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## ORIGINAL RESEARCH

# Sub-arctic mosses and lichens show idiosyncratic responses to combinations of winter heatwaves, freezing and nitrogen deposition

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## Abstract

Arctic ecosystems are increasingly exposed to extreme climatic events throughout the year, which can affect species performance. Cryptogams (bryophytes and lichens) provide important ecosystem services in polar ecosystems but may be physiologically affected or killed by extreme events. Through field and laboratory manipulations, we compared physiological responses of seven dominant sub-Arctic cryptogams (three bryophytes, four lichens) to single events and factorial combinations of mid-winter heatwave (6°C for 7 days), re-freezing, snow removal and summer nitrogen addition. We aimed to identify which mosses and lichens are vulnerable to these abiotic extremes and if combinations would exacerbate physiological responses. Combinations of extremes resulted in stronger species responses but included idiosyncratic species-specific responses. Species that remained dormant during winter (March), irrespective of extremes, showed little physiological response during summer (August). However, winter physiological activity, and response to winter extremes, was not consistently associated with summer physiological impacts. Winter extremes affect cryptogam physiology, but summer responses appear mild, and lichens affect the photobiont more than the mycobiont. Accounting for Arctic cryptogam response to multiple climatic extremes in ecosystem functioning and modelling will require a better understanding of their winter eco-physiology and repair capabilities.

## 1 | INTRODUCTION

The Arctic is experiencing more extreme weather events due to climate change, causing high mortality rates among species when events surpass survival thresholds (Walsh et al., 2020). Winter, in particular, is expected to experience more extreme events such as rain on snow, heatwaves (extreme winter warming [WW]), ground ice formation and loss of snow

cover (Liston & Hiemstra, 2011; Vikhamar-Schuler et al., 2016). Abrupt changes in winter snow cover and depth following winter thaw events (e.g., from -20°C to +5°C in 24 h) affect species survival as snow insulates against temperature extremes (Bokhorst, Pedersen, et al., 2016). In addition, loss of snow cover during a midwinter heatwave can induce physiological activity, while organisms are generally dormant, representing a deacclimation of winter hardiness (Bokhorst et al., 2010;

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Ögren, 1996; Schaberg et al., 1996). These weather extremes occur against a background of increasing nutrient availability from anthropogenic sources and wildfires (Ardyna et al., 2022; Karlsson et al., 2013; Lutsch et al., 2019; Melillo, 2021), which may influence ecosystem vulnerability to freeze damages. Mosses and lichens have high flexibility when it comes to freezing and drought stress compared to vascular plants due to a lack of vascular tissue and activity patterns linked to water availability instead of seasonality (Kranner et al., 2008; Lenne et al., 2010; Proctor et al., 2007). In principle, these attributes should make them less susceptible to extreme winter events, but various studies reported species-specific responses to snow as a limiting role for survival (Bokhorst, Convey, et al., 2016; Kuttim et al., 2019, e.g., Sonesson et al., 1994), icing conditions (Bjerke, 2011), and nitrogen in combination with freezing (Liu et al., 2017). Considering that mosses and lichens form an important part of polar, alpine and dryland terrestrial ecosystems where they impact the soil abiotic conditions (Blok et al., 2011; van Zuijlen et al., 2020), influence plant germination (Asplund & Wardle, 2017; Bokhorst et al., 2022), are a major component of the terrestrial carbon cycle (Street et al., 2013), provide shelter (Bokhorst et al., 2014), and are a food source for various animals (Kumpula et al., 2004), we need to identify the drivers behind cryptogam vulnerability to better understand sub-arctic vegetation changes and consequences for arctic ecosystems.

Winter climate extremes or changes in snow conditions primarily influence freezing intensity and water availability. The impacts of frost and drought are similar in that they limit free water availability for physiological activity (Lenne et al., 2010; Verhoeven et al., 2018). Cryptogams have the ability to quickly regain water, even from the atmosphere (Lange et al., 2001), to restart physiological activity (Bjerke et al., 2013; Kappen, 1993; Proctor et al., 2007). This ability allows for great physiological flexibility and their survival in high polar deserts (Kappen, 2000; Perera-Castro et al., 2021; Sancho et al., 2019). Moss vulnerability to freezing temperatures differs between species and, in part, depends on whether species grow in sites that are buffered to the most extreme low temperatures (Campbell & Rydin, 2019; Kuttim et al., 2019; Yin & Zhang, 2016), show physiological adaptations (Rütten & Santarius, 1992; Zhang & Zhang, 2020; Zuñiga-González et al., 2016) and the response may depend on frost and snow combinations (Yin et al., 2021). Therefore, we can expect that mosses, typically growing in habitats with deep snow cover, will be more vulnerable to winter heatwaves and freezing temperatures than those growing on exposed ridges with shallow snow depth. Generally, lichens are considered highly resistant to frost and drought (Kappen, 2000), but snow cover can have a strong impact on lichen survival as it creates high subnivean temperature and humidity that allows lichens to be physiologically active during the dark polar winters, which then results in a negative carbon balance (Benedict, 1991; Bidussi et al., 2016; Bokhorst, Convey, et al., 2016; Pannewitz et al., 2003; Sonesson et al., 1994). Hence, despite the general hardiness of lichens to polar climate extremes, they may be vulnerable to increased frequency of extreme winter events, such as heat waves followed by freezing. Overall, we expect mosses to be more vulnerable to extreme events than lichens (Bjerke et al., 2011). The occurrence and frequency of winter heatwaves are unpredictable but have been reported across

coastal and inland mountain regions of the sub-arctic as well as the high arctic (Bjerke, Treharne, et al., 2017; Bokhorst et al., 2009).

Basic physiological processes slow down or stop altogether in response to colder autumn temperatures as protection against freezing damage (Inouye, 2000), and cell membrane fatty acids play an important role in this freeze-acclimation process (Hughes & Dunn, 1996; Steponkus, 1984; Uemura et al., 2006). Many plants increase the unsaturated and longer fatty acid chains to limit membrane denaturation by freeze-dehydration (Dalmannsdóttir et al., 2001; Strimbeck et al., 2015; Uemura et al., 1995; Uemura & Steponkus, 1999) and similar patterns have been detected in some moss and lichen species (Chen et al., 2013). Mosses are known to build up freeze-tolerance but can also lose tolerance due to environmental cues (Lenne et al., 2010; Minami et al., 2005; Nagao et al., 2006). However, it is unclear if cryptogams can reacclimate these cold-hardening attributes after an abrupt midwinter warming event to re-establish freeze-tolerance. This mid-winter deacclimation and reacclimation, in response to a winter heatwave, may be affected by the nutritional status of plants (MacGillivray et al., 1995).

Atmospheric nitrogen (N) deposition is a global threat to biodiversity and ecosystem function, and despite policy measures, nitrogen deposition rates keep increasing (Ackerman et al., 2019) with noticeable biotic responses (Ardyna et al., 2022; Bergström & Jansson, 2006). Critically, even low-dose N deposition ( $5\text{--}10\text{ kg N ha}^{-1}\text{ year}^{-1}$ ) may lead to eutrophication and affect plant communities (Bobbink & Hettelingh, 2011; Choudhary et al., 2016). Cryptogams typically reflect the nitrogen status of their environment due to the lack of specialised tissue to regulate this (Asplund & Wardle, 2014; Palmqvist et al., 2002), and are known to capture a large proportion of the deposited N (Bokhorst, van Logtestijn, et al., 2019; Choudhary et al., 2016). Although N deposition is relatively low in polar regions, with the exception of local bird colonies (Bokhorst, Convey, & Aerts, 2019; Zwolicki et al., 2016), increased episodic N deposition is on the rise through long-distance transport from lower latitudes, originating from agricultural practices and wildfires (Ardyna et al., 2022; Forsius et al., 2010; Holtgrieve et al., 2011; Karlsson et al., 2013). Polar ecosystems are, therefore, subject to increasing temperature variability and episodic low-level nitrogen pulses, which can negatively impact vascular plants (Bokhorst et al., 2018; Phoenix et al., 2012; Power et al., 1998), and may likewise, pose a challenge for cryptogams (Liu et al., 2017).

To address this, we initiated a common garden experiment where the physiology of dominant sub-arctic mosses (*Hylocomium splendens*, *Polytrichum commune* and *Racomitrium lanuginosum*) and lichens (*Cladonia rangiferina*, *Nephroma arcticum*, *Peltigera aphthosa* and *Stereocaulon paschale*) was monitored during winter (March) and summer (August) when exposed to simulations of a heatwave (also known as an 'extreme WW event,' here simulated by exposure to  $6^{\circ}\text{C}$  for 7 days), re-freezing ( $-10^{\circ}\text{C}$  or  $-20^{\circ}\text{C}$ ), snow removal, summer nitrogen addition and a factorial combination of these treatments in sub-arctic Norway. We hypothesised that (1) species with increased physiological activity during the extreme WW event would be more vulnerable to re-freezing and result in reduced summer physiological activity; (2) a shift towards a greater dominance of longer chain and unsaturated fatty acids would take place as a protective measure against freezing damage following

**TABLE 1** Characteristics of the lichens (l) and mosses (m) selected for this study.

Species	Growth form	Green algal photobiont	Cyanobacterial photobiont	Habitat preferences
<i>Cladonia rangiferina</i> (l)	Fruticose	<i>Asterochloris</i>	None	On shallow, relatively dry soil in open forest or tundra
<i>Nephroma arcticum</i> (l)	Foliose	<i>Coccomyxa</i>	<i>Nostoc</i>	Rock screes and moist soil in forest or tundra
<i>Peltigera aphthosa</i> (l)	Foliose	<i>Coccomyxa</i>	<i>Nostoc</i>	On soil among mosses in shady, moist microhabitats in lowlands and in tundra.
<i>Stereocaulon paschale</i> (l)	Fruticose	<i>Asterochloris</i>	<i>Stigonema</i>	As <i>C. rangiferina</i> . Often on disturbed soil.
Leaf morphology				
<i>Hylocomium splendens</i> (m)	Vertically orientated shoots and sympodially formed annually produced feather-like segments	Small, ovate, with a single plate of cells		Mat-forming on nutrient-poor soils from shaded forest to open tundra
<i>Polytrichum commune</i> (m)	Erect shoots, with a water-conducting central cylinder	Thick, with parallel lamellae for droplet collection		On shallow nutrient-poor soils in open forest and tundra; moist abundant in dry environments
<i>Racomitrium lanuginosum</i> (m)	Dense, wide mats consisting of short, erect, tightly packed, brush-like shoots	Lanceolate, with a strong midrib and a long, thin hair point		Primarily on acidic rock surfaces, often in screes; more rarely on shallow soil and bogs

the extreme WW events; and (3) nitrogen addition would exacerbate mortality when extreme WW events impacted cryptogams.

## 2 | MATERIALS AND METHODS

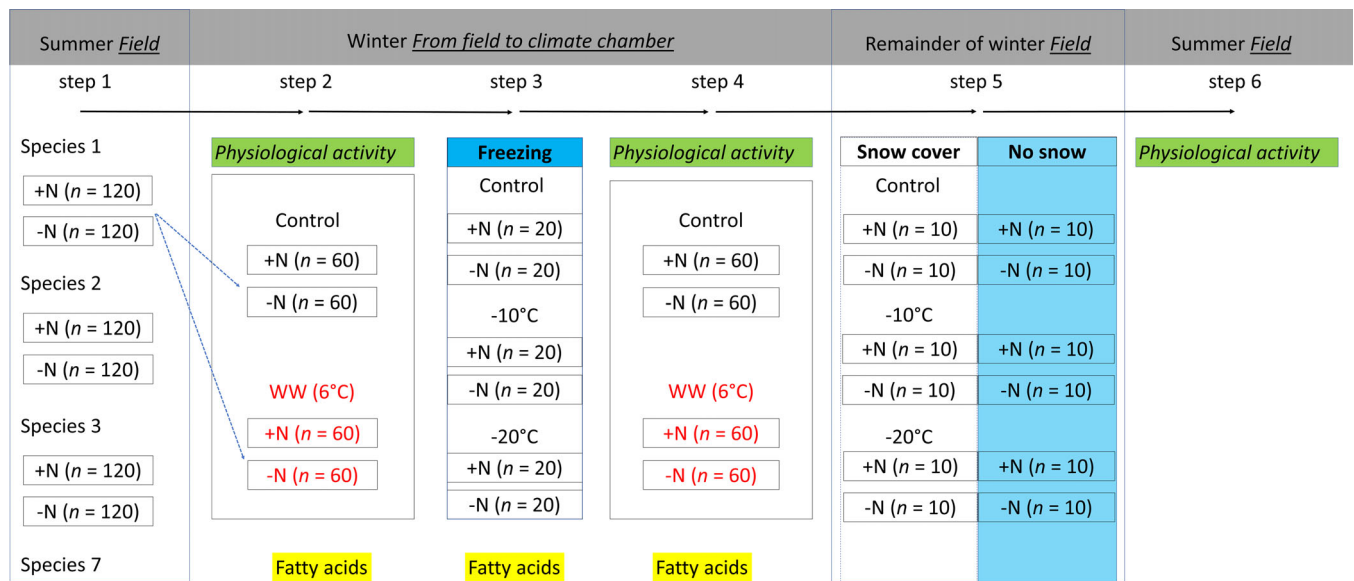
### 2.1 | Study species

As we expected to find contrasting vulnerability between mosses and lichens to extreme WW events, we aimed to compare the differences between dominant sub-arctic cryptogam species. As representatives of lichens, we used *C. rangiferina*, *N. arcticum*, *P. aphthosa* and *S. paschale* and for mosses, we used *H. splendens*, *P. commune* and *R. lanuginosum*. These species were selected because of their wide distributions and high abundance in sub-arctic environments, while at the same time, they differ from each other in habitat preferences, growth strategies and symbiotic associations (Table 1). From here on, genus names will be used to identify the different species. Species were obtained from different habitats across Troms region (Norway) and Norrbotten county (Sweden), but each species was collected from a specific location to avoid within-species habitat effects. A total of 1680 potted (5 × 5 × 5 cm) individuals ( $n = 280$  for each lichen and moss species) were used in the experiment with 20 individuals (pots) per species used as replicates for each treatment of which half received additional N ( $n = 10$  with additional N and  $n = 10$  without N).

### 2.2 | Experimental winter extremes and simulation of nitrogen deposition

To determine the vulnerability of sub-arctic cryptogams to extreme winter events, individual moss plugs or lichen thalli were collected

from the field during spring 2013 (see details below) and divided across three temperature treatments in the experimental garden at Holt (Tromsø, Norway, 69°40' N, 18°56' E). For the simulation of a winter heatwave, which from now on will be referred to as 'extreme WW,' and re-freezing events, we removed cryptogams from the experimental garden during March 2014 and exposed them to various temperature regimes under controlled conditions in climate chambers after which the cryptogams were returned to the experimental garden for the remainder of winter (Figure 1). To access the cryptogams, snow was removed from the top, and the cryptogams were divided across two dark climate chambers at 0.5 and 6.0°C for a week ( $n = 120$  replicate cryptogams per species for each temperature) (Figure 1). The 0.5°C temperature was considered an ambient 'treatment control' (TC) of the subnivean temperature, while the 6.0°C temperature represents the 'extreme WW' (Bokhorst et al., 2009). To quantify the impact of freezing on cryptogam physiological activity following 3 days at TC or WW, we split the 120 cryptogam samples per species across 3 groups: one with exposure to −10°C, one with exposure to −20°C, while the third remained at the respective TC (0.5°C) or WW (6.0°C) ( $n = 40$  per species for each treatment) (Figure 1). Cryptogam exposure to −10 or −20°C was done in dark climate chambers. The temperature in the climate chambers was gradually lowered from 0 to −10°C at 1 K h<sup>−1</sup> and to −20°C at 2 K h<sup>−1</sup>. After 2 days at the respective freezing temperature, cryptogams were returned to their starting temperature (0.5 or 6.0°C) for another 2 days. No water was added to cryptogams during the incubation in the climate chambers (Figure 1). Following these treatments, all cryptogams were returned to the experimental garden, but only half of each treatment received a new snow cover; meaning that for every temperature treatment combination, there was one set ( $n = 20$ ) insulated by snow (+S), while the other set was left exposed to variable ambient freezing conditions for the remainder of winter due to the



**FIGURE 1** Graphical representation of the experimental design from summer through winter. Individual mosses and lichens ( $n = 240$  per species) were divided across a nitrogen (N) addition group and a control without N (step 1). During winter, individuals were brought into the lab and exposed to control temperatures ( $0.5^{\circ}\text{C}$ , representing sub-nivean conditions) and extreme winter warming ( $6^{\circ}\text{C}$ ) for 3 days (step 2). Physiological activity was quantified during this period. Cryptogams were then exposed to refreezing or remained at their control or winter warming temperature (step 3). Upon return to their respective control or WW temperatures physiological activity was measured again (step 4), but from different experimental units. Cryptogams were placed back into the field (step 5). Half of each treatment received a new snow cover while the other half was left exposed to ambient conditions. All samples were collected during the following summer for final measurements of physiological activity (step 6). Samples for fatty acid analysis were collected during steps 2, 3 and 4.

lack of snow cover ( $-S$ ). As an example of the full experimental design for the  $0.5^{\circ}\text{C}$  treatments (TC) we now have: 'TC + S,' 'TC - S,' 'TC F -  $10^{\circ}\text{C}$  + S,' 'TC F -  $10^{\circ}\text{C}$  - S,' 'TC F -  $20^{\circ}\text{C}$  - S' and 'TC F -  $20^{\circ}\text{C}$  - S.' A group of potted cryptogams ( $n = 40$  replicate cryptogams per species) was left in the experimental garden as a control. Snow was removed from half of those ('C + S' and 'C - S'), at the same time as the samples from the extreme WW simulations were returned to the experimental garden.

To avoid crushing cryptogams with the manual build-up of a new snowpack, the cryptogams were first covered with 20 cm of fine light-weight hoarfrost collected from the surrounding snow surfaces, after which the snow level was brought back up to 80 cm with shovelled snow from the surrounding area. After the treatments, the cryptogams were left untouched for the remainder of winter and spring until collected for harvesting during summer (August) 2014. Cryptogam surface temperature (0–1 cm depth) was measured at hourly intervals from autumn (2013) to spring (2014) by temperature loggers (I-Button, Maxim integrated) placed in one replicate of *Polytrichum* per treatment. From these data, we quantified the minimum temperature, the number of freezing events (freeze–thaw cycles) and the spring thaw date.

To quantify the impact of nitrogen (N) addition in combination with extreme WW effects, half of each experimental treatment received additional N ( $5 \text{ kg N m}^{-2} \text{ year}^{-1}$ ) during the summer (2013) before the extreme WW events. N was applied by watering the cryptogams twice during summer (30 July and 15 August). The N treatments received 2 L of an ammonium nitrate ( $\text{NH}_4\text{-NO}_3$ ) solution in total, while the non-nitrogen cryptogams received an equal amount of tap water. The total

amount of water added was less than 1% of the mean annual precipitation falling in Tromsø (1031 mm). There were 10 replicates per species for each temperature–nitrogen treatment (Figure 1).

### 2.3 | Winter physiological activity

During the winter manipulations, we collected lichen thalli and moss shoots to quantify respiration rates, the potential activity of PSII (Fv/Fm) and potential cell membrane damage through electrolyte leakage. Samples were collected after 3 days at the respective acclimation temperature (TC and WW) and 24 h following the freezing treatments (Figure 1).

Cryptogam respiration was measured in complete darkness using a leaf gas exchange system (GFS-3000) with the measuring head set at  $0.5$  or  $6^{\circ}\text{C}$ , depending on the acclimation temperature (TC or WW) of the cryptogams, and 7000 ppm  $\text{H}_2\text{O}$ , with a 380-ppm base level of  $\text{CO}_2$ . We used Walz' cuvette 3010-V80, which is specifically designed for small, loose samples. For small tissues, we turned the cuvette upside-down so that the cryptogams were on top of the ventilated lid and thereby closer to the light source and emitting a stronger fluorescence signal. Cryptogam samples were collected after 3 days at their acclimation temperature ( $0.5$  or  $6^{\circ}\text{C}$ ) and following freezing (Figure 1). Tissue samples were measured within 5 min of collection to obtain a representative estimate of physiological activity between treatments. We used the mean respiration rates of nine measurements taken at 15 s intervals for each sample ( $n = 5$  per species for each treatment).



Fv/Fm was quantified for each cryptogam sample at the end of the CO<sub>2</sub> exchange measurements. All samples were immediately weighed after physiological activity measurements, oven-dried (70°C for 48 h) and reweighed to calculate water content. As the samples were not exposed to any light sources during sampling, we concluded that the cryptogams were properly dark-adapted for fluorescence measurements.

The electrolyte leakage method was used to assess the potential damage of cell membranes due to freezing (Caporn et al., 1994). Intact moss shoots and cut lichen thalli were sampled from each species before and after freezing ( $n = 10$  per species for each treatment). Samples were placed in a plastic tube with 30 ml distilled water, shaken briefly and stored at room temperature for 6 h. After this period, the conductivity of the solution was measured with a conductivity meter (HI 9835; Hanna Instruments) to quantify the initial electrolyte loss. The sealed tubes with cryptogam samples were then autoclaved (120°C for 4 h) to destroy the integrity of the cell membranes and to release all electrolytes to acquire the maximum conductivity of each sample. The ratio of the initial-to-the-maximum conductivity was calculated and compared across the treatments with high values indicative of a higher proportion of cell membrane damage.

## 2.4 | Fatty acid composition

We collected tissue samples after 3 days at the respective acclimation temperature (TC and WW), during freezing and 24 h following the freezing treatments (Figure 1). Samples were immediately frozen at -20°C, freeze-dried, ground, and later on analysed for fatty acids following the direct methylation procedure (Browse et al., 1986). Samples of 5–20 mg were dissolved in 1 ml methanolic hydrochloric acid (1 M) and an internal standard (heptadecanoic acid, C17:0) was added to a glass tube. The solution was heated to 80–100°C for 1 h, and after cooling, 0.4 ml hexane and 1 ml of 0.9% sodium chloride were added to each sample. The fatty acid methyl esters were extracted into the hexane phase by vigorous shaking on a benchtop shaker (10 s). The tubes were centrifuged for 10 min (at 212 g) to separate the phases completely, and a sample was then taken directly from the hexane phase. Samples were stored at -20°C until gas chromatography analyses were undertaken, according to procedures in Mæhre et al. (2013) using an Agilent 6890N equipped with a flame ionisation detector (Agilent Technologies Inc.) and a CP7419 capillary column (50 m × 250 μm × 0.25 μm nominal, Varian Inc., Middelburg, the Netherlands). The fatty acids were identified by comparing against the commercial fatty acid standards PUFA 1, 2 and 3 (Sigma-Aldrich Chemicals Co.) and the GLC standards 80, 411 and 412 (NuChec Prep. Inc.). Due to logistical constraints, fatty acid analysis was limited to control samples and extreme WW (during warming, during and after freezing) for *Cladonia* ( $n = 39$ ), *Stereocaulon* ( $n = 34$ ) and *Polytrichum* ( $n = 35$ ), while a near-complete analysis, across all treatments, was done for *Hylocomium* ( $n = 84$ ) and *Peltigera* ( $n = 140$ ). *Nephroma* and *Racomitrium* were not analysed for fatty acids.

## 2.5 | Summer activity

Cryptogams summer activity of respiration rates and potential activity of PSII (Fv/Fm) was quantified during August 2014 (Figure 1). The same methodology during the winter physiological activity was used, except that the measuring head temperature was now set to 10°C.

## 2.6 | Statistical analyses

ANOVA was used to test for any effects of 'TC' (samples that underwent winter laboratory control conditions) and 'control' (samples left untouched during winter) on cryptogam summer activity. These tests indicated overall small differences for summer Fv/Fm and respiration differences for *Nephroma*, *Hylocomium* and *Racomitrium* only. Therefore, 'control' and 'TC' results were grouped as full control replicates ( $n = 32$  for each species) for further analyses. We used a factorial ANOVA to compare the impact of extreme WW (present-absent), freezing (present-absent) and N addition (0 vs. 5 kg m<sup>-2</sup> year<sup>-1</sup>) on winter respiration, Fv/Fm, electrolyte leakage and water content. There was an overall declining respiration trend between the measurements done at the start of the winter measurements and at the end across all treatments. Therefore, we only used the final winter physiological measurements to test for any treatment effects on winter activity. Fatty acid composition, using the percentage of each fatty acid component in each sample, was analysed through principal component analyses and the resulting first two dimensions were used in factorial ANOVAs to identify any treatment effects. Changes in fatty acid composition (individual acids, the sum of saturated and unsaturated fatty acids and PC1 and PC2) across sampling time were included in the factorial ANOVAs. Because sampling at different time steps of the same sample was avoided, 'time' was treated as a fixed factor in the analyses. Analyses of cryptogam summer respiration, Fv/Fm and water content also included snow removal as a predictor variable. To limit the number of interaction factors and their interpretability, freezing intensity (no freezing, -10 or -20°C) was tested separately for the fatty acids, summer Fv/Fm and respiration. The number of significant interaction terms was limited and therefore, only the main effect responses are presented in tables and figures. Full interaction terms are presented in the supplementary files (Tables S2–S7). Significant, near-significant and meaningful interaction terms are mentioned in Section 3 where relevant. Respiration and Fv/Fm were correlated (Spearman rho) with water content for each treatment to identify if water may be an underlying factor of treatment effects. In all cases, homogeneity of variance was tested with Levene's test of equality and visual inspection of the residuals. Log-transformation was applied when necessary. All statistical analyses were carried out using R 3.3.0 (R-Core-Team, 2021).

**TABLE 2** Analysis of variance output (*F* and *P* values) of the winter potential activity of photosystem II (Fv/Fm), respiration and electrolyte leakage of cryptogams in response to extreme winter climate change treatments. The experimental treatments consisted of a factorial design including: nitrogen addition (N) during the previous growing season, extreme winter warming event (6°C for 1 week) and freezing. Analyses are based on *n* = 19–40 samples for each species across treatments. For clarity, significant treatment effects are highlighted in bold. There were few significant interaction terms and these are mentioned in the results text.

	Winter warming		Freezing		Nitrogen	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
<b>Fv/Fm</b>						
<i>Cladonia</i>	<b>6.4</b>	<b>0.014</b>	<b>15.3</b>	<b>&lt;0.001</b>	<b>11.1</b>	<b>0.002</b>
<i>Nephroma</i>	<b>20.8</b>	<b>&lt;0.001</b>	1.5	0.221	0.1	0.743
<i>Peltigera</i>	2.0	0.163	0.6	0.429	2.6	0.114
<i>Stereocaulon</i>	<b>8.1</b>	<b>0.006</b>	<b>4.5</b>	<b>0.039</b>	0.1	0.772
<i>Hylocomium</i>	0.6	0.462	<b>6.3</b>	<b>0.016</b>	<b>11.8</b>	<b>0.001</b>
<i>Polytrichum</i>	0.6	0.459	2.0	0.160	<b>7.2</b>	<b>0.010</b>
<i>Racomitrium</i>	0.1	0.844	0.0	0.967	1.6	0.208
<b>Respiration rate</b>						
<i>Cladonia</i>	2.5	0.122	0.4	0.512	0.6	0.450
<i>Nephroma</i>	<b>16.0</b>	<b>&lt;0.001</b>	0.6	0.453	0.3	0.571
<i>Peltigera</i>	<b>6.0</b>	<b>0.018</b>	0.3	0.571	<b>7.6</b>	<b>0.008</b>
<i>Stereocaulon</i>	<b>6.0</b>	<b>0.018</b>	0.1	0.796	2.3	0.135
<i>Hylocomium</i>	<b>19.9</b>	<b>&lt;0.001</b>	<b>4.9</b>	<b>0.031</b>	0.4	0.522
<i>Polytrichum</i>	0.1	0.789	0.3	0.611	2.8	0.098
<i>Racomitrium</i>	1.6	0.209	<b>5.3</b>	<b>0.026</b>	0.4	0.520
<b>Electrolyte leakage</b>						
<i>Cladonia</i>	<b>15.2</b>	<b>&lt;0.001</b>	<b>5.7</b>	<b>0.021</b>	0.5	0.504
<i>Nephroma</i>	<b>23.5</b>	<b>&lt;0.001</b>	0.0	0.887	1.1	0.300
<i>Peltigera</i>	<b>15.4</b>	<b>&lt;0.001</b>	0.3	0.583	0.1	0.756
<i>Stereocaulon</i>	0.2	0.647	2.2	0.141	0.6	0.445
<i>Hylocomium</i>	<b>23.2</b>	<b>&lt;0.001</b>	0.0	0.963	0.3	0.583
<i>Polytrichum</i>	<b>11.0</b>	<b>0.002</b>	<b>11.0</b>	<b>0.002</b>	0.6	0.455
<i>Racomitrium</i>	<b>4.8</b>	<b>0.033</b>	<b>8.8</b>	<b>0.005</b>	2.8	0.102

### 3 | RESULTS

#### 3.1 | Experimental abiotic conditions

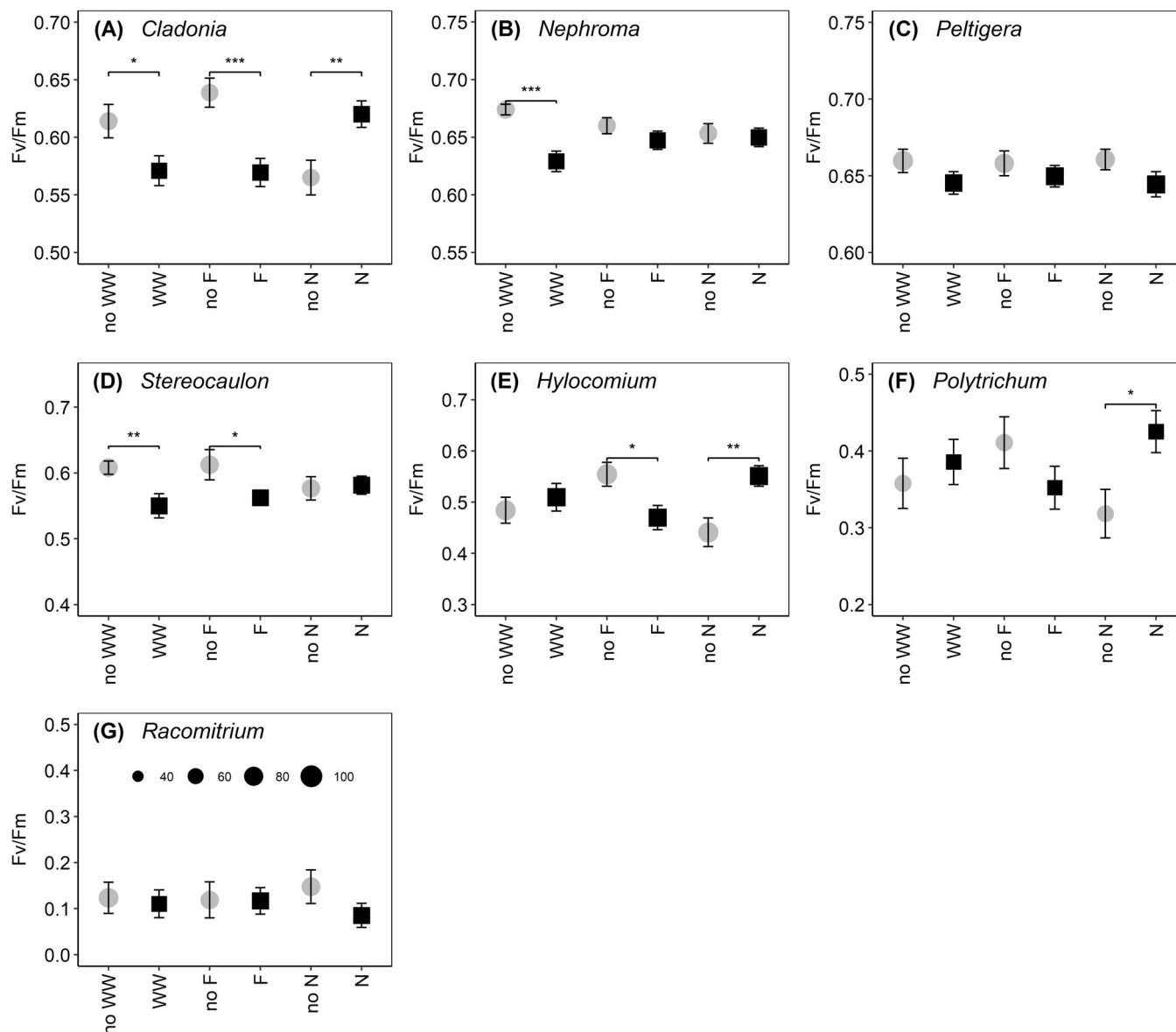
Cryptogam temperature rose to 5°C during extreme WW and dropped to –10 and –14°C in the –10 and –20°C freezing treatments, respectively (Figure S1). Snow removal resulted in an 8-day earlier melt out compared to plots with snow. There were no differences in temperature extremes or freeze–thaw cycle frequency due to snow removal as precipitation and snow drift covered exposed cryptogams.

There were significant differences in winter cryptogam water content between treatments but not consistently across treatments or species (Tables S1 and S2 and Figures S2–S5). Overall, there was no correlation between lichen winter physiological activity and water content, while in mosses, these were correlated in two cases (Table S1). Summer physiological activity was frequently positively correlated with cryptogam water content (Figures S6–S9). In cases of significant water content differences between treatments for a

species, which coincides with a significant physiological response to that treatment, this is mentioned.

#### 3.2 | Winter physiology

Potential activity of photosystem II (Fv/Fm) declined in response to extreme WW for *Cladonia* (7%), *Nephroma* (7%) and *Stereocaulon* (10%) but not for *Peltigera* or any of the mosses (Table 2, Figures 2 and 3). Freezing reduced Fv/Fm for *Cladonia* (11%), *Stereocaulon* (8%) and *Hylocomium* (15%), but a significant reduction of Fv/Fm in *Cladonia* was associated with a decline in water content (Table S1, Figure S2). Nitrogen increased winter Fv/Fm values of *Cladonia* (10%), *Hylocomium* (25%) and *Polytrichum* (34%). Winter Fv/Fm of *Peltigera* was reduced (5%) under extreme WW in combination with nitrogen compared to control values. Nitrogen in combination with extreme WW and freezing resulted in higher Fv/Fm values for *Hylocomium* (0.606) compared to extreme WW with freezing without additional



**FIGURE 2** Impact of extreme winter climate change on the potential winter activity of photosystem II (Fv/Fm) of lichen (A–D) and moss (E–G) species. Treatments included extreme winter warming (WW), experimental exposure to freezing (F) and a summer nitrogen treatment (N). Data points are the mean of  $n = 30$  for WW and N and  $n = 40$  for F with se as error bars. Significant treatment differences are indicated by asterisk \*  $< 0.05$ , \*\*  $< 0.01$ , \*\*\*  $< 0.001$ ; see also Table 2. Symbol size represents water content (%); see panel (G). Note the different scales on vertical axis.

nitrogen (0.377). *Hylocomium* winter Fv/Fm was positively correlated ( $\rho = 0.263$ ,  $P = 0.044$ ) with water content (Table S1), but there was no treatment effect on water content (Table S2). No further correlations were found for the other tested species. *Peltigera* water content was reduced under extreme WW (Table S2, Figure S2).

Winter respiration increased in response to extreme WW for *Nephroma* (155%), *Peltigera* (57%), *Stereocaulon* (100%) and *Hylocomium* (374%) (Table 2, Figures 3 and 4). Freezing increased winter respiration of *Hylocomium* (100%) and *Racomitrium* (88%). *Peltigera* winter respiration increased by 67% under nitrogen (coinciding with a higher water content of 81 vs. 87%) (Tables S1 and S2, Figure S3). Winter respiration of *Racomitrium* was positively correlated ( $\rho = 0.386$ ,  $P = 0.004$ ) with water content (Table S1) and water content was affected by experimental

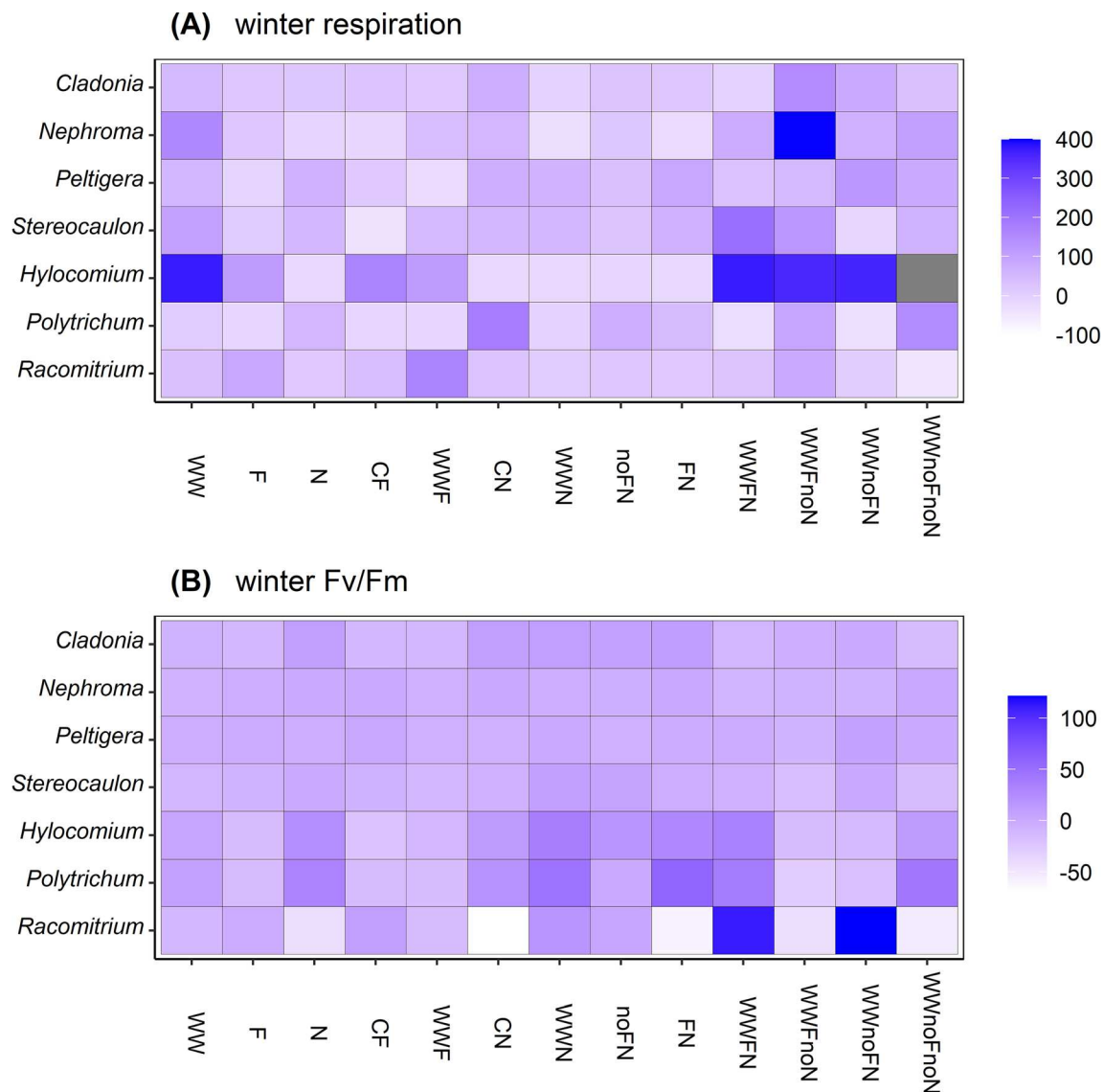
treatments such that water content was lower under extreme WW (18%) and higher under freezing (5%). There were no significant interaction terms with respect to cryptogam winter physiological activity.

Cryptogam electrolyte leakage was reduced (16–44%) in all species except *Stereocaulon* following exposure to extreme WW (Table 2). Freezing reduced electrolyte leakage in *Cladonia* (27%), *Polytrichum* (26%) and *Racomitrium* (26%). Nitrogen did not affect winter electrolyte leakage.

### 3.3 | Fatty acid composition

Membrane fatty acid composition differed between species (Figure S10). The principal component analyses indicate a separation

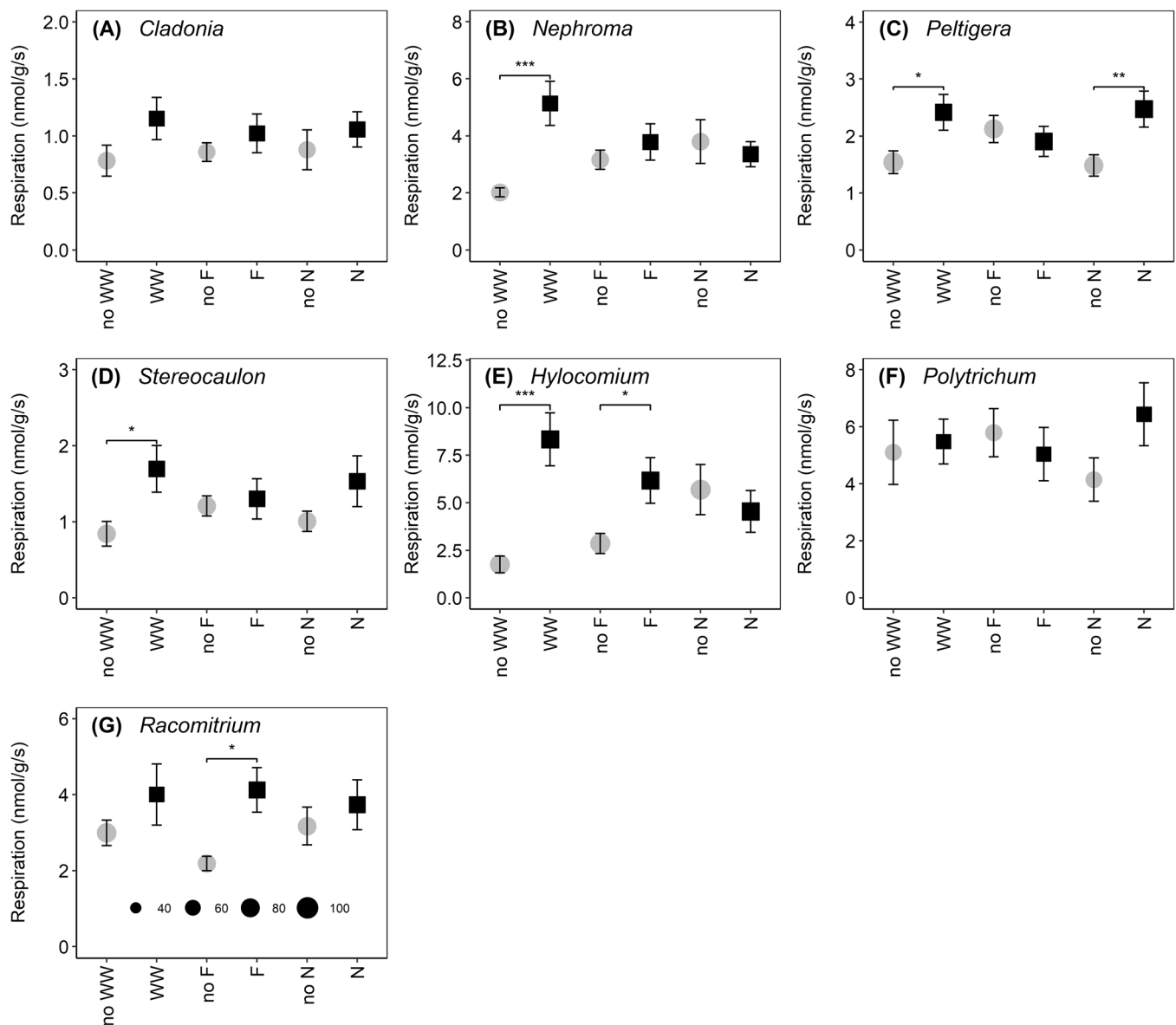




**FIGURE 3** Heatmap of cryptogam response to impact of extreme winter climate change on winter respiration (A) and Fv/Fm (B) values. Treatments included are extreme winter warming (WW), experimental exposure to freezing (F), and summer nitrogen addition (N). Colour intensity shows the percentage increase with respect to the treatments shown on the horizontal axis; for example, CF = % difference between control with and without freezing. Note the difference in colour scaling for panels A and B. Grey square indicative of >600% increase and omitted from the general colour scaling to better illustrate smaller changes.

in fatty acid composition for *Hylocomium*, *Polytrichum* and *Peltigera* in response to winter treatments, while no significant changes were found for *Cladonia* and *Stereocaulon* (Table S3). *Hylocomium* treatment differences were strongest between control and extreme WW with  $-20^{\circ}\text{C}$ . C15:1 and c17:1 were reduced by 50% under extreme WW with subsequent freezing, while c14:0, c15:0, c18:0, c18:1 n-12, c18:2 n-6 and c18:3 n-3 were highest when *Hylocomium* was exposed to extreme WW with subsequent freezing at  $-20^{\circ}\text{C}$  (Figure S11a). *Polytrichum* fatty acid composition differed between control and extreme WW with increased levels (17–29%) of c18:0, c18:2 n-6 and c18:3 n-3. In contrast, c13:1, c18:1 n-12 and c18:1 n-9 declined (20–64%) in response to extreme WW (Figure S11b). *Peltigera* fatty acid composition separated across three groups in response to the sampling timing and exposure to extreme WW and freezing (Figure S12). The first

*Peltigera* group ( $n = 40$ ) was associated with high values of specific fatty acids (c6:0, c10:0, c11:0, c14:1 C16:1 n-7 and C18:1 n-9) at the start of the experiment. These fatty acids declined over time (71–80%), in response to freezing (mostly at  $-20^{\circ}\text{C}$ ) (69–87%) and in response to extreme WW (51–70%). The second group ( $n = 40$ ) was associated with high values of fatty acids (c13:1, c18:3 n-6 and C20:3 n-6) during the experiment but did not show consistent responses to treatments. The third group ( $n = 60$ ) was associated with fatty acids (c18:1 n-7, c20:5 n-3 and c24:1) with higher content (three times) at the end of the experiment compared to the start. The proportion of C18:0 increased ninefold under extreme WW (without freezing) compared to control. c18:1 n-7, c20:5 n-3 and c24:1 increased (>170%) under extreme WW with subsequent freezing at  $-10^{\circ}\text{C}$  (but not at  $-20^{\circ}\text{C}$ ). c20:5 n-3 and c24:1 decreased by 91% in response to



**FIGURE 4** Impact of extreme winter climate change on winter respiration of lichen and moss species. Treatments included extreme winter warming (WW), experimental exposure to freezing (F), and a summer nitrogen treatment (N). Data points are the mean of  $n = 30$  for WW and N and  $n = 40$  for F; with SE as error bars. Significant treatment differences are indicated by asterisk \*  $< 0.05$ , \*\*  $< 0.01$ , \*\*\*  $< 0.001$ ; see also Table 2. Symbol size represents water content (%); see panel (G).

nitrogen in combination with freezing at  $-20^{\circ}\text{C}$ . There were no differences in the relative proportion of saturated versus unsaturated fatty acids in response to the treatments for any of the species.

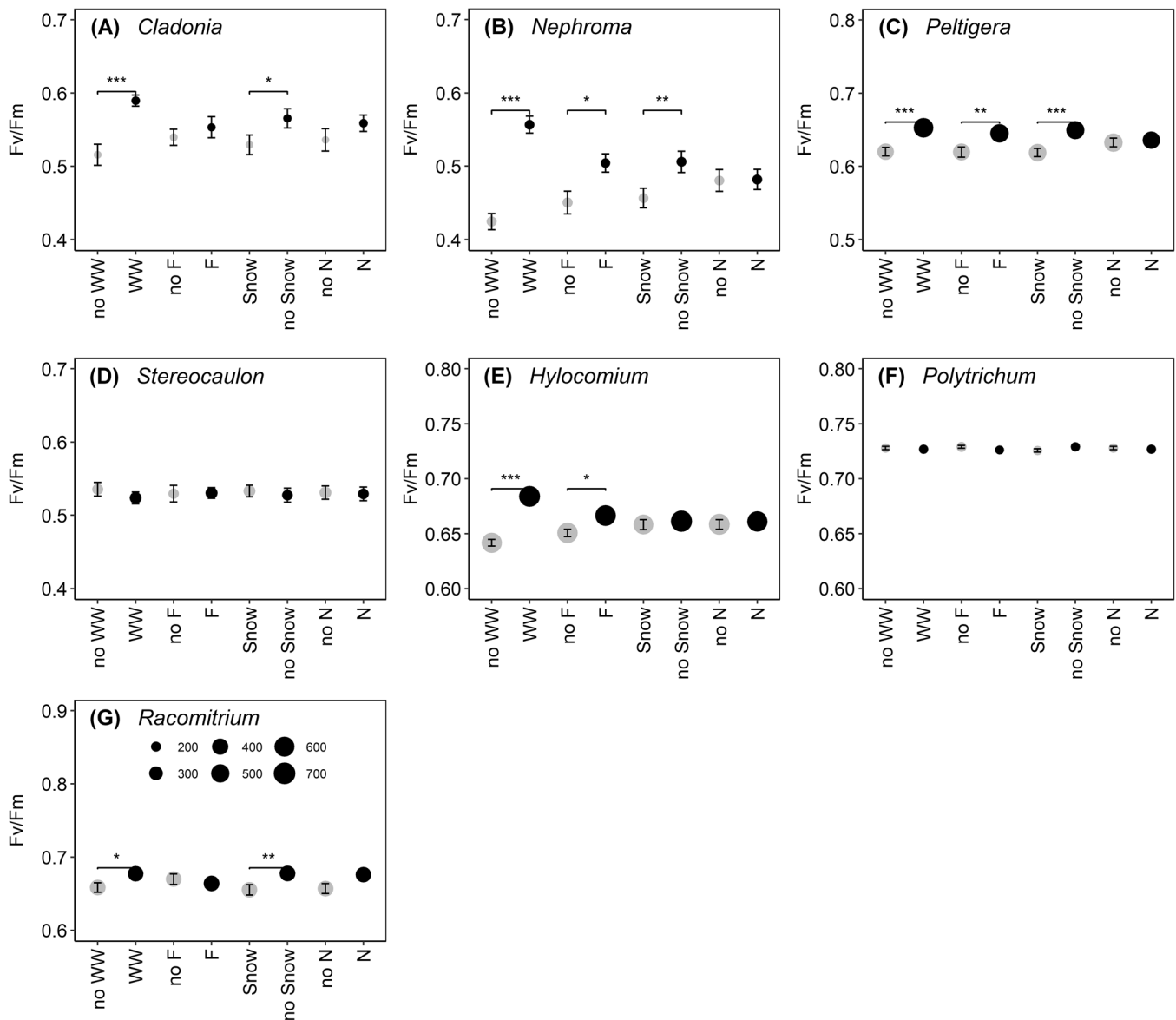
### 3.4 | Summer physiology

The summer activity of photosystem II (Fv/Fm) increased (3–31%) for most extreme WW treated species except *Stereocaulon* and *Polytrichum* (Figure 5, Table 3). Freezing enhanced summer Fv/Fm values of *Hylocomium* (2%), *Nephroma* (12%) and *Peltigera* (4%), while the other species were unaffected. Snow removal increased summer Fv/Fm of *Racomitrium* (3%), *Cladonia* (7%), *Nephroma* (11%) and *Peltigera* (5%),

while nitrogen did not affect Fv/Fm. Cryptogam summer respiration was unaffected by extreme WW for any of the tested species, while there were some species-specific responses to the effects of freezing, snow removal and nitrogen addition (Figure 6): *Cladonia* respiration was 12% lower following freezing. Snow removal reduced summer respiration of *Hylocomium* (10%). Nitrogen increased summer respiration of *Racomitrium* (10%) but reduced it for *Peltigera* (12%).

### 3.5 | Effect of treatment combinations

Freezing in combination with extreme WW reduced Fv/Fm values (10%) of *Stereocaulon*, whereas increased values (9%) were found



**FIGURE 5** Impact of extreme winter climate change on the potential summer activity of photosystem II (Fv/Fm) of lichen and moss species. Treatments included extreme winter warming (WW), experimental exposure to freezing (F), removal of the insulating snow layer (Snow) and a summer nitrogen treatment (N). Data points are the mean of  $n = 56$  for N and Snow; no WW and F ( $n = 64$ ), WW and no F ( $n = 48$ ) replicates with SE as error bars. Significant treatment differences are indicated by asterisk \*  $< 0.05$ , \*\*  $< 0.01$ , \*\*\*  $< 0.001$ ; see also Table 3. Symbol size represents water content (%); see panel (G).

when freezing was applied without extreme WW (Figure 7). Freezing and freezing in combination with extreme WW increased Fv/Fm of *Peltigera* (both 8%). Fv/Fm of *Hylocomium* was increased (8%) following extreme WW with freezing and reduced (3%) when freezing was applied without extreme WW. *Hylocomium* summer respiration declined (25%) following the combination of extreme WW with freezing, while summer respiration increased (15%) when freezing was applied without extreme WW. An opposite response was found for *Peltigera*, with a 28% increased summer respiration following extreme WW and freezing, while summer respiration declined by 16% following freezing.

Extreme WW with snow removal reduced *Polytrichum* summer respiration (15%,  $P = 0.052$ ). In contrast, snow removal reduced *Hylocomium* summer respiration (17%) in the absence of extreme WW. Summer Fv/Fm of *Racomitrium* and *Nephroma* were higher (8 and 19%, respectively) when snow cover was removed in the absence of freezing. *Peltigera* Fv/Fm was 7% higher when snow was removed in combination with freezing. There were no significant effects of freezing and snow removal on respiration rates.

Extreme WW combined with snow removal enhanced (43%) summer *Nephroma* Fv/Fm values in the absence of freezing. With freezing, summer *Nephroma* Fv/Fm values were still higher (32%) than the snow-covered samples without freezing. *Cladonia* summer respiration

**TABLE 3** Analysis of variance output (*F* and *P* values) of the summer potential activity of photosystem II (Fv/Fm) and respiration rates of cryptogams in response to extreme winter climate change treatments. The experimental treatments consisted of a factorial design including: nitrogen addition (N) during the previous growing season and winter treatments included: an extreme warming event (6°C for 1 week), freezing and snow removal. Analyses are based on *n* = 106–112 samples for each species *df* = 1, 96. For clarity, significant treatment effects are highlighted in bold. There were only a few significant interaction terms and these are mentioned in the results text.

Species	Winter warming		Freezing		Nitrogen		Snow	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
<b>Fv/Fm</b>								
<i>Cladonia</i>	17.9	<0.001	0.0	0.940	1.7	0.194	4.3	0.040
<i>Nephroma</i>	81.5	<0.001	4.9	0.030	0.0	0.931	11.6	0.001
<i>Peltigera</i>	23.9	<0.001	9.5	0.003	0.2	0.642	21.6	<0.001
<i>Stereocaulon</i>	1.0	0.314	0.1	0.755	0.0	0.912	0.3	0.581
<i>Hylocomium</i>	124.8	<0.001	5.8	0.018	0.5	0.481	0.7	0.416
<i>Polytrichum</i>	0.3	0.618	1.6	0.202	0.3	0.597	2.9	0.092
<i>Racomitrium</i>	5.2	0.024	1.2	0.281	5.4	0.022	7.6	0.007
<b>Respiration rate</b>								
<i>Cladonia</i>	3.1	0.081	7.1	0.009	0.7	0.399	0.4	0.537
<i>Nephroma</i>	0.1	0.785	0.0	0.980	2.1	0.154	1.5	0.223
<i>Peltigera</i>	1.6	0.208	0.2	0.690	7.1	0.009	0.3	0.565
<i>Stereocaulon</i>	0.5	0.471	1.7	0.193	0.1	0.814	0.1	0.721
<i>Hylocomium</i>	0.3	0.579	1.7	0.197	0.2	0.649	13.9	<0.001
<i>Polytrichum</i>	1.2	0.278	1.8	0.188	3.0	0.085	2.7	0.104
<i>Racomitrium</i>	1.0	0.332	0.0	0.965	5.7	0.019	2.8	0.097

was 25% higher under control with snow and without freezing compared to control without snow and freezing.

### 3.6 | Freezing intensity

There were some differences in summer physiological activity depending on freezing intensity in combination with other extremes (Table S7). Freezing to  $-20^{\circ}\text{C}$  reduced summer Fv/Fm (1%) of *Polytrichum*, but not following  $-10^{\circ}\text{C}$ . *Peltigera* summer Fv/Fm was enhanced (6%) following exposure to  $-20^{\circ}\text{C}$ . Extreme WW followed by freezing at  $-20^{\circ}\text{C}$  reduced summer Fv/Fm of *Stereocaulon* (13%), while Fv/Fm of *Racomitrium* increased (11%). Extreme WW in combination with  $-20^{\circ}\text{C}$  and snow removal enhanced (2%) *Polytrichum* summer Fv/Fm. Exposure to  $-20^{\circ}\text{C}$  reduced *Cladonia* summer respiration by 15% and snow removal reduced summer respiration (24%) when combined with exposure to  $-10^{\circ}\text{C}$ . Extreme WW in combination with  $-10^{\circ}\text{C}$  reduced *Hylocomium* summer respiration (21%) while without freezing, extreme WW resulted in increased respiration (27%).

### 3.7 | Nitrogen addition interactions with winter extremes

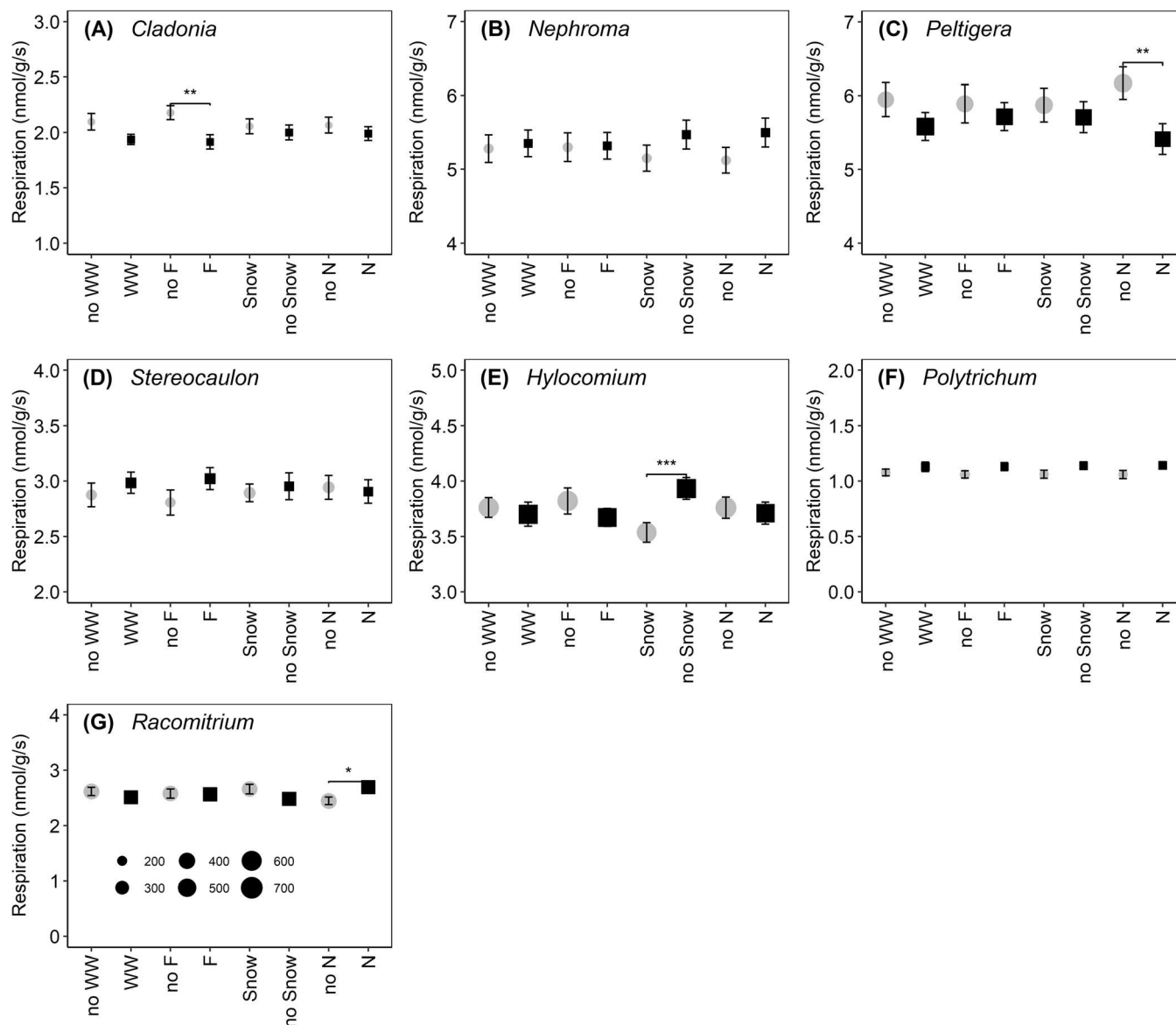
*Hylocomium* summer respiration showed a significant interaction for all four main factors (Table S6), but there was no consistent pattern among these. Snow cover among the control plots reduced (9%) summer *Peltigera* Fv/Fm, but this reduction was absent under added nitrogen, this pattern was not observed in combination with extreme WW. Nitrogen reduced *Peltigera* summer respiration

(20%) in the control plots but not in combination with extreme WW.

## 4 | DISCUSSION

Overall, our study shows that physiological responses to extreme winter events were highly species-specific in both winter and summer. In contrast to earlier findings, we did not observe a greater response by mosses compared to lichens (Bjerke et al., 2011; Bjerke, Bokhorst, et al., 2017). The overall cryptogam response to winter extremes in combination with summer nitrogen is lower than observed among vascular plants in other studies (Bokhorst et al., 2018; Henry et al., 2018), indicating that mosses and lichens are better adapted to handle such extreme events. However, the large response variability among the studied species suggests that more work is needed on cryptogam seasonal activity to understand cryptogam community responses to a future arctic climate with increasing frequency of extreme events.

There was no consistent support for our first hypothesis that increased physiological activity in response to a winter heatwave and following extremes negatively impact summer cryptogam physiology. Instead, the immediate responses of mosses and lichens to extreme WW were highly species-specific; though it is notable that all lichens but only one moss species showed signs of mid-winter reactivation. Physiological activity during winter can be costly for autotrophic organisms as photosynthetic capacity is limited; thus, winter suppression or dormancy can be beneficial (Ensminger et al., 2004; Gilmore & Ball, 2000; Longton, 1988; Sakai & Otsuka, 1970). Fungal components of lichens are known to show seasonal acclimation of their respiration rates (Lange & Green, 2005), but they can respond rapidly to changes

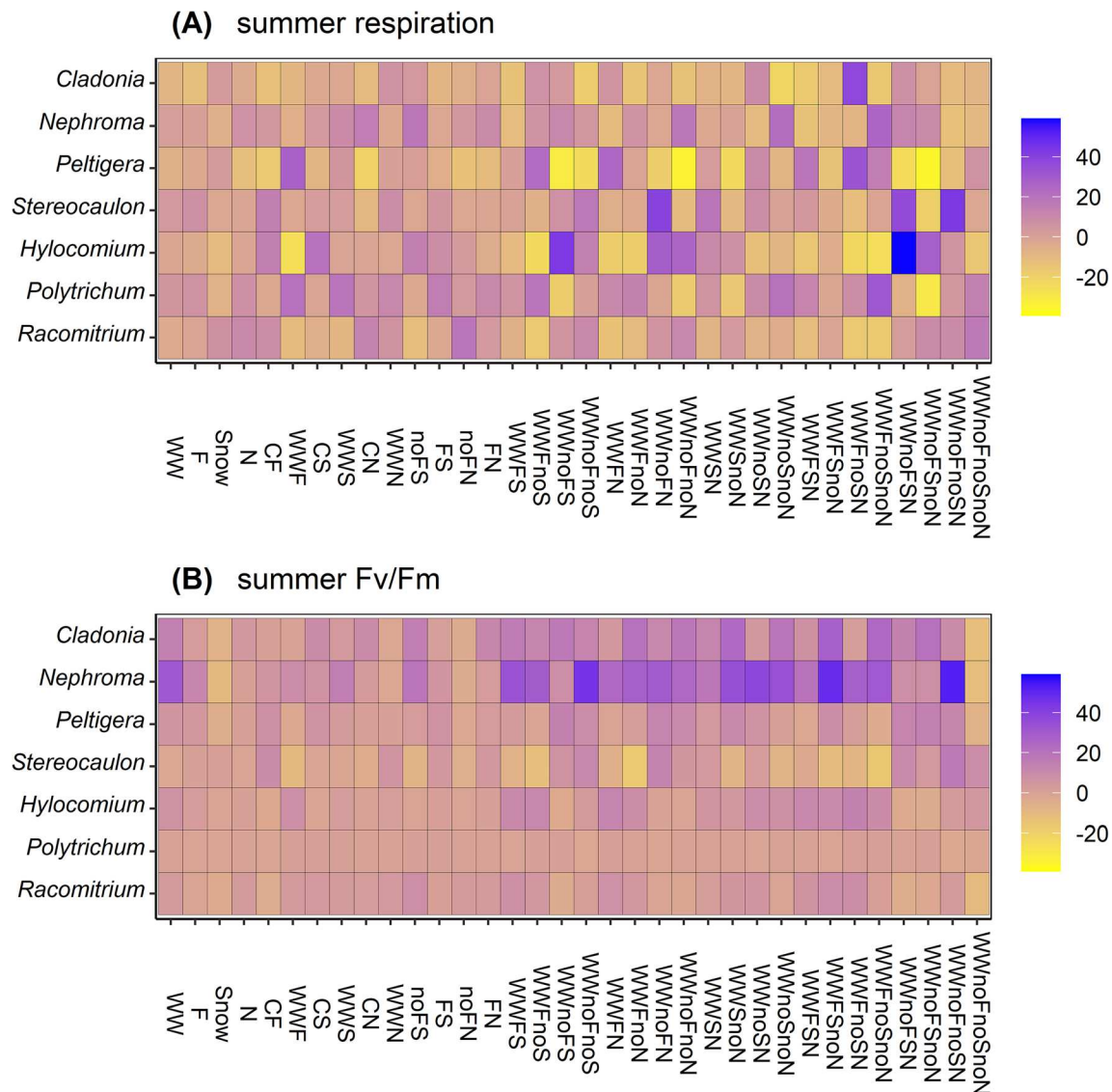


**FIGURE 6** Impact of extreme winter climate change on summer respiration of lichen and moss species. Treatments included extreme winter warming (WW), experimental exposure to freezing (F), removal of the insulating snow layer (Snow) and a summer nitrogen treatment (N). Data points are the mean of  $n = 56$  for N and Snow; no WW and F ( $n = 64$ ), WW and no F ( $n = 48$ ) replicates with  $\pm$  SE as error bars. Significant treatment differences are indicated by asterisk \*  $< 0.05$ , \*\*  $< 0.01$ , \*\*\*  $< 0.001$ ; see also Table 3. Symbol size represents water content (%); see panel (G).

in abiotic conditions (Bjerke, 2011; Bjerke et al., 2013). Winter lichen activity of photosystem II (Fv/Fm values) was overall quite high, irrespective of photobiont taxa (Table 1), indicating that they were primed for physiological activity as a result of sufficient thallus hydration (Vivas et al., 2017). However, lichen Fv/Fm declined during extreme WW, indicating that the photobiont was performing less well as a result of this treatment. Likewise, freezing decreased the Fv/Fm of some lichens while respiration was unaffected. This disbalance in response to extreme WW in the fungal and algal parts of the lichen suggests that acclimation or response to extremes requires attention to both bionts (Bjerke, 2011), as also indicated by results on Antarctic lichens exposed to gradual warming and freezing (Colesie et al., 2018;

Sadowsky & Ott, 2012). Lichen responses to the combination of winter extremes were highly variable, differed between the photobiont (Fv/Fm) and the mycobiont (respiration), and were mediated by changes in fatty acid composition. *Peltigera* showed increased proportions of longer-chained fatty acids such as c18:1 n-7, c20:5 n-3 and c24:1 in response to extreme WW and freezing to  $-10^{\circ}\text{C}$ , these changes were associated with increased summer respiration. The absence of these changes in fatty acid composition (among control samples exposed to  $-10^{\circ}\text{C}$ ) was associated with reduced summer respiration, suggesting that these fatty acids play a role in freezing protection for *Peltigera*. Unsaturated fatty acids, as the ones listed above, can play an important role in membrane integrity during stressful





**FIGURE 7** Heatmap of cryptogam response to impact of extreme winter climate change on summer respiration (A) and Fv/Fm (B) values. Treatments included extreme winter warming (WW), experimental exposure to freezing (F), snow removal (S) and a summer nitrogen treatment (N). Colour intensity shows the percentage increase with respect to the treatments shown on the horizontal axis; for example, CF = % difference between control with and without freezing.

events (Hughes & Dunn, 1996; Steponkus, 1984; Uemura et al., 2006; van Dooremalen et al., 2011). However, *Cladonia* and *Stereocaulon* responded to winter extremes, with consequences for their summer physiology, but without apparent changes in their winter fatty acid composition. There was no consistent response to winter deacclimation between species growing in open and disturbed sites, such as *Cladonia* and *Stereocaulon* (Table 1), compared to species growing in more sheltered sites with a thicker insulating snow cover, such as *Nephroma* and *Peltigera*, indicating that habitat characteristics do not play a large role in shaping lichen response to winter extremes.

The response in mosses during winter was highly variable, with little to no response in terms of potential activity of photosystem II. While respiration was greatly increased in *Hylocomium*, there was little response in the other two mosses. Both *Polytrichum* and

*Racomitrium* had suppressed Fv/Fm values during winter and showed limited respiration response to the extreme winter events, clearly suggesting that these mosses remained dormant throughout the winter treatments. Suppression of winter physiology can be an important protective mechanism against frost (Lenne et al., 2010; Lovelock et al., 1995; Schlenz et al., 2003) that can occur during polar winters. The habitat preferences of *Polytrichum* and *Racomitrium* contrast to those of *Hylocomium* (Hedenäs et al., 2014). While *Polytrichum* and *Racomitrium* often grow on rocks or shallow soil in wind-exposed areas that dry out quickly, *Hylocomium* prefers forest floor or leesides that are covered in snow for most of the winter (Table 1). Survival during winter in exposed sites with high temperature fluctuations requires a high resistance to reactivation, or deacclimation, during periods of thaw, while survival in wind-protected sites with high snow

accumulation that may thaw out late in spring or early summer often are associated with quick responses to warming, both in non-vascular and vascular plants (Bjerke, Bokhorst, et al., 2017; Oberbauer & Starr, 2002; Schlensoeg et al., 2004; Snell et al., 2007). Thus, adaptation to different winter habitats may be the reason for the contrasting winter responses of *Hylocomium* versus *Polytrichum* and *Racomitrium* reported here, as also observed in desert ecosystems (Yin & Zhang, 2016).

Changes in membrane fatty acids may also have mediated moss response to winter extremes (Batsale et al., 2021; Beike et al., 2014). Reduction in c15:1 and c17:1 in response to extreme WW with freezing was associated with reduced *Hylocomium* summer respiration. Similarly, c14:0, c15:0, c18:0, c18:1 n-12, c18:2 n-6 and c18:3 n-3 were highest when *Hylocomium* was exposed to extreme WW with  $-20^{\circ}\text{C}$ , but without a significant summer response, suggesting that these fatty acid changes may have helped reduce the impact of freezing intensity. *Polytrichum* fatty acid composition differed between control and extreme WW with increased levels (17–29%) of c18:0 and c18:2 n-6 and c18:3 n-3. In contrast, c13:1, c18:1 n-12 and c18:1 n-9 declined in response to extreme WW. However, there were no treatment differences for summer physiological activity by *Polytrichum*, suggesting that these fatty acids may have protected this moss, or that it was able to recover from any winter damage during spring. These findings provide indications that changes in membrane fatty acid composition can play a role in protection against winter extremes, but more work is needed to resolve which changes are crucial for which species in relation to freezing intensity. Surprisingly little work has been done on fatty acid composition of mosses and lichens in relation to abiotic stress as most work has focused on abscisic acid production and dehydration capabilities (Takezawa, 2018).

Basal physiological activity, measured through respiration, was largely unaffected by extreme WW, freezing or snow removal, suggesting resistance or rapid recovery (Solhaug et al., 2018). However, combinations of extreme WW with freezing resulted in reduced respiration rates as also shown for *Hylocomium* in previous studies (Bjerke et al., 2011; Bjerke, Bokhorst, et al., 2017). The potential activity of summer photosystem II (Fv/Fm) was enhanced for many of the tested species to various extreme event treatments indicating that this part of the photosynthetic apparatus appears to benefit, and may influence cryptogam capability to cope with extreme winter climatic events. Fen bryophytes have shown negative response in Fv/Fm to snow removal (Kuttim et al., 2019), which is in contrast to our results and indicative of habitat-specific responses to winter climate change as also observed among *Sphagnum* species (Campbell & Rydin, 2019). Hydration status greatly affects seasonal variation in moss CO<sub>2</sub> flux rates with largest variation among hydric mosses (Davey & Rothery, 1996) which is in line with the contrasting response between *Hylocomium* versus *Polytrichum* and *Racomitrium*.

Overall, species that showed a lack of response during winter also did not show any response to the treatments during summer (*Polytrichum*), indicating that a ‘safe strategy’ to remain dormant in winter leads to no negative effects in summer. The remaining species were, with some variation, responsive to extreme winter events but this did

not consistently lead to treatment effects in the following summer season. A more ‘flexible strategy’ allows for opportunistic use of ad hoc suitable conditions irrespective of season, but this may come at a cost as seen in the reduced summer physiological response to treatment combinations (*Cladonia*, *Hylocomium* and *Stereocaulon*). These cryptogam strategies reflect the same challenges faced by vascular plants at the treeline and in snow covered ecosystems where plants are selected for freezing resistance or late phenological development to avoid spring freezing damage (Körner, 2012; Neuner, 2014; Wipf, 2010).

There were some species-specific responses in winter and summer physiology (both positive and negative) to summer nitrogen additions, but there was very little evidence for nitrogen-enhancing effects of the winter extremes, as hypothesised. Freezing in combination with high N deposition ( $>20\text{ kg N ha}^{-1}$ ) has been implicated with reduced activity of moss PSII (Liu et al., 2017), but this was at a much higher N level than used in our experiment. Cryptogam responses to nitrogen deposition is typically highly variable and dependent on the amount of nitrogen deposited (Jónsdóttir et al., 1995; Koranda et al., 2007; Olsson & Kellner, 2006; Pearce & van der Wal, 2002), but can enhance moss growth at levels similar to those used in this study (Armitage et al., 2012). Similarly, lichens typically show great flexibility to cope with either high or low nitrogen deposition (Dahlman et al., 2002). We had anticipated some vulnerability among cryptogams to nitrogen in combination with extreme WW and freezing, and although there were some significant treatment interactions with nitrogen for *Hylocomium*, *Polytrichum*, *Racomitrium* and *Peltigera*, the overall response was low. Therefore, our work suggests that relatively low nitrogen loads ( $5\text{ kg N ha}^{-1}$ ) will not greatly affect cryptogam physiological responses to extreme winter climate change. This is in accordance with the old and new empirical critical loads of 5 to  $10\text{ kg N ha}^{-1}$  of nitrogen (CL<sub>emp</sub>N) for arctic, alpine and subalpine scrub habitats (S2) and taiga woodlands (T3F-G) – similar to the vegetation of the site the different specimens were collected from (Bobbink et al., 2010; Bobbink et al., 2022). For example, *Polytrichum* and *Racomitrium* having a CL<sub>emp</sub>N lying below  $10\text{ kg N ha}^{-1}\text{ year}^{-1}$  (Jones et al., 2002), while *Hylocomium* and *Peltigera* are more sensitive since those species may be negatively influenced with CL<sub>emp</sub>N of  $5\text{ kg N ha}^{-1}\text{ year}^{-1}$  (Dahlman et al., 2002). Mäkipää and Heikkinen (2003) reported that the relative abundance of *Peltigera aptosa*, which has cyanobacteria as its photobiont and expected to be N sensitive, decreased significantly in Finland between 1951 and 1986, and again in 1995 due to N-deposition.

All tested cryptogams survived multiple winter extremes with only minor changes in physiological performance in late summer. This contrasts greatly with past work showing that vascular plants often show considerable mortality following such extremes (Bokhorst et al., 2009; Bokhorst et al., 2018). Greater frequency and intensity of extreme events may facilitate mosses and lichens outperforming vascular plants under a future Arctic climate. Nonetheless, strong negative impacts of winter extremes on cryptogams are reported (Bjerke, 2011; Bjerke et al., 2011; Bjerke, Bokhorst, et al., 2017) and this seems to be linked to winter activity. In addition, linkages

between habitat preferences and responses to winter extremes appear to be stronger in mosses than in lichens. A better understanding of cryptogam seasonal activity patterns can provide handholds for a mechanistic understanding of cryptogam communities in a future Arctic with more weather extremes.

## AUTHOR CONTRIBUTIONS

Stef Bokhorst and Jarle W. Bjerke designed and conducted the experimental work. All authors wrote or commented on the main text and supplementary notes. Hanne K. Mæhre was responsible for the fatty acid analyses. Laura Jaakola arranged and directed climate laboratory facilities.

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## CONFLICT OF INTEREST

The authors declare no conflicts of interests for this publication.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author

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## SUPPORTING INFORMATION

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

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