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1 **Persistent reduction of segment growth and photosynthesis in a**
2 **widespread and important sub-Arctic moss species after cessation**
3 **of three years of experimental winter warming**

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6
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19
20 **Summary**

21 **1.** Winter is a period of dormancy for plants of cold environments. However, winter
22 climate is changing, leading to an increasing frequency of stochastic warm periods
23 (winter warming events) and concomitant reductions in snow cover. These conditions
24 can break dormancy for some plants and expose them to freeze-and-thaw stress.

25 Mosses are a major component of high latitude ecosystems, yet the longer-term
26 impacts of such winter warming events on mosses remain unknown.

27 **2.** In order to determine the longer-term legacy effects of winter warming events on
28 mosses, we undertook a simulation of these events over three consecutive winters in a
29 sub-Arctic dwarf shrub-dominated open woodland. The mat-forming feathermoss
30 *Hylocomium splendens* (the most abundant cryptogam in this system), is one of the
31 most widespread Arctic and boreal mosses and plays a key functional role in
32 ecosystems. We studied the ecophysiological performance of this moss during the
33 summers of the experimental period (2007-2009) and in the following years (2010-
34 2013).

35 **3.** We show that the previously reported warming-induced reduction in segment growth
36 and photosynthesis during the experimental years was persistent. Four years after the
37 last event, photosynthesis and segment growth were still 30 and 36 % lower than
38 control levels, which was only a slight improvement from 44 and 43 % four years
39 earlier. Winter warming did not affect segment symmetry. During the years after the
40 last simulated event, in both warmed and control plots, chlorophyll fluorescence and
41 segment growth, but not net photosynthesis, increased slightly. The increases were
42 probably driven by increased summer rainfall over the study years, highlighting the
43 sensitivity of this moss to rainfall change.

44 **4.** Overall, the legacy effects shown here demonstrate that this widespread and important
45 moss is likely to be significantly disadvantaged in a future sub-Arctic climate where
46 frequent winter warming events may become the norm. Given the key importance of
47 mosses for soil insulation, shelter and carbon sequestration in high-latitude regions,
48 such persistent impacts may ultimately affect important ecosystem functions.

49

50 **Key-words:** Arctic, bryophyte, climate change, extreme events, feathermoss, *Hylocomium*,
51 snowmelt, warmer winters

52

53 **Introduction**

54

55 Understanding the impacts of climate change on ecosystems is complex for many reasons.
56 Firstly, there are many drivers of change and each driver, for example increasing temperature,
57 has numerous interacting facets such as diurnal, seasonal and extremes. Secondly, there are
58 practical issues that hinder research, such as difficult species groups and winter research. In
59 this paper, we focus on a cryptogam (an understudied group), the winter period (under-
60 represented in field studies) and extreme warming events, that are both difficult to observe
61 and even more difficult to predict (Bokhorst *et al.* 2016; Phoenix & Bjerke 2016).

62 Climate in northern ecosystems is changing more than the global average and more
63 abruptly during winter than during summer (Walsh *et al.* 2011). Despite this, there are far
64 fewer reports on moss responses to winter climate change than to summer warming. A recent
65 literature search and review of ecosystem responses to winter climate change (Cooper 2014)
66 found only four reports related to cryptogams, only two of these (Dorrepaal *et al.* 2004;
67 Bjerke *et al.* 2011) dealt with mosses, and only the latter treated effects of stochastic warm
68 periods (winter warming events).

69 Warm winters are often characterized by one or several warming events of variable
70 duration preceded and followed by naturally cold, freezing periods (Putkonen & Roe 2003;
71 Bokhorst *et al.* 2008; Bjerke *et al.* 2014; Hansen *et al.* 2014). During warming events, snow
72 cover melts completely or partly, exposing the ground vegetation to subsequent ambient
73 temperatures, which may be 15-30 degrees below freezing and fluctuate much more than in
74 the subnivean environment (Pauli *et al.* 2013). The dehardening of vascular plants during such

75 events leads to increased susceptibility to freezing on the return of ambient sub-zero winter
76 temperatures. Therefore, many vascular plants are severely damaged after winter warming
77 events, as shown both by analyses of natural events and field experiments (Bokhorst *et al.*
78 2009, 2010, 2012a; Bjerke *et al.* 2014; Cooper 2014).

79 The clonal mat-forming feathermoss *Hylocomium splendens* (Hedw.) Schimp. is the
80 only moss that has been studied in relation to winter warming events (Bjerke *et al.* 2011). It is
81 one of the most common and widespread mosses of the circumboreal forest and Arctic tundra,
82 and it has scattered occurrences elsewhere, for instance at high altitudes close to the Equator
83 (Rohrer 2004; GBIF 2015). It is probably one of the most abundant moss species in the world.
84 As other mat-forming mosses, it plays a crucial functional role in its ecosystems. It
85 contributes to soil insulation and stability, preservation of permafrost, sheltering for small
86 organisms, carbon balance, nitrogen fixation, and ecosystem conservation through hampering
87 of seed establishment (Chapin & Bledsoe 1992; Hörnberg, Ohlson & Zackrisson 1997;
88 Beringer *et al.* 2001; Heijmans, Arp & Chapin 2004; Cornelissen *et al.* 2007; Bjerke *et al.*
89 2011, 2013; Yoshikawa *et al.* 2002; Lang *et al.* 2012; Bokhorst *et al.* 2014). It is therefore of
90 major functional importance in its ecosystems.

91 Segment growth and photosynthetic activity of *H. splendens* was severely reduced (up
92 to 52 %) after three consecutive winters with short-lasting warming events that exposed the
93 moss to ambient temperatures (Bjerke *et al.* 2011, 2013). Negative effects were evident
94 already during the summer following the first winter warming event. Photosynthetic rates and
95 segment growth rates of previously winter-warmed mosses were 48 % to 52 % lower than in
96 control plots. During the winter warming events, photosynthetic rates were at optimal rates,
97 viz. similar to or higher than the rates achieved in summer. This clearly shows that the moss
98 was activated during the winter warming events. *Hylocomium splendens* and other mosses
99 initiate the growth of young, freeze-susceptible shoot apices in the early growing season

100 (Clausen 1964; Hudson & Brustkern 1965). Temperatures at -7.6 °C within 24 h after
101 warming was ceased in the winter warming study, followed by temperatures as low as -18 °C
102 two weeks later (Bokhorst *et al.* 2008, 2009, 2010) probably caused freezing damage to the
103 moss shoot apices, which resulted in reductions in growing season photosynthesis and
104 segment growth far beyond the year-to-year variation found in populations with stable winter
105 climates (Bjerke *et al.* 2011). Death of apices promotes the sympodial growth form over the
106 monopodial form. Although the two growth forms are genetically determined to some extent,
107 they are both found in the same area and respond to environmental cues (Ross *et al.* 2001).
108 This shows that mosses are under threat from winter warming events. However, impacts of
109 winter warming beyond the immediate short-term responses reported above (Bjerke *et al.*,
110 2011) remain unknown.

111 Here, we follow up on the short-term responses in the experimental period reported in
112 Bjerke *et al.* (2011) with a longer-term assessment of the moss growth and photosynthesis
113 responses in the four years after the warming simulations, to determine the persistence of the
114 observed negative effects arising from the events. We also studied in more detail the
115 morphology of moss segments by resurveying all moss samples taken from the experimental
116 years and following years to test whether declining vitality is associated with developmental
117 instability. Fluctuating asymmetry (FA), as a measure of developmental instability, is widely
118 used as a symptom of stress in vascular plant leaves and animals (Palmer & Strobeck 1992,
119 2003; Kozlov *et al.* 1996). However, FA in mosses is a poorly studied subject; to our
120 knowledge, the applicability of FA as a stress signal in mosses has not been assessed
121 previously.

122 We hypothesized that surviving individuals of *H. splendens* from three consecutive
123 winter warming events (Bjerke *et al.* 2011) would increase photosynthetic and segment
124 growth rates and reach the same level as in control plots within the first years after the last

125 winter warming event. Furthermore, we hypothesized that retarded growth also would be
126 associated with increasing FA of moss segments, and that this trait would disappear during
127 recovery of normal segment growth rates.

128 During the recovery years of 2012 and 2013, our study site and the entire Abisko region
129 (Swedish sub-Arctic) was affected by a caterpillar outbreak of the geometrid moth *Epiritta*
130 *autumnata* (Bjerke *et al.* 2014; Bokhorst *et al.* 2015). The caterpillars defoliated many
131 vascular plants, and this led to a decline in the cover of dwarf shrubs and an increase of moss
132 cover in the years of 2012 and 2013 (Bokhorst *et al.* 2015). These summers were also much
133 wetter than the summers during the experimental period (Table 1; see also Bokhorst *et al.*
134 2015). Since these environmental changes took place after the study was initiated, we did not
135 phrase any *a priori* hypotheses of their impacts on the ecophysiology of *H. splendens*.
136 However, knowing that summer rainfall stimulates moss growth (Potter *et al.* 1995, Sonesson
137 *et al.* 2002), that more space would be available for horizontal growth due to vascular plant
138 damage, and that more throughfall of nutrients would reach the cryptogam layer (Bokhorst *et*
139 *al.* 2015); we hypothesized *a posteriori* that the ecophysiological performance of *H.*
140 *splendens* would benefit from the caterpillar outbreak and the increasing precipitation rates.

141

142

143 **Material and methods**

144

145 FIELD SITE AND EXPERIMENTAL SIMULATIONS

146 Simulations of winter warming events in the field were conducted on a sub-Arctic heathland
147 in open mountain birch (*Betula pubescens*) forest close to the Abisko Scientific Research
148 Station (ANS) in northern Sweden (68° 21' N, 18° 49' E) during March 2007, 2008 and 2009.
149 Details of the research site and experimental design are described in Bokhorst *et al.* (2008,
150 2009) and Bjerke *et al.* (2011). In brief, the experiment consisted of 18 plots (2.1 m × 1.0 m),

151 consisting of 6 control plots that remained under their natural snow cover throughout the
152 winter; 6 that were exposed to a week-long winter warming event called ‘canopy warming’,
153 using infrared heating lamps (800 W emitting at 3 μm ; HS 2408, Kalglo Electronics Co.,
154 Bethlehem, USA); and 6 where warming from infrared heating lamps was combined with soil
155 warming from cables at 5 cm soil depth called ‘canopy and soil warming’. Soil warming
156 cables (LS-TXLP, Nexans, Norway, producing 120 W m^{-2}) were switched on two days after
157 the lamps to simulate the delay in soil thaw during a real event. Complete snow melt and
158 exposure of the vegetation was achieved within two to three days.

159 Temperature was monitored with thermistors placed in each plot at canopy height and at
160 the soil surface, with logging at 6-h intervals recorded on a data logger (CR10 X, Campbell
161 Scientific, UK). Details on the temperature effects of the treatments are provided in Bokhorst
162 *et al.* (2011, 2012b). In summary, temperatures rose on average to 5 °C during the events,
163 while for the remainder of winter, temperatures fluctuated concurrently with the ambient
164 conditions (ranging from -17 °C to 4 °C) due to the reduced snow cover (initially none, and
165 then thin coverage as new snow covered the exposed vegetation), meaning there was little to
166 no insulation from air temperatures. In contrast, control plots remained well insulated under
167 snow and experienced temperatures between -7 °C and 0 °C until snowmelt. Weather data
168 presented are from the meteorological station at ANS, ca. 200 m from our study site.

169

170 PHOTOSYNTHESIS AND CHLOROPHYLL FLUORESCENCE

171 Gas exchange (CO_2) was measured in June or July during the summers following the
172 experimental years from 2007 to 2009 and during the summers of 2012 and 2013.
173 Measurements were made with a portable gas exchange fluorescence system (GFS-3000,
174 Heinz Walz GmbH, Effeltrich, Germany) using moss segments with optimal water content, as
175 described in Bjerke *et al.* (2011). Only first-year and second-year segments of *H. splendens*

176 were used, as older segments have reduced photosynthetic rates (Callaghan *et al.* 1978, 1997).
177 Each moss sample consisted of *c.* 5 cut shoots. Assimilation rates were calculated on a per-
178 dry-weight basis. One sample was analysed from each plot at each occasion. A saturating
179 photosynthetic photon flux density (PPFD) of $800 \mu\text{mol m}^{-2} \text{s}^{-1}$ was used during
180 measurements of net photosynthesis. CO₂ concentration was set to 380 ppm and cuvette
181 humidity to 9000 ppm H₂O. Cuvette temperatures were adjusted to ambient noon
182 temperatures at the time of measurement.

183 Using the PAM-fluorometer of the GFS-3000, chlorophyll fluorescence, as maximal
184 quantum efficiency of photosystem II, i.e. F_v/F_m (Maxwell & Johnson 2000), was measured
185 on dark-adapted, naturally moist or wetted samples consisting of first- and second-year
186 segments.

187

188 SEGMENT GROWTH AND ASYMMETRY

189 Segments were collected at one point in time during the growing seasons following the winter
190 warming events of 2007, 2008 and 2009, and also during the growing seasons of 2010, 2011
191 and 2013. These were air-dried, and lengths of second-year segments were measured with
192 digital callipers along the main stem. Total width, i.e. left (L) plus right (R) sides of the
193 segment, was measured on the same second-year segments at the place where the shoot
194 segment was widest, typically near the segment base, perpendicular to the stem. Samples were
195 collected in June or early July, except for in 2010, when they were collected in late August.

196 Width of each side (i.e. R and L separately) was subsequently measured on samples
197 from 2007, 2008, 2009 and 2011. This latter dataset was first tested for directional
198 asymmetry, i.e. when one side is consistently larger than the other in the same direction
199 (Palmer & Strobeck 2003). Thereafter, we tested for treatment effects on fluctuating
200 asymmetry (FA). As FA has not been tested on mosses previously, we screened the utility of

201 various FA indices. The most commonly used indices all rendered similar results, and we here
202 present results for the index for trait difference divided by trait mean $[|R-L|/((R+L)/2)]$.

203

204 DATA PUBLISHED PREVIOUSLY

205 The ecophysiological data from the experimental years and the shoot segment lengths from
206 the years 2008-2010 were first reported in Bjerke *et al.* (2011), there with separate values for
207 the two types of warming treatment data, while we here pool these data (see below). The
208 following data have not been reported previously: the ecophysiological data from 2012 to
209 2013, segment lengths from 2011 and 2013, all data on segment width, length-to-width ratios
210 and FA.

211

212 DATA ANALYSES

213 All tests were run with SPSS Statistics 22 (IBM Corporation, Armonk, NY, USA).

214 Differences between the two warming treatments were first tested with a series of Student's *t*-
215 tests. For most of the measured parameters (except FA), there were no significant differences
216 between the two treatments, and the two types of warming treatment data could be pooled
217 (canopy warming, and canopy and soil warming), here called 'winter warming'. Time
218 differences, overall treatment differences and their interactions, and pairwise comparisons of
219 specific years were tested with the repeated-measures ANOVA procedure of SPSS. The
220 Tukey HSD test was used for multiple comparisons of treatment effects. Year-to-year
221 comparisons from the repeated-measures ANOVA were also checked with paired student's *t*-
222 tests, and these two tests rendered almost identical results. Student's *t*-tests were also applied
223 to test for differences between left and right segment widths.

224 Data sets containing more than one randomly chosen data point per replicate (plot) were
225 first surveyed using a nested ANOVA design in order to check whether the variation within
226 the replicates was larger than between replicates. As the variation within plots in all cases was

227 very low as compared to the variation between plots, the mean value per replicate was used in
228 further ANOVA analyses. Data were tested for normality and heterogeneity of variance prior
229 to ANOVAs and correlations. No transformation of data was necessary.

230

231

232 **Results**

233

234 Negative effects of the simulated winter warming events on net photosynthesis (NP) were still
235 evident in *Hylocomium splendens* in the summers 3-4 years after the last simulated event, i.e.
236 in 2012 and 2013 (Fig. 1a). The average reduction from controls was 32 % in 2012 and 30 %
237 in 2013, whilst in 2009, the summer after the last winter warming event, the reduction was 44
238 %.

239 Even if NP of both treatments (warming and control) reached minimum rates in 2009
240 (Fig. 1a), pairwise comparisons show that NP in controls did not vary in time, except for the
241 pair 2009 and 2012 ($P = 0.010$). The only between-year differences for NP in warmed plots
242 were that 2009 had lower values than the other years ($P < 0.033$).

243 The warming events did not affect chlorophyll fluorescence in any of the years, but
244 fluorescence increased steadily with time ($P < 0.001$) and was 26 % higher in 2013 than in
245 2007 (Fig. 1b).

246 In the years following the experimental period, second-year segments of the warmed
247 plots were significantly smaller than segments from the controls (Fig. 2). In 2013, segments
248 from the warmed plots were 36 % shorter (Fig. 2a) and 25 % narrower (Fig. 2b) than the
249 control samples. The highest relative reduction was found in 2009 after the third winter
250 warming event when segments were 43 % shorter and 38 % narrower.

251 Segment length in the warmed plots increased by 27-52 % from the two last years of
252 manipulation (2008-2009) to the recovery years of 2011 and 2013 and became more similar to
253 the length of controls (Fig. 2a). However, segments were still 25 % shorter in the last recovery
254 year than in the first year of manipulation (2007 vs. 2013: $P = 0.001$). Segment size also
255 varied in control plots, but with few significant between-year differences (Fig. 2).

256 The length-to-width ratio varied much between years (Fig. 2c). Treatment effects on this
257 ratio were minor and only significant in two of the six years (2008 and 2013). Width and
258 length were correlated both for controls and warmed plot samples, and regression lines have
259 almost identical slopes and intercepts, but many samples deviated much from the general
260 trend, rendering relatively low correlations (Fig. 3).

261 The right sides of segments were 3.8 % narrower than the left sides (paired samples
262 from all years and treatments: $t = 3.65$, $P < 0.001$). There was no treatment effect on R-L
263 (repeated-measures ANOVA: treatment: $F = 1.26$, $P = 0.280$; time: $F = 0.37$, $P = 0.772$; time
264 \times treatment: $F = 0.43$, $P = 0.733$; data not shown).

265 The FA index varied between years and treatments (Fig. 4). The canopy and
266 canopy+soil warming treatments showed contrasting trends and were therefore not pooled. In
267 2007, moss segments from the canopy warming treatment had higher asymmetry than from
268 the soil and canopy warming treatment, but none of those differed from controls. The only
269 time when one of the warming treatments differed from controls was in 2009 when mosses
270 from the soil and canopy warming treatment were 44 % more asymmetric than mosses from
271 the controls.

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275 **Discussion**

276

277 This is the first study to determine the effects of winter warming events on a common and
278 important moss species, both immediately after the events and in the recovery years that
279 followed. We did not find support for the hypothesis that the negative effects of the warming
280 treatment would disappear in the years following the experimental period, as photosynthesis
281 and segment growth rates remained lower compared to control plots even four years after the
282 final winter warming event simulation. These persistent impacts on physiology and segment
283 growth following the extreme winter warming events suggest that *H. splendens* may be
284 disadvantaged in a future sub-Arctic climate where high frequency of winter warming events
285 may become the norm. More broadly, this suggests the potential for large consequences for
286 high-latitude systems where bryophytes are often a major component and where the greatest
287 increases in extreme winter events may be expected (Bjerke *et al.* 2014), and where they can
288 have a strong influence on ground temperature and moisture, evapotranspiration, cycling of
289 nutrients and water, permafrost thaw and ecosystem carbon balance (Turetsky *et al.* 2012).

290 Size of new clonal shoots and segments of *H. splendens* is strongly size-dependent
291 (Økland 1995); viz. developing shoots and segments receive limited nutrients from small
292 mother individuals. Hence, new shoots and segments have limited abilities to grow larger than
293 their mother shoots/segments. This may be a primary reason for the persistent difference
294 between size and photosynthetic rates of previously damaged shoots in warmed plots and
295 undamaged shoots in control plots.

296 High chlorophyll fluorescence rates in the warmed plots during all study years show
297 that intact photosynthesizing cells were highly active, even in shoots with retarded growth and
298 reduced photosynthesis. The decoupling of photosynthesis and chlorophyll fluorescence
299 indicates that only certain parts of the segments were stressed, and that the unaffected parts

300 continued to photosynthesize at normal rates. This suggests an on-or-off mechanism whereby
301 in affected cells, the photosynthetic apparatus is badly damaged and turned off thereby not
302 emitting any chlorophyll signal, while in unaffected cells, photosynthesis continues as normal
303 and makes up the entire chlorophyll signal detected by the fluorometer. The observed
304 decoupling of photosynthesis and fluorescence rates has been seen in other stress studies,
305 though these are not well understood (e.g. Taulavuori *et al.* 2000; Nabe *et al.* 2007; Granath,
306 Wiedermann & Strengbom 2009). Chlorophyll fluorescence imaging (Chen *et al.* 2015) could
307 render further insight into this decoupling.

308 We did not find support for the hypothesis that retarded segment growth would be
309 associated with increasing asymmetry, as even the most stressed shoots of *H. splendens*
310 showed only modest developmental instability, as measured by the FA index. This may
311 largely be due to the directional asymmetry of segment width, with left sides being inherently
312 wider than right sides. Instead of increasing asymmetry with increasing stress, both width and
313 length were equally reduced in stressed shoots. It may be that FA indices developed for
314 vascular plant leaves and other organisms (Palmer & Strobeck 1992, 2003; Kozlov *et al.*
315 1996) do not work well for mosses. More moss species under variable stress regimes should
316 be studied to test if FA is a useful indicator of stress for mosses. It is unknown if different
317 structures of a moss show contrasting symmetry responses to stress. Traits not showing
318 directional asymmetry should in such case be searched for (Palmer & Strobeck 2003). In
319 addition, the ratio of monopodial to sympodial segments could be studied to detect the
320 environmental effects on these two contrasting growth forms that co-occur but vary in ratios
321 between sub- and high-Arctic areas (Ross *et al.* 2001).

322 Despite the adverse effects of experimental winter warming, the abundance of *H.*
323 *splendens* did not change during the years of manipulations (Bokhorst *et al.* 2011), but
324 increased during the recovery years by 50 % compared to the starting conditions of 2007

325 (Bokhorst *et al.* 2015). The increase in moss cover was assumed to be driven by optimal
326 moisture conditions due to increased rainfall during the growing seasons, nutrient input from
327 frass from the large caterpillar outbreak and increasing open space following the severe
328 caterpillar-induced dieback of dwarf shrubs (Bokhorst *et al.* 2015). Chlorophyll fluorescence
329 and segment length in both warmed and control plots increased from the years of
330 manipulation (2008-2009) to the last recovery year (2013). Although the difference between
331 NP of controls and warmed plots was high in all years, absolute values varied much between
332 years, which probably was caused by variation in moisture and temperature in the days prior
333 to analysis (Bjerke *et al.* 2011, 2013). NP measured once per season may therefore not be a
334 good trait for between-year comparisons, but still, it is likely that the increase from 2009 to
335 2012 for both treatments was driven by increasing precipitation rates. We conclude that our *a*
336 *posteriori* hypothesis that the ecophysiological performance of *H. splendens* would benefit
337 from the caterpillar outbreaks and the increasing precipitation rates was partly supported. It is
338 also noted though, that while the shrub damage from *E. autumnata* should create conditions
339 conducive to *H. splendens* recovery, the recovery in segment growth was relatively modest
340 and may well have been even weaker had the shrub canopy not been removed by the
341 caterpillar outbreak.

342 Other multi-year studies also report on recent increases in *H. splendens*, and these
343 studies may contribute to elucidate the drivers of increasing cover, chlorophyll fluorescence
344 and segment length. At another birch forest site near our field study, *H. splendens* showed
345 some indications of increases in cover from 1991 to 2009 (Callaghan *et al.* 2013), while mat-
346 forming mosses show an increasing trend at Norwegian forest monitoring sites (Aarrestad *et*
347 *al.* 2014; Framstad 2015). This has been assumed to be due to delayed onset of snow season,
348 which provides opportunity for moss growth in autumn after vascular plant growth has ceased
349 (Framstad 2015). *Hylocomium splendens* shows high photosynthetic activity at temperatures

350 between 0 and 5 °C at relatively low light intensities (Sonesson, Gehrke & Tjus 1992; Bjerke
351 *et al.* 2013). Other cryptogams also take advantage of mild autumns (Bjerke *et al.* 2013; Lund
352 *et al.* 2014). Thus, warmer and longer autumns may in fact stimulate cryptogams more than
353 vascular plants, and this may drive vegetation changes that contrast to what is expected based
354 on the results of summer warming simulations that do not extend into the autumn. In Abisko,
355 September was 1.5 °C warmer during the years from 2011 to 2013 than during the
356 experimental years. Thus, it may be that autumn weather in the years after the simulated
357 winter warming events also contributed to the increases in segment growth, chlorophyll
358 fluorescence and cover.

359 Framstad (2015) also hypothesizes that rodent outbreaks facilitate moss growth through
360 thinning of the vascular plant cover and denudation of soil. Near our experimental field site,
361 there have been recent outbreaks of rodents (Callaghan *et al.* 2013). However, we have not
362 observed rodent activity within our field plots, thus rodents do not seem to have played a role
363 in the increase of cryptogams there.

364 Overall, this study has demonstrated that the widespread and important moss *H.*
365 *splendens* is likely to be significantly impacted by winter warming events. Moreover, it is of
366 concern that the damage to photosynthesis and segment growth persisted even four years after
367 the last event and that these legacy effects remained of similar magnitude to the responses
368 immediately after the events. Such impacts may ultimately affect important ecosystem
369 functions given the importance of mosses for these, including ecosystem carbon balance, in
370 high-latitude regions.

371

372

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385

386 **Data accessibility**

387 All data used in this manuscript are present in the main article and in the supporting
388 information.

389

390

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539
540

541 **Tables and Figures**

542

543 **Legends**

544

545 **Table 1.** Monthly precipitation rates (mm) of summers (June-July) during the experimental
546 period (2007-2009) and during the following years (2010-2013).

547

548 **Fig. 1.** Ecophysiological performance of *Hylocomium splendens* in the growing seasons
549 following the simulated winter warming events (2007-2009) and during the summers 3-4
550 years after the last winter warming event (2012-2013). (a) Net photosynthetic rates at optimal
551 water content (treatment: $F = 38.08$, $P < 0.001$, time: $F = 3.75$, $P = 0.008$, treatment \times time: $F =$
552 0.45 , $P = 0.771$); (b) photosystem II efficiency of dark-adapted samples (treatment: $F =$
553 3.66 , $P = 0.077$, time: $F = 26.56$, $P < 0.001$, treatment \times time: $F = 0.225$, $P = 0.923$), $n = 4-6$
554 per treatment and time combination (some plots with low abundance and hence not sampled
555 in all occasions). Treatment years are left and recovery years right of the dashed vertical line.
556 Error bars are ± 1 SE. Asterisks indicate treatment effects: * = $P < 0.05$; ** = $P < 0.01$; *** =
557 $P < 0.001$. Letters placed on bars reflect time differences within the control (black letters) and
558 warming (white letters) treatments, respectively. Bars sharing the same letter are not
559 significantly different.

560

561 **Fig. 2.** Size of second-year shoot segments of *Hylocomium splendens* measured in the
562 growing seasons following the winter warming events (2007-2009) and during the summers 1,
563 2 and 4 years after the last winter warming event (2010, 2011 and 2013). (a) Segment length
564 (treatment: $F = 53.78$, $P < 0.001$, time: $F = 11.98$, $P < 0.001$, treatment \times time: $F = 3.23$, $P =$
565 0.011). (b) Segment width (treatment: $F = 51.42$, $P < 0.001$, time: $F = 7.77$, $P < 0.001$,

566 treatment \times time: $F = 2.30$, $P = 0.052$). (c) Length-to-width ratio (treatment: $F = 0.51$, $P =$
567 0.485 , time: $F = 8.69$, $P < 0.001$, treatment \times time: $F = 1.67$, $P = 0.151$). $n = 4-6$ per
568 treatment and time combination (some plots with low abundance and hence not sampled in all
569 occasions). Treatment years are left and recovery years right of the dashed vertical line. Error
570 bars are ± 1 SE. Asterisks indicate treatment effects: * = $P < 0.05$; ** = $P < 0.01$; *** = $P <$
571 0.001 . Letters placed on bars reflect time differences within the control (black letters) and
572 warming (white letters) treatments, respectively. Bars sharing the same letter are not
573 significantly different.

574

575 **Fig. 3.** Relationship between length and width of second-year shoot segments. Shoots from
576 (a) control plots ($r^2 = 0.38$, $P < 0.001$), and (b) winter warming simulation plots ($r^2 = 0.37$, P
577 < 0.001).

578

579 **Fig. 4.** Fluctuating asymmetry of second-year shoot segments of *Hylocomium splendens*
580 measured on dried samples from the growing seasons following the winter warming events
581 (2007-2009) and from the growing season 2 years after the last winter warming event (2011)
582 (treatment $F = 2.20$, $P = 0.150$, time: $F = 1.27$, $P = 0.297$, time \times treatment: $F = 2.59$, $P =$
583 0.033). $n = 6$ for each treatment. Treatment years are left and recovery years right of the
584 dashed vertical line. Error bars are ± 1 SE. Different letters indicate significant differences (P
585 < 0.05) between treatments. n.s. = not significant.

586

587 **Table 1.**

588

Period	June	July	June-July
2007	14	47	60
2008	55	33	88
2009	16	18	34
Mean 2007-2009	28	33	60
2010	45	52	96
2011	62	46	108
2012	30	94	123
2013	40	76	116
Mean 2011-2013	44	76	116
Increase from 2007-2009 to 2011-2013 (%)	55	121	91

589