost damage. Rather, frost damage increased with higher mean temperature and lower frost degree sums, showing that freezing temperatures closer to 0°C may result in more frost damage than extreme freezing temperatures. During spring, a shift from below-zero temperatures in winter to above-zero during the growing season leads to extended periods of near-zero temperatures that enable more fluctuations around the freezing point (Ernakovich et al., 2014). In our study, higher numbers of diurnal freeze–thaw cycles were abundant during periods with minimum temperatures reaching 0°C while mean temperatures were already slightly above 0°C (Figure S4). However, the number of freeze–thaw cycles during the preceding 14 days preceding damage assessment showed no relationship with frost damage, nor did the number of freeze–thaw

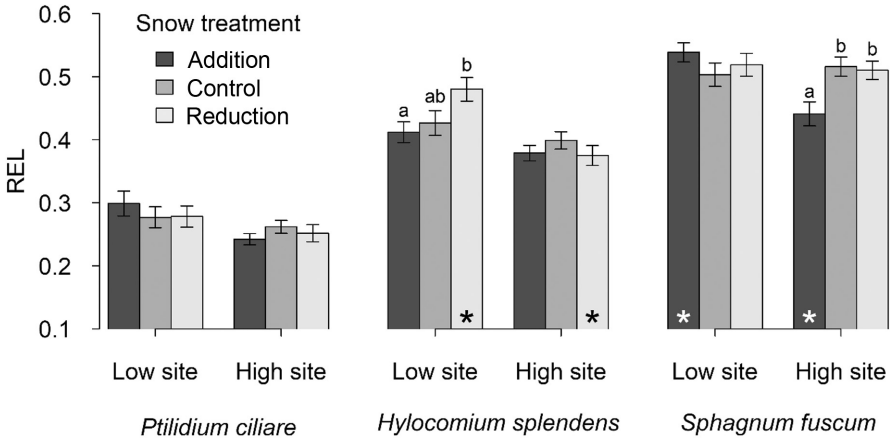

FIGURE 1 Mean (±SE) of relative electrolyte leakage (REL) as a measure of frost damage in bryophytes across species, sites and snow treatments, averaged across sampling occasions and blocks (*n* = 30). Significant effects ($p < 0.05$) are indicated with different lower-case letters for snow treatments within sites and species and with asterisks for site differences within species and snow treatments.

TABLE 2 Results of linear mixed effects models testing for the effects of site, snow treatment (snow), species and their two-way interactions on various microclimate variables calculated from hourly measurements taken at 0–5 cm depth inside the bryophyte cores, from 16 April to and including 26 May 2015; mean temperature (Mean T), absolute minimum temperature (Min T), frost degree sums (FDSUMS), no. of frost days (FRDAYS), no. of diurnal freeze–thaw cycles (DFT), and mean moisture (mean VWC). Significant effects are in bold and denoted with *($p < 0.05$), **($p < 0.01$) and ***($p < 0.001$).

		Mean T		Min T		FDSUMS		FRDAYS		DFT		Mean VWC	
	df	*F*	*p*	*F*	*p*	*F*	*p*	χ^2	*p*	χ^2	*p*	*F*	*p*
Site	1	121.63	**<0.001*** **	86.70	**<0.001*** **	88.20	**<0.001*** **	225.42	**<0.001*** **	0.153	0.695	8.18	**0.035***
Snow	2	11.69	**0.006****	37.61	**<0.001****	16.95	**0.002****	0.89	0.642	48.07	**<0.001*** **	0.43	0.667
Species	2	2.05	0.155	0.90	0.423	0.94	0.408	0.02	0.991	9.84	**0.007****	0.16	0.856
Site × Snow	2	9.17	**0.011***	0.47	0.644	1.30	0.331	1.19	0.553	7.73	**0.021***	0.41	0.682
Site × Species	2	0.05	0.949	3.74	**0.042***	2.00	0.162	0.40	0.818	6.55	**0.038***	1.65	0.216
Snow × Species	4	1.14	0.369	1.50	0.241	1.67	0.197	0.43	0.980	5.93	0.205	1.17	0.351

cycles differ between the high and the low site. Not only the frequency of freeze–thaw events (Kennedy, 1993) but also the rate of cooling may be important, and it has even been suggested that direct freezing injury may be unlikely to occur at the relatively slow rates of cooling experienced in nature (Dilks & Proctor, 1975). Since we clearly observed frost damage in our bryophytes, other and more complex effects of spring climate may be responsible for the higher frost damage at the low site.

As such, the positive relationship between frost damage and mean temperature and the negative relationship between frost damage and frost degree sums (i.e. higher frost damage at higher temperatures and less frost) suggests that frost events may be more damaging not only when occurring at the generally milder, lower site, but also as spring proceeds and the general climate becomes warmer with time. Indeed, increasing frost damage over time was observed in 2016, but not in 2015 (Figure S3). An increase in frost hardiness has been linked to elevated sugar concentrations in bryophytes in winter, and vice versa, elevated temperatures caused a decrease in sugar concentrations and frost hardiness (Rütten & Santarius, 1992b). Furthermore, young moss shoots have been found to be more susceptible to frost than mature shoots (Rütten & Santarius, 1992a). This suggests that bryophytes may be particularly sensitive to frost events in spring, as young shoots start to develop and elevated temperatures decrease frost hardiness, at a time when temperatures may still drop below zero occasionally. Given the milder climate at the low site, and the corresponding earlier snow melt-out, development of young shoots and deactivation of frost hardiness may start earlier, and occasional frost events may therefore have a stronger impact than at the high site.

4.2 | Frost damage responses to changes in snow cover depend on general climate and taxon

Snow treatments had clear effects on the subnival microclimate, while their effects on frost damage were weaker and depended on bryophyte taxon and site. Our second hypothesis was therefore partly confirmed, as snow addition resulted in lower frost damage compared with control and snow reduction but not consistently across all taxa and sites. *Sphagnum fuscum*, which suffered most frost damage of the three taxa, benefited from snow addition at the high site. The high site generally had lower snow cover than the low site (Lett et al., 2017) and snow addition was therefore likely to give more pronounced protective effects here. Meltwater influx was likely increased in snow addition plots, even though there was no overall treatment effect on mean moisture content in the bryophyte cores throughout the experimental period. Since *Sphagnum* species are adapted to moist conditions, increased meltwater influx may have protected *S. fuscum* from frost dehydration, as frost damage decreased with increasing moisture.

Hylocomium splendens suffered the highest frost damage under snow reduction at the low site. Less wind exposure at the low site meant that the snow layer in control plots was deeper than at the

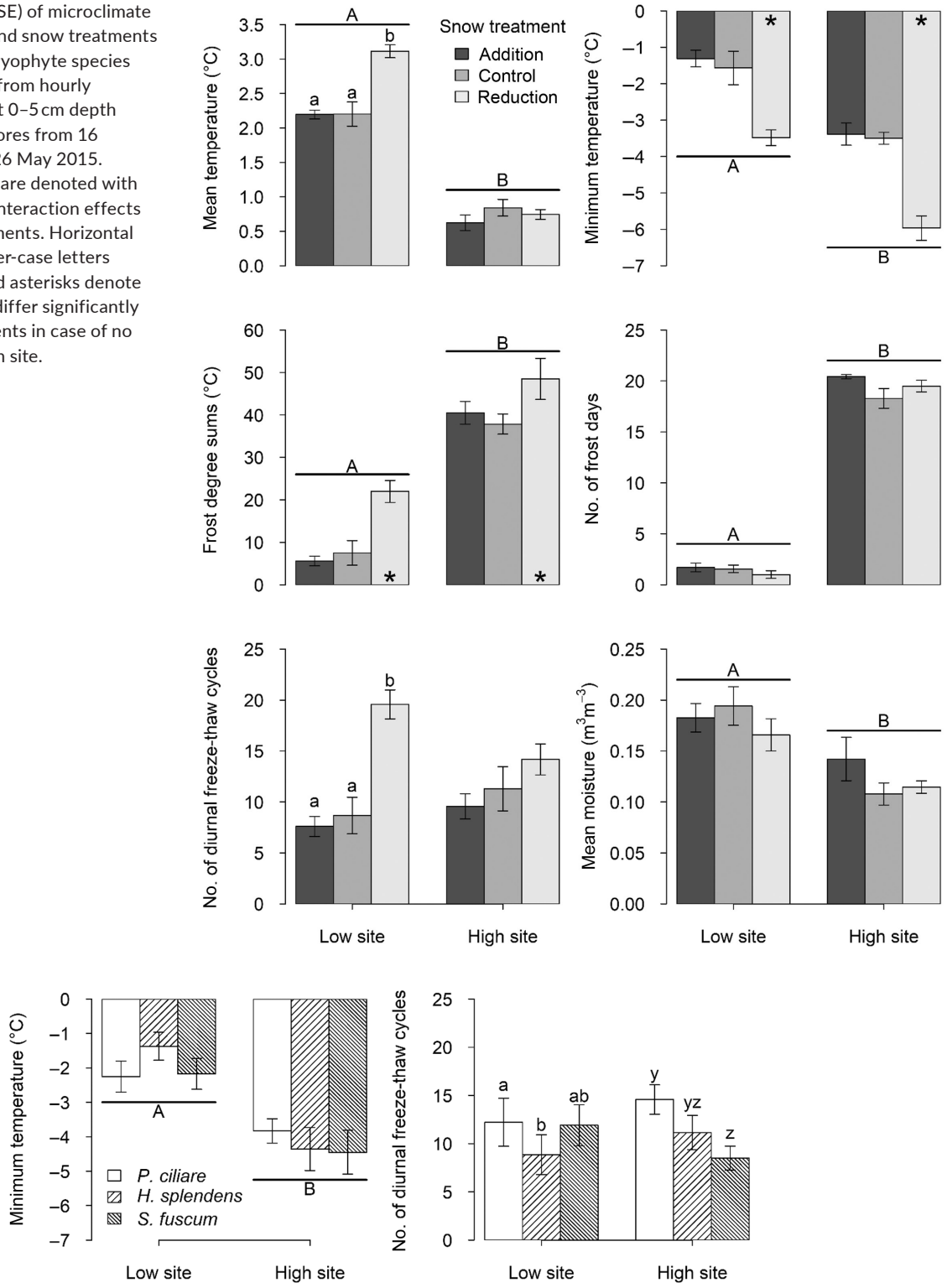

FIGURE 2 Mean (±SE) of microclimate variables across sites and snow treatments and averaged across bryophyte species and blocks, calculated from hourly measurements taken at 0–5 cm depth inside the bryophyte cores from 16 April to and including 26 May 2015. Significant differences are denoted with lower-case letters for interaction effects of site and snow treatments. Horizontal bars and different upper-case letters denote site effects, and asterisks denote snow treatments that differ significantly from the other treatments in case of no interaction effects with site.

FIGURE 3 Mean (±SE) of minimum temperature and no. of diurnal freeze–thaw cycles variables across sites and bryophyte species (*Ptilidium ciliare*, *Hylocomium splendens* and *Sphagnum fuscum*), averaged over snow treatments and blocks, calculated from hourly measurements taken at 0–5 cm depth inside the bryophyte cores from 16 April to and including 26 May 2015. Significant differences are denoted with lower-case letters for interaction effects of site and species (given as species differences within sites) and with horizontal bars and upper-case letters for site effects.

high site (as shown in Lett et al., 2017), and snow reduction therefore had a stronger effect on the microclimate there than at the high site. However, our observation suggests that low snow cover in a warmer spring climate can be harmful for *H. splendens*, which is in line with observed damage to the photosynthetic performance in *H. splendens* after a simulated winter warming event (Bjerke et al., 2011). One

TABLE 3 Results of linear mixed effects models testing for the effect of separate microclimatic variables on relative electrolyte leakage (REL) in bryophyte cores, using a random effect structure of bryophyte core (species) nested within snow treatment, snow treatment nested within block and block nested within site. Microclimatic variables were calculated over the 14 days prior to each REL sampling in 2015 and 2016. Marginal and conditional coefficients of determination (R^2) are given for each model to provide a measure of the variance explained by the fixed effect and the entire model (fixed and random effects), respectively. Significant effects are in bold and denoted with *($p < 0.05$); plus (+) and minus (−) indicate whether significant relations were positive or negative.

	+/−	Marg. R^2	Cond. R^2	*p*
Mean temperature	+	0.02	0.58	**0.037***
Minimum temperature		0.01	0.57	0.153
Frost degree sums	−	0.02	0.58	**0.027***
No. of frost days (average temp. <0°C)		<0.01	0.56	0.797
No. of diurnal freeze–thaw cycles (DFT)		<0.01	0.56	0.437
No. of days since last DFT		<0.01	0.57	0.723
Mean moisture	−	0.04	0.75	**0.021***

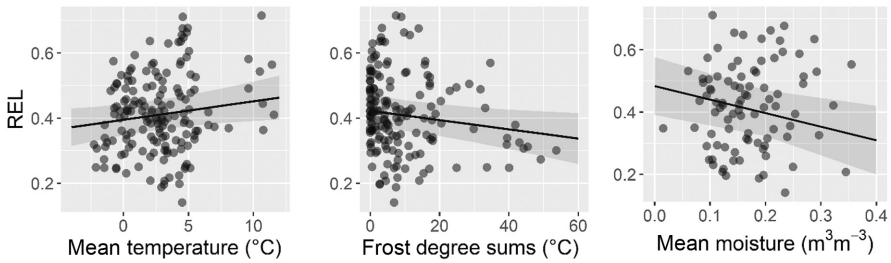

FIGURE 4 Observed (dots) and predicted (line) values of relative electrolyte leakage (REL; measure of frost damage) with 95% confidence levels (shaded area) from separate mixed effects models (as given in Table 1) using mean temperature, frost degree sums or mean moisture as independent effects. Microclimate variables were calculated over the 14 days prior to each REL sampling in 2015 and 2016.

mechanism for damage can be the low snow cover combined with a relatively high number of freeze–thaw cycles in the exposed shoots, as also seen in vascular plants (Kreyling, Haei, et al., 2012). However, the combination of exposure to frost in a milder climate could also mean that bryophytes are more photosynthetically active and excessive light may result in slightly higher damage under reduced snow cover for *H. splendens*. High-light intensities can be especially damaging to bryophytes in early spring when temperatures are low (Küttim et al., 2019). As a pleurocarpous, weft-forming species that is often less pigmented and thus potentially less light-protected than the other taxa, *H. splendens* may be more vulnerable to excessive light.

Bryophyte taxa responded differently to the snow treatments and sites, in line with their habitat preferences and assumed desiccation tolerance and confirming our third hypothesis. Studies on cryopreservation of bryophytes have demonstrated a positive correlation between desiccation tolerance and freezing tolerance (Burch, 2003; Segreto et al., 2010), which could explain the large differences in overall frost damage among the taxa in the present study. *Sphagnum* species are adapted to high moisture habitats and are equipped with adaptations such as hyaline cells, which makes their tissue especially good at holding on to moisture (Hájek & Beckett, 2008). *Sphagnum* species therefore have much lower desiccation tolerance than other moss lineages (Hájek & Vicherová, 2014; Vitt et al., 2014). In accordance, *S. fuscum* exhibited the highest frost damage in our study. *Sphagnum fuscum* is a mire species forming hummocks in peatlands and in the wetter parts of tundra ecosystems, which is further demonstrated by its high indicator value for moisture (Ellenberg & Leuschner, 2010). Despite *S. fuscum* being one of the more desiccation-tolerant peat mosses, a comparison of different *Sphagnum* species revealed that *S. fuscum* had relatively low frost tolerance, even compared with hollow species that generally experience milder conditions in nature (Balagurova et al., 1996). On the other end, *P. ciliare* can grow in more exposed sites than the other taxa and thus may be helped by its high tolerance for desiccation (Wood, 2007) to withstand freezing better. This could explain why frost damage was unaffected by site and snow treatment in this species.

Despite their ecological differences, it should be noted that the species included in the study represent very different evolutionary lineages. The large differences in frost damage between these taxa could therefore originate from a higher functional level, which may be consistent within lineages regardless of ecological preferences. The large differences in frost damage between unmanipulated shoots of *H. splendens* and *P. ciliare* support this idea, but more work is needed to answer this question.

4.3 | Bryophytes in a changing winter climate

Previous work has shown that the long-term absence of snow cover results in strong declines in bryophyte biomass, which was attributed to frost damage (Kreyling, Haei, et al., 2012). Although we did not find such strong effects, the site and snow treatment had clear impact on the microclimate in our study, but microclimate variables only explain little of the frost damage responses we observed (as shown by the low marginal coefficients of determination). Importantly, microclimate in the actual shoot is challenging to measure but might differ substantially from the bryophyte mat conditions that we measured (Rice et al., 2018). It therefore remains partly unsettled under exactly which circumstances bryophytes are most susceptible to frost damage.

Experimental studies in controlled environments have shown that freeze–thaw events cause photoinhibition and sucrose leakage in bryophytes (Kennedy, 1993; Lovelock et al., 1995; Melick & Seppelt, 1992; Rütten & Santarius, 1992a). Various field experimental studies found positive effects of snow addition and negative effects of snow removal on bryophytes, highlighting the importance of insulating snow cover against frost. For instance, winter and early spring snow addition had protective effects on *S. fuscum* capitula and increased biomass production in the subsequent growing season (Dorrepaal et al., 2004), while winter warming and snow removal led to severe damage in bryophytes, with consequences reaching far into the following growing season (Bjerke et al., 2011; Küttim et al., 2019). Our study further adds to these findings that bryophytes in nature experience frost damage when subjected to harsh conditions, while unmanipulated shoots exhibited much lower frost damage. Yet, the consequences for survival and growth after frost damage remain to be studied. Although other studies have reported long-lasting effects (Bjerke et al., 2011; Küttim et al., 2019), bryophyte shoot growth during summer in our study indicates that frost damage does not necessarily have to be detrimental.

The bryophyte cores were transplanted ca. 21 months before the first frost damage measurements were taken, but the lower frost damage in 2016 compared to 2015 may suggest that bryophytes were still acclimatizing during this study. Previous work has shown that acclimatization, that is formation of a new photosynthetic apparatus, occurred in 2 years for bryophytes that were transplanted over a large geographic distance (Kallio & Saarnio, 1986). In our study, the bryophyte cores were transplanted from a separate collection site to a similar elevation (the high site) and a lower elevation (the low site), but the higher shoot growth over summer indicates that bryophytes were not better acclimatized to the high site conditions compared with the low site. Instead, shoot growth was higher at the low site with the more benign climate. The opposite patterns for shoot growth and frost damage at the two sites suggest that the frost damage responses are likely the result of the factors that we aimed to test, rather than a site acclimatization effect. Furthermore, the lower frost damage in unmanipulated shoots of *H. splendens* and *P. ciliare* suggests that all bryophyte cores in the field common gardens experienced more severe frost damage than the unmanipulated shoots.

4.4 | Conclusion

Our study indicates that warmer winters may have negative impacts on tundra bryophytes and that snow cover can modify the effect of winter conditions in some taxa. Climate predictions show that precipitation is expected to increase in many but not all regions in the Arctic (AMAP, 2019) and more precipitation may fall as rain (Bintanja & Andry, 2017). Future snowfall and snow cover patterns are also highly variable in the landscape (Litaor et al., 2008). The differences in frost damage responses among bryophyte taxa indicate that species naturally occurring in more exposed sites may be more robust towards changes in overall climate and snow cover, likely because they are more desiccation-tolerant and can recover photosynthesis upon rehydration (Deltoro et al., 1999). *Sphagnum* and other wetland taxa that have low desiccation tolerance will likely experience the most frost damage unless protected under sufficient snow cover, which may not always be the case for hummock-forming species such as *S. fuscum*. Despite high levels of frost damage in late winter, our growth measurements indicate that bryophyte performance does not necessarily have to be constrained in the following growing season. The fact that the three taxa included in this study have widespread distributions and can also be found in warmer climates suggests that these species may be plastic enough to cope with future climate changes. For instance, subalpine populations may slowly replace the alpine populations of *H. splendens* (Cronberg, 2004). Nevertheless, extreme events such as winter warming resulting in reduced snow cover, rain-on-snow events, or early snowmelt followed by frost events, will likely affect bryophytes negatively. Under a future climate scenario, higher frost damage combined with other climate-related stresses might therefore reduce fitness of bryophyte colonies (Bjerke et al., 2011; Bragazza, 2008; He et al., 2016; Kreyling, Haei, et al., 2012). Compromised bryophyte fitness may in turn impact ecosystem processes such as carbon and nutrient cycling, water retention, seedling establishment and trophic interactions (Elumeeva et al., 2011; Lett et al., 2020; Turetsky et al., 2012), with consequences for the appearance and functioning of the tundra landscape.

AUTHOR CONTRIBUTIONS

Signe Lett and Ellen Dorrepaal conceived the idea; Kristel van Zuijlen and Marlene Kassel conducted field and laboratory work in 2015 and 2016, respectively, with supervision of Signe Lett and Ellen Dorrepaal; Marlene Kassel wrote a first version of the manuscript; Kristel van Zuijlen analysed the data and wrote the final manuscript with help from Signe Lett; all authors contributed to the writing and approved the final manuscript.

ACKNOWLEDGEMENTS

We thank the editor and three anonymous reviewers for their helpful comments on the manuscript. For valuable help in the field and at the research station, we thank Jan Borgelt, Alisa Brandt, Charly Géron, Simone Santos, Max Schuchardt, Laurenz Teuber and Elias Viippola. This study benefited from funding by Kempestiftelserna (Grant number JCK-1112), Centrum för Miljövetenskaplig Forskning (CMF), Vetenskapsrådet (VR), and a Wallenberg Academy Fellowship to E.D. Finally, we thank the Swedish Polar Research Secretariat and SITES for the support of the work done at the Abisko Scientific Research Station.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/1365-2745.14236.

DATA AVAILABILITY STATEMENT

Data associated with this article are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.79cnp5j2q (van Zuijlen et al., 2023).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Maximum shoot growth.

Figure S1. Relative electrolyte leakage across years.

Figure S2. Relative electrolyte leakage of unmanipulated shoots.

Figure S3. Relative electrolyte leakage across sampling occasions.

Figure S4. Microclimatic variables across sampling occasions.

How to cite this article: van Zuijlen, K., Kassel, M., Dorrepaal, E., & Lett, S. (2024). Frost damage measured by electrolyte leakage in subarctic bryophytes increases with climate warming. *Journal of Ecology*, *112*, 220–232. https://doi.org/10.1111/1365-2745.14236