

1 **Contrasting sensitivity to extreme winter warming events**  
2 **of dominant sub-Arctic heathland bryophyte and lichen**  
3 **species**

4

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21

22 **Running headline: Bryophyte and lichen sensitivity to winter warming**

23

## 24 **Summary**

- 25       **1.** Climate change in northern high latitudes is predicted to be greater in winter rather  
26       than summer, yet little is known about the effects of winter climate change on northern  
27       ecosystems. Among the unknowns are the effects of an increasing frequency of acute,  
28       short-lasting winter warming events. Such events can damage higher plants exposed to  
29       warm, then returning cold, temperatures after snow melt and it is not known how  
30       bryophytes and lichens, which are of considerable ecological importance in high-  
31       latitude ecosystems, are affected by such warming events. However, even  
32       physiological adaptations of these cryptogams to winter environments in general are  
33       poorly understood.
- 34       **2.** Here we describe findings from a novel field experiment that uses heating from  
35       infrared lamps and soil warming cables to simulate acute mid-winter warming events  
36       in a sub-Arctic heath. In particular, we report the growing season responses of the  
37       dominant lichen, *Peltigera aphthosa*, and bryophyte, *Hylocomium splendens*, to  
38       warming events in three consecutive winters.
- 39       **3.** While summertime photosynthetic performance of *P. aphthosa* was unaffected by the  
40       winter warming treatments, *H. splendens* showed significant reductions of net  
41       photosynthetic rates and growth rates (of up to 48% and 52% respectively). Negative  
42       effects were evident already during the summer following the first winter warming  
43       event.
- 44       **4.** While the lichen develops without going through critical phenological stages during  
45       which vulnerable organs are produced, the moss has a seasonal rhythm, which  
46       includes initiation of growth of young, freeze-susceptible shoot apices in the early  
47       growing season; these might be damaged by breaking of dormancy during warm  
48       winter events.

49       **5. Synthesis.** Different sensitivities of the bryophyte and lichen species were unexpected,  
50       and illustrate that very little is known about the winter ecology of bryophytes and  
51       lichens from cold biomes in general. In sharp contrast to summer warming  
52       experiments that show increased vascular plant biomass and reduced lichen biomass,  
53       these results demonstrate that acute climate events in mid-winter may be readily  
54       tolerated by lichens, in contrast to previously observed sensitivity of co-occurring  
55       dwarf shrubs, suggesting winter climate change may compensate for (or even reverse)  
56       predicted lichen declines resulting from summer warming.

57

58   Key-words: Arctic, climate change, ecophysiology, extreme events, *Hylocomium splendens*,  
59   lichenized ascomycete, moss, *Peltigera aphthosa*, plant–climate interactions, warming  
60   experiment

61

62

## 63 **Introduction**

64 The circumpolar region is characterized by vegetation rich in lichens and bryophytes  
65 (Callaghan *et al.* 2005). In addition to their significant contribution to biodiversity in high-  
66 latitude ecosystems (e.g. Elvebakk & Bjerke 2006), bryophytes, lichens and their associated  
67 cyanobacteria play major functional roles by contributing considerably to ecosystem carbon  
68 and nitrogen sequestration, soil insulation, soil stability and preservation of permafrost  
69 (Oechel & van Cleve 1986; Longton 1988; Chapin & Bledsoe 1992; Heijmans *et al.* 2004).  
70 However, bryophyte- and lichen-rich ecosystems in the circumpolar region are under threat.  
71 Large areas of heath vegetation rich in bryophytes and lichens have deteriorated due to  
72 overgrazing by reindeer and caribou, increasing land use and air pollution (Tømmervik *et al.*  
73 2004; Rees *et al.* 2008; Myking *et al.* 2009). In addition, enhanced plant growth due to  
74 increasing growing season temperatures and precipitation rates (Cornelissen *et al.* 2001; van  
75 Wijk *et al.* 2004; Tømmervik *et al.* 2004; Walker *et al.* 2006) and increasing deposition rates  
76 of reactive nitrogen (van Wijk *et al.* 2004; Fremstad *et al.* 2005) induce shifts in higher plant–  
77 cryptogam interactions, leading to reduced lichen and bryophyte cover. These factors are  
78 predicted to increase in impact in the future (Callaghan *et al.* 2005), thus causing declines of  
79 cryptogam biomass and biodiversity in circumpolar regions, with potentially important  
80 ecological and socio-economic impacts. Effects on reindeer husbandry, for example, could be  
81 considerable, given that lichens constitute a major component of the winter diet of reindeer  
82 and caribou. Incorporating the loss of lichen biomass in their modelling, Rees *et al.* (2008)  
83 estimate that reindeer numbers in Norway and Sweden must be reduced by 50–60% by 2080  
84 in order to maintain self-sustainable populations.

85 Most currently used climate-related models for predicting future vegetation changes (e.g.  
86 Kaplan & New 2006; Sitch *et al.* 2007; Wolf *et al.* 2008) are based on directional climate  
87 trends, e.g. of regional warming and humidification. However, there is growing evidence that

88 discrete or extreme climate events play an important role in shaping and changing ecosystems  
89 worldwide (e.g. Jentsch *et al.* 2007; Barrett *et al.* 2008; Bokhorst *et al.* 2008, 2009; Post *et al.*  
90 2009). For instance, acute climate events, e.g. a week-long period with above-zero  
91 temperatures during mid-winter or summer ‘heat waves’ in the Arctic, can have  
92 disproportional influence over ecosystems relative to the short temporal scale over which they  
93 occur (Barrett *et al.* 2008).

94 Detection of acute climate events in climate records and prediction of the future frequency  
95 of such events are challenging, particularly because many regions do not have satisfactory  
96 monitoring systems for carrying out extreme-value climate statistics, and because standard  
97 outlier eliminations tend to hide rare events from climate time series (Jentsch *et al.* 2007).  
98 Despite these challenges, several studies suggest that discrete wintertime warming events  
99 have become and will become even more frequent in circumpolar regions (Visbeck *et al.*  
100 2001, Callaghan *et al.* 2010). In these events, warm spells can cause partial or complete snow  
101 melt over large areas (Bonsal *et al.* 2001; Putkonen & Roe 2003). The loss of the insulating  
102 snow cover combined with the return of subzero temperatures after such warming events may  
103 lead to extensive plant damage. Simulations of 1-week long winter warming events in the sub-  
104 Arctic, using infrared heating lamps suspended above the vegetation run with and without soil  
105 warming cables (to further investigate the impacts of soil thaw), had major negative impacts  
106 on survival, phenology and reproduction of ericoid dwarf shrubs, suggesting that such events  
107 may potentially severely impact the biodiversity and productivity of these systems (Bokhorst  
108 *et al.* 2008, 2009, 2010). Also, a natural event in December 2007 in the sub-Arctic confirmed  
109 these damaging impacts in some higher plants (Bokhorst *et al.* 2009). Currently though, the  
110 impacts on cryptogams remain unknown.

111 Given the importance of lichens, bryophytes and their associated cyanobacteria for sub-  
112 Arctic ecosystems, there is a clear need to determine how these cryptogams are affected by

113 such winter warming events. Using the same field experimental facility as described by  
114 Bokhorst *et al.* (2008, 2009), we aimed to quantify the effects of three discrete warming  
115 events (winters of 2007, 2008 and 2009) on the most abundant lichen, *Peltigera aphthosa*, and  
116 bryophyte, *Hylocomium splendens*, in this area. While long periods of mild temperatures are  
117 required to break the winter dormancy of many sub-Arctic vascular plants, past work suggests  
118 that cold-adapted lichens and bryophytes are reactivated relatively rapidly when temperature,  
119 light and humidity conditions rise above threshold limits (Kallio & Saarnio 1986; Lange  
120 2003; Kappen & Valladares 2007; Glime 2007; Bjerke in press).

121       Given the ability of bryophytes and lichens to switch rapidly between states of metabolic  
122 rest and activity as dictated by fluctuations in the external environment, we hypothesize that  
123 photosynthetic performance and growth rates during growing seasons following winter  
124 warming events would not be negatively affected, and that these cryptogams can therefore  
125 tolerate winter warming events better than the dwarf shrubs with which they co-occur (cf.  
126 Bokhorst *et al.* 2008, 2009). We also hypothesize that the impacts of canopy only warming  
127 and canopy and soil warming will be the same, as the cryptogams are not in direct contact  
128 with the soil.

129

## 130 **Materials and methods**

### 131 SITE DESCRIPTION AND WARMING TREATMENT

132 The study was performed in sub-Arctic heathland vegetation close to the Abisko Scientific  
133 Research Station in northern Sweden (68° 21' N, 18° 49' E). The field layer is dominated by  
134 dwarf shrubs, in particular the deciduous *Vaccinium myrtillus* L. and the evergreen *V. vitis-*  
135 *idaea* L. and *Empetrum nigrum* L. *s.l.* The ground layer is dominated by feather mosses,  
136 especially *Hylocomium splendens* (Hedw.) Schimp., and by foliose cyanolichens, especially  
137 *Peltigera aphthosa* (L.) Willd. Other bryophytes and lichens are more scattered.

138 The experiment—which simulates warming based on real events occurring previously in  
139 the Abisko region—is described in Bokhorst *et al.* (2008, 2009). In brief, it consists of 18  
140 plots (2.1 × 1.0 m) representing six control plots and six each of two warming treatments,  
141 canopy warming and canopy and soil warming. For canopy warming plots, 4 infrared heating  
142 lamps were suspended (70 cm apart) in parallel from wooden frames. To further investigate  
143 the effects of increased soil thaw, the same set-up was used in six additional plots, but these  
144 were further warmed by soil heating cables producing 120 W m<sup>-2</sup> at 5 cm soil depth and  
145 running parallel at 20 cm distance from each other (canopy and soil warming). Soil warming  
146 cables were switched on 2 days after the lamps to simulate the delay in soil thaw during a real  
147 event. Control plots received no warming treatment and remained insulated under the natural  
148 winter snow cover. To monitor temperature, thermistors were placed in each plot at canopy  
149 height and at the soil surface and 5 cm depth. Temperatures were logged at 6-h intervals and  
150 recorded on a data logger.

151 Simulation of discrete winter warming events started at the beginning of March in 2007,  
152 2008 and 2009. Each event lasted 7 days during which the lamps were kept at 50 cm distance  
153 from the snow surface and lowered accordingly as the snow depth decreased. This approach  
154 ensured gradual snow thaw, taking two to three days to thaw the full depth of snow in each

155 plot. As vegetation became exposed, lamps were kept at 50 to 70 cm above the soil surface to  
156 maintain canopy warming (the lower lamp heights were needed during higher wind speeds  
157 and lower ambient temperatures). Temperatures from the thermistors were monitored to  
158 ensure warming was realistic and within the bounds of temperatures recorded for real events.  
159 Thermocouple measurements of vegetation surface temperatures were also made. At the end  
160 of the warming treatment, heating lamps were removed from the frames to avoid shading  
161 effects of the lamps during the following growing season. Plots were then left untouched for  
162 the remaining period of winter before data collection in spring and summer (late May–  
163 August).

164

#### 165 SPECIES

166 *Hylocomium splendens* and *Peltigera aphthosa* are the dominant cryptogams in the ground  
167 flora. These two species are most abundant in mesic heath vegetation, which, under normal  
168 winter conditions, is covered by snow for 5-7 months. *Hylocomium splendens* occurred in all  
169 18 plots, but in some plots with very low abundance, while *P. aphthosa* was absent from three  
170 plots, reducing the number of replicates to 4 and 5 for canopy and canopy-and-soil warming,  
171 respectively. Cyanobacteria grow as epiphytes on the stems of *H. splendens* and in well-  
172 defined colonies, called cephalodia, in the thallus of *P. aphthosa*, which is tripartite, i.e. it  
173 consists of three major bionts; the fungus, the green alga and the cyanobacterium.

174 Measurements of the cyanobacteria's nitrogen fixation rates are presented in Appendix S1 in  
175 Supporting Information.

176

#### 177 PHOTOSYNTHESIS AND CHLOROPHYLL FLUORESCENCE

178 Gas exchange measurements were taken in June or July during the growing seasons after  
179 winter warming events. Measurements were made with the portable gas exchange  
180 fluorescence system GFS-3000 (Heinz Walz GmbH, Effeltrich, Germany). Since

181 photosynthesis in poikilohydric organisms is strongly affected by water status, repeated  
182 measurements were made on each sample, starting with very humid samples that experienced  
183 photosynthetic depression. Samples for gas exchange were randomly selected and carefully  
184 removed from the plots, placed on paper towels in small containers and sprayed with water to  
185 achieve full hydration. The samples were left overnight to fully recover from desiccation.  
186 Samples were re-hydrated in the morning before initiation of measurements. Only first-year  
187 and second-year segments of *H. splendens* were used, as older segments have reduced  
188 photosynthetic rates (Oechel & van Cleve 1986). Each moss sample consisted of c. 5 cut  
189 shoots. Samples of *P. aphthosa* consisted of one ellipsoid lobe without apothecia, c. 2.5 cm  
190 wide and 4 cm long. Samples were allowed to slowly air dry between measurements, which  
191 were made until assimilation rates showed that optimal water content was passed.  
192 Immediately after each measurement, samples were dried completely and weighed.  
193 Assimilation rates were then calculated on a per-dry-weight basis. One sample each of *P.*  
194 *aphthosa* and *H. splendens* was analysed from each plot at each occasion.

195 A saturating photosynthetic photon flux density (PPFD) of  $800 \mu\text{mol m}^{-2} \text{s}^{-1}$  was used  
196 during measurements of net photosynthesis.  $\text{CO}_2$  concentration was set to 380 ppm and  
197 cuvette humidity to 9000 ppm  $\text{H}_2\text{O}$ . Cuvette temperatures were adjusted to ambient noon  
198 temperatures at the time of measurement. Samples were stored at the same temperatures as the  
199 one at which they were dried between the measurements. They were equilibrated in the  
200 cuvette for 20 min each time, and exchange rates were measured repeatedly during the last 10  
201 min of this period.

202 Using the PAM-fluorometer of the GFS-3000, maximal quantum efficiency of PS II, i.e.  
203  $F_v/F_m$  (Maxwell & Johnson 2000), was measured on dark-adapted, naturally moist or wetted  
204 samples during the growing seasons after the winter warming events. Measurements on *H.*  
205 *splendens* were taken on second-year plus first-year developing segments (Callaghan *et al.*

206 1978). Measurements on *P. aphthosa* were taken on young lobe parts without any, or with  
207 very few, visible cephalodia.

208

#### 209 SEGMENT GROWTH

210 Shoots of *H. splendens* collected at one point in time during the growing seasons following  
211 the winter warming events of 2008 and 2009, and also during the first recovery growing  
212 season (first season following a winter without an event simulation—2010), were dried, and  
213 lengths of first-year and second-year segments were measured with digital callipers.

214

#### 215 DATA ANALYSES

216 Differences between treatments at any one measurement event were tested with a fixed one-  
217 way ANOVA design. Time differences and overall treatment differences, and their  
218 interactions, were tested with repeated-measures ANOVA. The Tukey HSD test was used for  
219 multiple comparisons of treatment effects. *F*- and *P*-values presented in the text are from the  
220 repeated-measures ANOVA unless otherwise stated.

221 Data sets containing more than one randomly chosen data point per replicate (plot) were  
222 first surveyed using a nested ANOVA design in order to check whether the variation within  
223 the replicates was larger than between replicates. As the variation within plots in all cases was  
224 very low as compared to the variation between plots, the mean value per replicate was used in  
225 further ANOVA analyses. Data sets were tested for normality and heterogeneity. No  
226 transformation of data was necessary. No significant or near-significant ( $P < 0.10$ )  
227 interactions between treatment and time were detected for any of the analysed parameters.  
228 Therefore, time-treatment interaction terms are not referred to in the Results. All tests were  
229 done using the SPSS 16 and 17 statistical packages for Windows (SPSS Inc., Chicago, IL,  
230 USA).

231

## 232 **Results**

### 233 TREATMENT EFFECTS ON CANOPY AIR AND CRYPTOGAM SURFACE TEMPERATURES

234 The initiation of the canopy warming and canopy and soil warming treatments caused an  
235 immediate rise in soil and canopy air temperatures (see Bokhorst *et al.* 2008, 2009, 2010 for  
236 detailed Figures). In all years, both types of warming treatment resulted in complete snow  
237 melt and full exposure of the ground vegetation after 3 days, as typically observed during a  
238 natural warming event (Bokhorst *et al.* 2009). Leaf canopy temperatures were on average  
239 between 5-10 °C during the warming simulations. Canopy and soil surface temperatures did  
240 not differ between the canopy-only and the canopy-with-soil warming treatments, whereas the  
241 soil warming in the canopy and soil warming treatment increased soil temperatures by 4 °C at  
242 5 cm depth compared to control plots (see Fig 2b in Bokhorst *et al.* 2008). Surface  
243 temperatures of *H. splendens* and *P. aphthosa*, measured by leaf thermocouples, were on  
244 average 8.6 for both species  $\pm$  0.6 and 0.7 °C standard error, respectively.

245 After the warming periods (following switching off of the lamps and cables), temperatures  
246 in the warmed plots immediately declined and returned to ambient. However, the small  
247 amount of snow that accumulated in the warmed plots following the warming periods was not  
248 sufficient to re-insulate the ground cover, resulting in much colder canopy temperatures (with  
249 a minimum at -18 °C) than in plots covered by snow (controls, minimum temperature of -8  
250 °C) for the remainder of winter until spring.

251

### 252 *PELTIGERA APHTHOSA*

253 The warming treatments of 2007, 2008 and 2009 did not affect net photosynthetic rates and  
254 PS II efficiency in the following growing seasons (Fig. 1). There was some variation in net  
255 photosynthetic rates between growing seasons, but this variation was also seen in the controls  
256 (Fig. 1a). There was no time effect on PS II efficiency. The warming treatments did not affect

257 the nitrogen fixation rates of *P. aphthosa* and the associated lichen *Nephroma arcticum* (Fig.  
258 S1).

259

#### 260 *HYLOCOMIUM SPLENDENS*

261 Growing season net photosynthetic rates in *H. splendens* were reduced by an average of 44%  
262 over the three years in the canopy warming treatment and 35% in the canopy and soil  
263 warming treatment (Fig. 2a). In 2007, net photosynthetic rates were significantly lower in the  
264 canopy warming treatment than in the control ( $P = 0.03$ ). While the canopy and soil warming  
265 treatment was not statistically different from the canopy warming treatment or the control, it  
266 none-the-less had a mean value much closer to that of the canopy warming treatment. In 2008  
267 and 2009, both warming treatments had significantly lower rates than the controls, while there  
268 were no significant differences between the two treatments (Fig. 2a). The relative reductions  
269 and corresponding one-way  $P$ -values for 2007, 2008 and 2009 were 38% (0.03), 48% (0.01)  
270 and 47% (0.008) in the canopy warming treatment, and 32% (0.15), 42% (0.04) and 44%  
271 (0.02) in the canopy and soil warming treatment. There were no treatment or time effects on  
272 PS II efficiency (Fig. 2b).

273 Growth of shoot segments in the warmed plots was severely reduced following the second  
274 and third winter warming events, while there were no significant differences between the two  
275 warming treatments (Fig. 3). Following the second event (2008), second-year segment growth  
276 was reduced by 33-35% in the treatment plots (Fig. 3b, left panel). However, first-year  
277 segment growth in 2008 was more variable and only marginally affected (Fig. 3a, left panel;  
278 warming treatments analysed separately:  $F_{2,15} = 2.68$ ,  $P = 0.101$ ; warming treatments pooled:  
279  $F_{1,16} = 4.54$ ,  $P = 0.049$ ). In 2009, both first-year and second-year segments suffered from a  
280 significant, serious growth reduction in winter-warmed plots (50-52% and 40-45% reduction,  
281 respectively; Fig. 3, middle panels). These effects persisted during the first recovery growing  
282 season, i.e. the growing season of 2010, with a 34-38% reduction in first-year segments, and a

283 41-42% reduction in second-year segments in treated plots compared to controls (Fig. 3, right  
284 panels).

285 The nitrogen fixation rate of epiphytic cyanobacteria growing on *H. splendens* was not  
286 affected by the warming treatments (Fig. S1).

287

## 288 **Discussion**

289 Very little is known about the winter ecology of bryophytes and lichens in general, and their  
290 responses to winter climate change in particular are more or less unknown (Glime 2007;  
291 Bjerke 2009, in press) with most cold- and frost-related research on bryophytes and lichens  
292 being performed either in the laboratory or during the snow-free season in cold biomes.  
293 Whereas some winter climate change studies have tested the effects of altered snow  
294 conditions on bryophyte- and lichen-containing vegetation (Dorrepaal *et al.* 2003; Wahren *et*  
295 *al.* 2005; Scott *et al.* 2007), this study is the first to test the effects of winter warming events  
296 on these important functional groups.

297 Our findings are consistent with the hypothesis that lichens and their associated  
298 cyanobacteria are tolerant of short-lived winter warming events as seen from the general  
299 absence of winter warming impacts on chlorophyll fluorescence, net photosynthetic rates and  
300 nitrogen fixation rates in the dominant *P. aphthosa*. In contrast, the dominant bryophyte *H.*  
301 *splendens* was clearly not tolerant of winter warming events, showing significant reductions  
302 both in net photosynthetic rates and growth rates. The reasons for the contrasting sensitivity  
303 are not fully understood, although such differences may be driven by contrasting growth form  
304 and seasonal rhythm between lichens and bryophytes from mesic habitats.

305 While radial growth in lichens is undertaken by an almost indefinite number of hyphal  
306 cells (e.g. Armstrong 2003), growth in mosses is restricted to a few regions of meristematic  
307 cells (e.g. La Farge-England 1996). The huge difference in number of growing points  
308 suggests that the buffering capacity of lichens is much higher than that of mosses against  
309 damage from external stress. This may explain why only the moss was affected by the winter  
310 warming treatments.

311 Many bryophytes, *H. splendens* included, go through phenological stages with initiation  
312 of growth of new segments early in the growing season, completion of growth during the

313 growing season and instigation of frost resistance mechanisms in autumn (Callaghan *et al.*  
314 1978; Longton 1988; Rütten & Santarius 1992). There is, to our knowledge, no evidence of  
315 active growth of sub-Arctic or alpine heath mosses during the cold season, despite the  
316 potential for slightly positive net photosynthetic rates under rather low light intensities in  
317 autumn and after snow melt in spring (Kallio & Saarnio 1986; Larsen *et al.* 2007). Breaking  
318 of dormancy during the winter warming events is the likely driver for the observed mortality  
319 of the vascular plants (Bokhorst *et al.* 2010). Mosses probably also experience breaking of  
320 dormancy, and reactivation in mid-winter may disturb the seasonal timing in *H. splendens*,  
321 initiating development of new segments. Young shoot apices appear to be especially  
322 susceptible to freezing damage (Clausen 1964; Hudson & Brustkern 1965), and indeed this  
323 was also observed in bryophytes living in cold Antarctic environments (Longton & Holdgate  
324 1967; Collins 1976; Kennedy 1993). Temperatures at  $-7.6$  °C within 24 h after warming was  
325 switched off in our study, followed by temperatures as low as  $-18$  °C two weeks later  
326 (Bokhorst *et al.* 2008, 2009, 2010) probably caused freezing damage to the moss shoot apices,  
327 which was manifest as reductions in growing season photosynthesis and growth. The recorded  
328 reductions in *H. splendens* photosynthesis and growth seem to be far beyond the year-to-year  
329 variation found in populations with stable winter climates (cf. Callaghan *et al.* 1978, 1997;  
330 Økland 1997).

331 Lichens, on the other hand, do not have any clear phenological stages that need to be  
332 completed before the onset of winter (Benedict 1990; Lange 2003; Hahn *et al.* 1993; Kappen  
333 & Valladares 2007). When reactivated, lichens apparently continue growth processes at the  
334 point they were stopped before anabiosis, and there are no vulnerable organs in lichens which  
335 are susceptible to freezing damage, like the young, frost-sensitive moss shoot apices. Hence,  
336 in contrast to *H. splendens*, the lack of clear phenological stages contributes to explaining  
337 why *P. apthosa* was unaffected by the mid-winter warming.

338 We have interpreted the reduced vitality of *H. splendens* as being a result of the freezing  
339 stress that they were exposed to after the warming events. However, as winter-adapted  
340 cryptogams have reduced heat tolerance (Tegler & Kershaw 1981; Longton 1988), one may  
341 consider that the warming *per se* also had negative effects. There are, however, to our  
342 knowledge, no reports that might suggest that *H. splendens* would be less tolerant than *P.*  
343 *aphthosa* to winter heat. Moreover, the warming temperatures they were exposed to during  
344 the events are similar to the temperature regimes immediately after snow melt in spring (and  
345 not followed by severe freezing), and these natural conditions do not seem to harm the  
346 cryptogams in any way, suggesting that the damage to *H. splendens* was largely caused by the  
347 severe freezing temperatures following the warming events. The responses seen here for *H.*  
348 *splendens* may be restricted to continental regions in contrast to more oceanic regions where  
349 extreme winter warming events are unlikely to be followed by very low temperatures (< -15  
350 °C). It is currently unclear if the bryophyte response seen here is driven by the disruption of  
351 the developmental program or simply by deep-freezing due to the absence of snow cover.  
352 Further studies incorporating different regimes of mid-winter snow melting and re-covering  
353 with snow across oceanic to continental gradients should elucidate the mechanisms behind the  
354 susceptibility of *H. splendens* to these winter warming events.

355 There were no significant treatment effects on the PS II efficiency of any of the tested  
356 species. It is interesting that PS II efficiency in *H. splendens* was not affected by the warming  
357 treatments, while net photosynthesis was. Damage to PS II is often the first manifestation of  
358 stress (Maxwell & Johnson 2000). However, PS II efficiency is not always a true indicator of  
359 plant stress. Several stress studies, both on vascular plants and bryophytes, have found strong  
360 effects on growth and photosynthetic rates, but no or only small effects on PS II efficiency  
361 (e.g. Taulavuori *et al.* 2000; Nabe *et al.* 2007; Granath *et al.* 2009). Although the mechanisms  
362 are not fully understood, these reports and our results suggest that gas exchange and growth

363 rates are more precise indicators of plant performance under various types of stress  
364 conditions.

365 Activity of organisms underneath winter snow cover is probably less dormant than  
366 generally assumed. Indeed, the microbial community composition is known to change during  
367 winter (Schadt *et al.* 2003), considerable ecosystem carbon fluxes occur (Larsen *et al.* 2007;  
368 Liptzin *et al.* 2009) and some plants actively grow new roots underneath the snow layer to  
369 access nutrients (Onipchenko *et al.* 2007). Bryophytes and lichens are also among the  
370 organisms which can be active during favourable winter conditions. However, the contrasting  
371 sensitivities of the dominant bryophyte and lichen in our study suggest that this strategy may  
372 not be successful under all winter snow conditions. The contrasting sensitivities further  
373 suggest that many more species-specific studies on cryptogam winter activity are required if  
374 we are to understand their roles in winter ecosystem processes, which also affect growing  
375 season processes (Cornelissen *et al.* 2007).

376

#### 377 CONCLUDING REMARKS

378 We have shown here that acute, extreme warming events in the sub-Arctic can strongly affect  
379 the ecophysiology and growth of the dominant bryophyte *H. splendens*. This bryophyte  
380 therefore appears sensitive to winter warming, as are the dwarf shrubs in this widely  
381 distributed Arctic/sub-Arctic ecosystem (Bokhorst *et al.* 2008, 2009, 2010). If such events  
382 become more frequent, as a result of climate change, this may have large consequences for  
383 productivity of the affected species, and may ultimately induce community shifts (Hobbs *et*  
384 *al.* 2007; Bokhorst *et al.* 2008). Only one lichen species, *Peltigera aphthosa*, has so far been  
385 investigated in detail. The contrasting sensitivities of the dominant bryophyte and lichen  
386 species were unexpected, and highlight that the general understanding of cryptogams'  
387 adaptive mechanisms to winter climate and snow cover is still poor. If most lichen species are  
388 tolerant to winter warming events, then lichens may be favoured by such events at the

389 expense of competing dwarf shrubs and bryophytes. If so, some of the trends for declines in  
390 lichen-dominated ecosystems seen in recent decades (Cornelissen *et al.* 2001; Tømmervik *et*  
391 *al.* 2004; van Wijk *et al.* 2004; Walker *et al.* 2006; Rees *et al.* 2008; Myking *et al.* 2009) may  
392 be compensated for, which is in sharp contrast to the current view that global change will  
393 enhance vascular plant biomass at the expense of lichens (Cornelissen *et al.* 2001; van Wijk *et*  
394 *al.* 2004). Furthermore, the consequences for reindeer husbandry—where lichens are a major  
395 food source—may be much less negative than hitherto predicted (Rees *et al.* 2008). Whereas  
396 climate-change-driven extension of the growing season can affect lichens negatively, climate-  
397 change-driven discrete winter warming events may have positive effects on lichens. The  
398 former of these two climate change elements have, to date, received far more attention  
399 (Jentsch *et al.* 2007; Post *et al.* 2009). Our current study shows that acute climate events in  
400 mid-winter may be equally important as summer warming for regulation of high-latitude  
401 ecosystem functioning and community composition. Overall, the changes place a considerable  
402 challenge to predicting vegetation change in a future Arctic where winter will warm more  
403 than summer.

404

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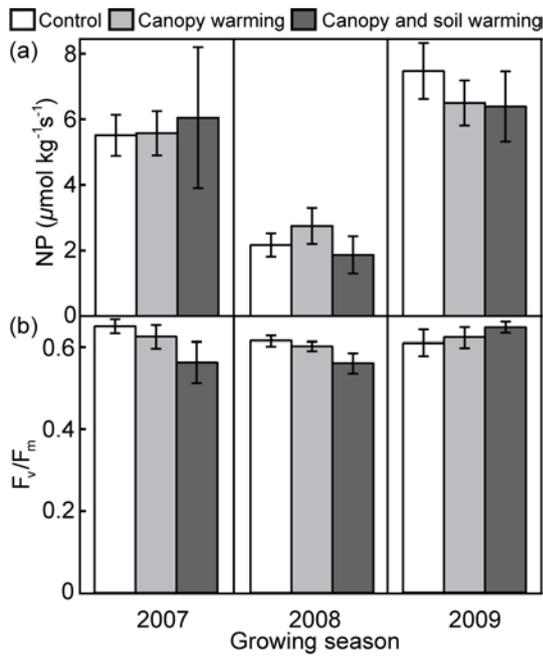
582 **SUPPORTING INFORMATION**

583 Additional supporting information may be found in the online version of this article:

584 **Appendix S1** *Nitrogen fixation potential.*

585 **Figure S1** *Nitrogen fixation rates of Peltigera aphthosa, Hylocomium splendens and*  
586 *Nephroma arcticum.*

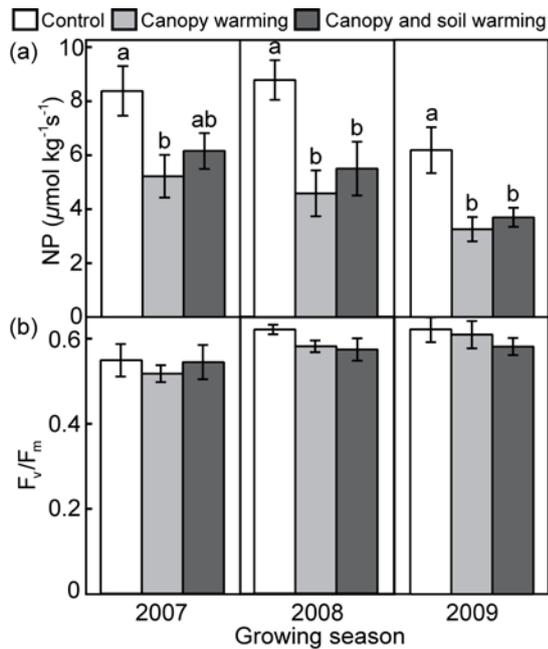
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590 **Fig. 1.** Ecophysiological performance of *Peltigera aphthosa* in the growing seasons following  
 591 winter warming events. (a) Net photosynthetic rates at optimal water content (treatment:  $F_{2,9}$   
 592 = 2.9,  $P = 0.11$ , time:  $F_{2,18} = 31.8$ ,  $P = 0.000$ ); (b) photosystem II efficiency of dark-adapted  
 593 samples (treatment:  $F_{2,10} = 0.2$ ,  $P = 0.85$ , time:  $F_{2,20} = 4.3$ ,  $P = 0.028$ ).  $n = 4-5$  per treatment.  
 594 Error bars are  $\pm 1$  SE.

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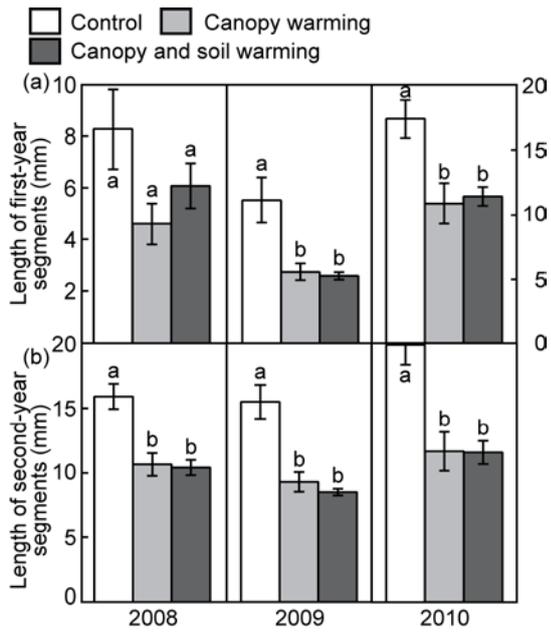
598 **Fig. 2.** Ecophysiological performance of *Hylocomium splendens* in the growing seasons

599 following winter warming events. (a) Net photosynthetic rates at optimal water content

600 (treatment:  $F_{2,15} = 15.5$ ,  $P = 0.000$ , time:  $F_{2,30} = 7.8$ ,  $P = 0.002$ ); (b) photosystem II efficiency601 of dark-adapted samples (treatment:  $F_{2,13} = 1.2$ ,  $P = 0.34$ , time:  $F_{2,26} = 4.1$ ,  $P = 0.03$ )  $n = 4-6$ 

602 per treatment and time combination (some plots with low abundance and hence not sampled

603 in all occasions). Error bars are  $\pm 1$  SE. Different letters indicate significant differences ( $P <$ 604  $0.05$ ) between treatments.



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**Fig. 3.** Lengths of shoot segments of *Hylocomium splendens* measured in the growing seasons following the winter warming events of 2008 and 2009, and during the first recovery season of 2010. (a) First-year segments, which were under development. Left scale is for 2008 and 2009, right scale is for 2010 (samples were collected later in the growing season in 2010, hence the different scales). (b) Second-year segments. Different letters above bars indicate significant differences between treatments. First-year segments: treatment:  $F_{2,15} = 11.8$ ,  $P = 0.001$ , time:  $F_{2,30} = 75.5$ ,  $P = 0.000$ . Second-year segments: treatment:  $F_{2,15} = 41.0$ ,  $P = 0.000$ , time:  $F_{2,30} = 7.71$ ,  $P = 0.002$ .  $n = 6$  for each treatment. Error bars are  $\pm 1$  SE. Different letters indicate significant differences ( $P < 0.05$ ) between treatments.