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

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Summary

1. Winter is a period of dormancy for plants of cold environments. However, winter climate is changing, leading to an increasing frequency of stochastic warm periods (winter warming events) and concomitant reductions in snow cover. These conditions 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.

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Introduction

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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

3

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

(Clausen 1964; Hudson & Brustkern 1965). Temperatures at −7.6 °C within 24 h after

warming was ceased in the winter warming study, followed by temperatures as low as −18 °C

two weeks later (Bokhorst et al. 2008, 2009, 2010) probably caused freezing damage to the

moss shoot apices, which resulted in reductions in growing season photosynthesis and

segment growth far beyond the year-to-year variation found in populations with stable winter

climates (Bjerke et al. 2011). Death of apices promotes the sympodial growth form over the

monopodial form. Although the two growth forms are genetically determined to some extent,

they are both found in the same area and respond to environmental cues (Ross et al. 2001).

This shows that mosses are under threat from winter warming events. However, impacts of

winter warming beyond the immediate short-term responses reported above (Bjerke et al.,

2011) remain unknown.

Here, we follow up on the short-term responses in the experimental period reported in

Bjerke et al. (2011) with a longer-term assessment of the moss growth and photosynthesis

responses in the four years after the warming simulations, to determine the persistence of the

observed negative effects arising from the events. We also studied in more detail the

morphology of moss segments by resurveying all moss samples taken from the experimental

years and following years to test whether declining vitality is associated with developmental

instability. Fluctuating asymmetry (FA), as a measure of developmental instability, is widely

used as a symptom of stress in vascular plant leaves and animals (Palmer & Strobeck 1992,

2003; Kozlov et al. 1996). However, FA in mosses is a poorly studied subject; to our

knowledge, the applicability of FA as a stress signal in mosses has not been assessed

previously.

We hypothesized that surviving individuals of H. splendens from three consecutive

winter warming events (Bjerke et al. 2011) would increase photosynthetic and segment

growth rates and reach the same level as in control plots within the first years after the last

winter warming event. Furthermore, we hypothesized that retarded growth also would be

associated with increasing FA of moss segments, and that this trait would disappear during

recovery of normal segment growth rates.

During the recovery years of 2012 and 2013, our study site and the entire Abisko region

(Swedish sub-Arctic) was affected by a caterpillar outbreak of the geometrid moth *Epiritta*

autumnata (Bjerke et al. 2014; Bokhorst et al. 2015). The caterpillars defoliated many

vascular plants, and this led to a decline in the cover of dwarf shrubs and an increase of moss

cover in the years of 2012 and 2013 (Bokhorst et al. 2015). These summers were also much

wetter than the summers during the experimental period (Table 1; see also Bokhorst et al.

2015). Since these environmental changes took place after the study was initiated, we did not

phrase any a priori hypotheses of their impacts on the ecophysiology of H. splendens.

However, knowing that summer rainfall stimulates moss growth (Potter et al. 1995, Sonesson

et al. 2002), that more space would be available for horizontal growth due to vascular plant

damage, and that more throughfall of nutrients would reach the cryptogam layer (Bokhorst et

al. 2015); we hypothesized a posteriori that the ecophysiological performance of H.

splendens would benefit from the caterpillar outbreak and the increasing precipitation rates.

Material and methods

FIELD SITE AND EXPERIMENTAL SIMULATIONS

Simulations of winter warming events in the field were conducted on a sub-Arctic heathland

in open mountain birch (Betula pubescens) forest close to the Abisko Scientific Research

Station (ANS) in northern Sweden (68° 21' N, 18° 49' E) during March 2007, 2008 and 2009.

Details of the research site and experimental design are described in Bokhorst et al. (2008,

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 μmol m^{-2} s^{-1} was used during

180   measurements of net photosynthesis. CO_2 concentration was set to 380 ppm and cuvette

181   humidity to 9000 ppm H_2O. 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.

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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   × 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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Discussion

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

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

High chlorophyll fluorescence rates in the warmed plots during all study years show that intact photosynthesizing cells were highly active, even in shoots with retarded growth and reduced photosynthesis. The decoupling of photosynthesis and chlorophyll fluorescence 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

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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.

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373   **Acknowledgements**

385

386   Data accessibility

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

388   information.

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540

Tables and Figures

Legends

Table 1. Monthly precipitation rates (mm) of summers (June-July) during the experimental

period (2007-2009) and during the following years (2010-2013).

Fig. 1. Ecophysiological performance of Hylocomium splendens in the growing seasons

following the simulated winter warming events (2007-2009) and during the summers 3-4

years after the last winter warming event (2012-2013). (a) Net photosynthetic rates at optimal

water content (treatment: $F = 38.08$, $P < 0.001$, time: $F = 3.75$, $P = 0.008$, treatment $\times$ time: F

$= 0.45$, $P = 0.771$); (b) photosystem II efficiency of dark-adapted samples (treatment: $F =$

3.66, $P = 0.077$, time: $F = 26.56$, $P < 0.001$, treatment $\times$ time: $F = 0.225$, $P = 0.923$), $n = 4$–6

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

in all occasions). Treatment years are left and recovery years right of the dashed vertical line.

Error bars are ± 1 SE. Asterisks indicate treatment effects: $* = P < 0.05$; $** = P < 0.01$; $*** =$

$P < 0.001$. Letters placed on bars reflect time differences within the control (black letters) and

warming (white letters) treatments, respectively. Bars sharing the same letter are not

significantly different.

Fig. 2. Size of second-year shoot segments of Hylocomium splendens measured in the

growing seasons following the winter warming events (2007-2009) and during the summers 1,

2 and 4 years after the last winter warming event (2010, 2011 and 2013). (a) Segment length

(treatment: $F = 53.78$, $P < 0.001$, time: $F = 11.98$, $P < 0.001$, treatment $\times$ time: $F = 3.23$, $P =$

0.011). (b) Segment width (treatment: $F = 51.42$, $P < 0.001$, time: $F = 7.77$, $P < 0.001$,

566 treatment × 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 × 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 × 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