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

Jarle W. Bjerke[* a], Stef Bokhorst [a b], Terry V. Callaghan [c d e] & Gareth K. Phoenix [c]

[a] Norwegian Institute for Nature Research (NINA), FRAM – High North Research Centre for Climate and the Environment, PO Box 6606 Langnes, NO-9296 Tromsø, Norway

[b] Department of Ecological Science, VU University Amsterdam, De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands

[c] Department of Animal and Plant Sciences, University of Sheffield, Western Bank, Sheffield S10 2TN, UK

[d] Royal Swedish Academy of Sciences, Lilla Frescativägen 4A, 114 18 Stockholm, Sweden

[e] Department of Botany, National Research Tomsk State University, 36 Lenin Ave., Tomsk 634050, Russia

* Corresponding author. E-mail: jarle.bjerke@nina.no

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.

49

52

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 ambient temperatures,

73   which may be 15-30 degrees below freezing and fluctuate much more than in the subnivean

74   environment (Pauli *et al.* 2013). The dehardening of vascular plants during such events leads

75 to increased susceptibility to freezing on the return of ambient sub-zero winter temperatures.

76 Therefore, many vascular plants are severely damaged after winter warming events, as shown

77 both by analyses of natural events and field experiments (Bokhorst *et al.* 2009, 2010, 2012a;

78 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 in

147 open mountain birch (*Betula pubescens*) forest close to the Abisko Scientific Research Station

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

149 of the research site and experimental design are described in Bokhorst *et al.* (2008, 2009) and

150 Bjerke *et al.* (2011). In brief, the experiment consisted of 18 plots (2.1 m × 1.0 m), consisting

151   of 6 control plots that remained under their natural snow cover throughout the winter; 6 that

152   were exposed to a week-long winter warming event called 'canopy warming', using infrared

153   heating lamps (800 W emitting at 3 μm; HS 2408, Kalglo Electronics Co., Bethlehem, USA);

154   and 6 where warming from infrared heating lamps was combined with soil warming from cables

155   at 5 cm soil depth called 'canopy and soil warming'. Soil warming cables (LS-TXLP, Nexans,

156   Norway, producing 120 W m^{-2}) were switched on two days after the lamps to simulate the delay

157   in soil thaw during a real event. Complete snow melt and exposure of the vegetation was

158   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, while

163   for the remainder of winter, temperatures fluctuated concurrently with the ambient conditions

164   (ranging from −17 °C to 4 °C) due to the reduced snow cover (initially none, and then thin

165   coverage as new snow covered the exposed vegetation), meaning there was little to no

166   insulation from air temperatures. In contrast, control plots remained well insulated under snow

167   and experienced temperatures between −7 °C and 0 °C until snowmelt. Weather data presented

168   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, Heinz

174   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-dry-

178 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 measurements

180 of net photosynthesis. CO_2 concentration was set to 380 ppm and cuvette humidity to 9000 ppm

181 H_2O. Cuvette temperatures were adjusted to ambient noon temperatures at the time of

182 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

9

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 in

229 prior 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   × 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.

272

273

274

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

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

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

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

(Bokhorst *et al.* 2015). The increase in moss cover was assumed to be driven by optimal

moisture conditions due to increased rainfall during the growing seasons, nutrient input from

frass from the large caterpillar outbreak and increasing open space following the severe

caterpillar-induced dieback of dwarf shrubs (Bokhorst *et al.* 2015). Chlorophyll fluorescence

and segment length in both warmed and control plots increased from the years of

manipulation (2008-2009) to the last recovery year (2013). Although the difference between

NP of controls and warmed plots was high in all years, absolute values varied much between

years, which probably was caused by variation in moisture and temperature in the days prior

to analysis (Bjerke *et al.* 2011, 2013). NP measured once per season may therefore not be a

good trait for between-year comparisons, but still, it is likely that the increase from 2009 to

2012 for both treatments was driven by increasing precipitation rates. We conclude that our *a*

posteriori hypothesis that *H. splendens* would benefit from the caterpillar outbreaks and the

increasing precipitation rates was partly supported. It is also noted though, that while the

shrub damage from *E. autumnata* should create conditions conducive to *H. splendens*

recovery, the recovery in segment growth was relatively modest and may well have been even

weaker had the shrub canopy not been removed by the caterpillar outbreak.

Other multi-year studies also report on recent increases in *H. splendens*, and these

studies may contribute to elucidate the drivers of increasing cover, chlorophyll fluorescence

and segment length. At another birch forest site near our field study, *H. splendens* showed

some indications of increases in cover from 1991 to 2009 (Callaghan *et al.* 2013), while mat-

forming mosses show an increasing trend at Norwegian forest monitoring sites (Aarrestad *et*

al. 2014; Framstad 2015). This has been assumed to be due to delayed onset of snow season,

which provides opportunity for moss growth in autumn after vascular plant growth has ceased

(Framstad 2015). *Hylocomium splendens* shows high photosynthetic activity at temperatures

between 0 and 5 °C at relatively low light intensities (Sonesson, Gehrke & Tjus 1992; Bjerke

350 *et al.* 2013). Other cryptogams also take advantage of mild autumns (Bjerke *et al.* 2013; Lund

351 *et al.* 2014). Thus, warmer and longer autumns may in fact stimulate cryptogams more than

352 vascular plants, and this may drive vegetation changes that contrast to what is expected based

353 on the results of summer warming simulations that do not extend into the autumn. In Abisko,

354 September was 1.5 °C warmer during the years from 2011 to 2013 than during the

355 experimental years. Thus, it may be that autumn weather in the years after the simulated

356 winter warming events also contributed to the increases in segment growth, chlorophyll

357 fluorescence and cover.

358   Framstad (2015) also hypothesizes that rodent outbreaks facilitate moss growth through

359 thinning of the vascular plant cover and denudation of soil. Near our experimental field site,

360 there have been recent outbreaks of rodents (Callaghan *et al.* 2013). However, we have not

361 observed rodent activity within our field plots, thus rodents do not seem to have played a role

362 in the increase of cryptogams there.

363   Overall, this study has demonstrated that the widespread and important moss *H.*

364 *splendens* is likely to be significantly impacted by winter warming events. Moreover, it is of

365 concern that the damage to photosynthesis and segment growth persisted even four years after

366 the last event and that these legacy effects remained of similar magnitude to the responses

367 immediately after the events. Such impacts may ultimately affect important ecosystem

368 functions given the importance of mosses for these, including ecosystem carbon balance, in

369 high-latitude regions.

370

371

384

Data accessibility

386 All data used in this manuscript are present here.

387

388

References

390

391 Aarrestad, P.A., Bjerke, J.W., Hak, C., Lunder, H., Myklebost, H.E., Westergaard, K.B. &

392   Åström, J. (2014) Statoil's environmental monitoring program for Snøhvit: Monitoring of

393   vegetation and soil – reanalysis in 2013. *NINA Report*, **1017,** 71 pp. (In Norwegian, with

394   English abstract).

395 Beringer, J., Lynch, A.H., Chapin, F.S. III, Mack, M. & Bonan, G.B. (2001) The

396   representation of Arctic soils in the land surface model: The importance of mosses.

397   *Journal of Climate*, **14**, 3324–3335.

398   Bjerke, J.W., Bokhorst, S., Zielke, M., Callaghan, T.V. & Phoenix, G.K. (2011) Contrasting

399   sensitivity to extreme winter warming events of dominant sub-Arctic heathland bryophyte

400   and lichen species. *Journal of Ecology*, **99**, 1481–1488.

401   Bjerke, J.W., Bokhorst, S., Callaghan, T.V., Zielke, M. & Phoenix, G.K. (2013) Rapid

402   photosynthetic recovery of a snow-covered feather moss and *Peltigera* lichen during sub-

403   Arctic midwinter warming. *Plant Ecology & Diversity*, **6**, 383–392.

404   Bjerke, J.W., Karlsen, S.R., Høgda, K.A., Malnes, E., Jepsen, J.U., Lovibond, S., Vikhamar-

405   Schuler, D. & Tømmervik, H. (2014) Record-low primary productivity and high plant

406   damage in the Nordic Arctic Region in 2012 caused by multiple weather events and pest

407   outbreaks. *Environmental Research Letters*, **9**, 084006. 14 pp.

408   Bokhorst, S., Bjerke, J.W., Callaghan, T.V., Melillo, J., Bowles, F.W. & Phoenix, G.K.

409   (2008) Impacts of extreme winter warming in the sub-Arctic: growing season responses of

410   dwarf-shrub heathland. *Global Change Biology*, **14**, 2603–2612.

411   Bokhorst, S.F., Bjerke, J.W., Tømmervik, H., Callaghan, T.V. & Phoenix, G.K. (2009)

412   Winter warming events damage sub-Arctic vegetation: consistent evidence from an

413   experimental manipulation and a natural event. *Journal of Ecology*, **97**, 1408–1415.

414   Bokhorst, S., Bjerke, J.W., Davey, M.P., Taulavuori, K., Taulavuori, E., Laine, K. *et al.*

415   (2010) Impacts of extreme winter warming events on plant physiology in a sub-Arctic

416   heath community. *Physiologia Plantarum*, **140**, 128–140.

417   Bokhorst, S., Bjerke, J.W., Tømmervik, H., Preece, C. & Phoenix, G.K. (2012a) Ecosystem

418   response to climatic change: the importance of the non-growing season. *Ambio*, **41**, suppl.

419   3, 246–255.

420   Bokhorst, S., Phoenix, G.K., Bjerke, J.W., Callaghan, T.V., Huyver-Brugman, F. & Berg, M.

421   (2012b) Extreme winter warming events more negatively impact small rather than large

422   soil fauna: shift in community composition explained by traits not taxa. *Global Change*

423   *Biology*, **18**, 1152–1162.

424   Bokhorst, S., Wardle, D.A., Nilsson, M.-C., Gundale, M.J. (2014) Impact of understory

425   mosses and dwarf shrubs on soil micro-arthropods in a boreal forest chronosequence.

426   *Plant and Soil*, **379**, 121-133.

427   Bokhorst, S., Phoenix, G.K., Berg, M.P., Callaghan, T.V, Kirby-Lambert, C. & Bjerke, J.W.

428   (2015) Climatic and biotic extreme events moderate long-term responses of above- and

429   belowground sub-Arctic heathland communities to climate change. *Global Change*

430   *Biology*, **21**, 4063–4075.

431   Bokhorst, S., Pedersen, S.H., Brucker, L., Anisimov, O., Bjerke, J.W., Brown R.D. *et al.*

432   (2016) Changing Arctic snow cover: A review of recent developments and assessment of

433   future needs for observations, modelling and impacts. *Ambio*, DOI: 10.1007/s13280-016-

434   0770-0.

435   Callaghan, T.V., Collins, N.J. & Callaghan, C.H. (1978) Strategies of growth and population

436   dynamics of tundra plants. 4. Photosynthesis, growth and reproduction of *Hylocomium*

437   *splendens* and *Polytrichum commune* in Swedish Lapland. *Oikos*, **31**, 73–88.

438   Callaghan, T.V., Carlsson, B.A., Sonesson, M. & Temesvary, A. (1997) Between-year

439   variation in climate-related growth of circumarctic populations of the moss *Hylocomium*

440   *splendens*. *Functional Ecology*, **11**, 157–165.

441   Callaghan, T.V., Jonasson, C., Thierfelder, T., Zhenlin, Y., Hedenås, H., Johansson, M. *et al.*

442   (2013) Ecosystem change and stability over multiple decades in the Swedish sub-Arctic:

443   complex processes and multiple drivers. *Philosophical Transactions of the Royal Society*

444   *B-Biological Sciences*, **368**, 20120488. 18 pp.

445   Chapin, D.M. & Bledsoe, C.S. (1992) Nitrogen fixation in Arctic plant communities. *Arctic*

446   *Ecosystems in a Changing Climate: An Ecophysiological Perspective* (eds F.S. Chapin,

R.L. Jefferies, J.F. Reynolds, G.R. Shaver, J. Svoboda & E.W. Chu), pp. 301–319. Academic Press Inc., San Diego.

Chen, Y.E., Cui, J.-M., Yang, J.-C., Zhang, Z.-W., Yuan, M., Song, C. *et al.* (2015) Biomonitoring heavy metal contaminations by moss visible parameters. *Journal of Hazardous Materials*, **296**, 201–209.

Clausen, E. (1964) The tolerance of hepatics to desiccation and temperature. *Bryologist*, **67**, 411–417.

Cooper, E.J., (2014) Warmer shorter winters disrupt Arctic terrestrial ecosystems. *Annual Review of Ecology, Evolution, and Systematics*, **45**, 271-295.

Cornelissen, J.H.C., van Bodegom, P.M., Aerts, R., Callaghan, T.V., van Logtestijn, R.S.P., Alatalo, J. *et al.* (2007) Global negative vegetation feedback to climate warming responses of leaf litter decomposition rates in cold biomes. *Ecology Letters*, **10**, 619–627.

Dorrepaal, E., Aerts, R., Cornelissen, J.H.C., Callaghan, T.V. & van Logtestijn, R.S.P. (2004) Summer warming and increased winter snow cover affect *Sphagnum fuscum* growth, structure and production in a sub-Arctic bog. *Global Change Biology*, **10**, 93–104.

Framstad, E. (ed) (2015) Terrestrial Ecosystems Monitoring in 2014: Ground vegetation, small mammals and birds. Summary of results. *NINA Report*, **1186**, 74 pp. (In Norwegian, with English abstract).

GBIF (Global Biodiversity Information Facility) (2015) *Hylocomium splendens* W. P. Schimper. GBIF ID 2679696. Retrieved 10 November from http://www.gbif.org/species/2679696.

Granath, G., Wiedermann, M.M. & Strengbom, J. (2009) Physiological responses to nitrogen and sulphur addition and raised temperature in *Sphagnum balticum*. *Oecologia*, **161**, 481–490.

471   Hansen, B.B., Isaksen, K., Benestad, R., Kohler, J., Pedersen, Å.Ø., Loe, L.E. *et al.* (2014)

472   Warmer and wetter winters: characteristics and implications of an extreme weather event in

473   the High Arctic. *Environmental Research Letters*, **9**, 114021. 10 pp.

474   Heijmans, M.M.P.D., Arp, W.J. & Chapin, F.S. (2004) Carbon dioxide and water vapour

475   exchange from understory species in boreal forest. *Agricultural and Forest Meteorology*,

476   **123**, 135–147.

477   Hörnberg, G., Ohlson, M. & Zackrisson, O. (1997) Influence of bryophytes and microrelief

478   conditions on *Picea abies* seed regeneration patterns in boreal old-growth swamp forests.

479   *Canadian Journal of Forest Research*, **27**, 1015–1023.

480   Hudson, M.A. & Brustkern, P. (1965) Resistance of young and mature leaves of *Mnium*

481   *undulatum* L. to frost. *Planta*, **66**, 135–155.

482   Kozlov, M.V., Wilsey, B.J., Koricheva, J. & Haukioja, E. (1996) Asymmetry of birch leaves

483   increases under pollution impact. *Journal of Applied Ecology*, **33**, 1489–1495.

484   Lang, S.I., Cornelissen, J.H.C., Shaver, G.R., Ahrens, M., Callaghan, T.V., Molau, U. *et al.*

485   (2012) Arctic warming on two continents has consistent negative effects on lichen

486   diversity and mixed effects on bryophyte diversity. *Global Change Biology*, **18**, 1096–

487   1107.

488   Lund, M., Bjerke, J.W., Drake, B., Engelsen, O., Hansen, G., Parmentier, F.-J. *et al.* (2015)

489   Low impact of dry conditions on the CO_2 exchange of a northern-Norwegian blanket bog.

490   *Environmental Research Letters*, **10**, 025004. 13 pp.

491   Maxwell, K. & Johnson, G.N. (2000) Chlorophyll fluorescence – a practical guide. *Journal of*

492   *Experimental Botany*, **51**, 659–668.

493   Nabe, H., Funabiki, R., Kashino, Y., Koike, H. & Satoh, K. (2007) Responses to desiccation

494   stress in bryophytes and an important role of dithiothreitol-insensitive non-photochemical

495   quenching against photoinhibition in dehydrated states. *Plant & Cell Physiology*, **48**,

496   1548–1557.

497   Økland, R.H. (1995) Population biology of the clonal moss *Hylocomium splendens* in

498   Norwegian boreal spruce forests. I. Demography. *Journal of Ecology*, **83**, 697–712.

499   Palmer, A.R. & Strobeck, C. (1992) Fluctuating asymmetry as a measure of developmental

500   stability: Implications of non-normal distributions and power of statistical tests. *Acta*

501   *Zoologica Fennica*, **191**, 57–72.

502   Palmer, A. R. & Strobeck, C. (2003) Fluctuating asymmetry analyses revisited.

503   *Developmental instability: causes and consequences* (ed M. Polak), pp. 279–319. Oxford

504   University Press, Oxford.

505   Pauli, J.N., Zuckerberg, B., Whiteman, J.P. & Porter, W. (2013) The subnivium: a

506   deteriorating seasonal refugium. *Frontiers in Ecology and the Environment*, **11**, 260–267.

507   Phoenix, G.K. & Bjerke, J.W. (2016) Arctic browning: extreme events and trends reversing

508   Arctic greening. *Global Change Biology*, in press.

509   Potter, J.A., Press, M.C., Callaghan, T.V. & Lee, J.A. (1995) Growth responses of

510   *Polytrichum commune* and *Hylocomium splendens* to simulated environmental change

511   in the subarctic. *New Phytologist*, **131**, 533–541.

512   Putkonen, J. & Roe, G. (2003) Rain-on-snow events impacts soil temperatures and affect

513   ungulate survival. *Geophysical Research Letters*, **30**, 1188. 4 pp.

514   Rohrer J.R. 2004. *Hylocomium. Bryophyte Flora of North America, Vol. 2* (ed R.H. Zander),

515   retrieved 10 November 2015 from www.efloras.org.

516   Ross, S.E., Callaghan, T.V., Sheffield, E. & Sonesson, M. (2001) Variation and control of

517   growth form in the moss *Hylocomium splendens*. *Journal of Bryology*, **23**, 283–292.

518   Sonesson, M., Gehrke, C. & Tjus, M. (1992) CO_2 environment, microclimate and

519   photosynthetic characteristics of the moss *Hylocomium splendens* in a sub-Arctic habitat.

520   *Oecologia*, **92**, 23–29.

521   Sonesson, M., Carlsson, B.A., Callaghan, T.V., Halling, S., Björn, L.O., Bertgren, M. *et al.*

522   (2002) Growth of two peat-forming mosses in subarctic mires: species interactions and

523   effects of simulated climate change. *Oikos*, **99**, 151–160.

524   Taulavuori, K., Taulavuori, E., Sarjala, T., Savonen, E.M., Pietilainen, P., Lahdesmaki, P. *et*

525   *al.* (2000) *In vivo* chlorophyll fluorescence is not always a good indicator of cold

526   hardiness. *Journal of Plant Physiology*, **157**, 227–229.

527   Turetsky, M.R., Bond-Lamberty, B., Euskirchen, E., Talbot, J., Frolking, S., McGuire, A.D.

528   *et al*. (2012) The resilience and functional role of moss in boreal and Arctic ecosystems.

529   *New Phytologist*, **196**, 49–67.

530   Walsh, J.E., Overland, J.E., Groisman, P.Y. & Rudolf, B. (2011) Arctic Climate: Recent

531   Variations. *Water, Ice and Permafrost in the Arctic (SWIPA): Climate Change and the*

532   *Cryosphere* (eds C. Symon, H. Thing, J. Pawlak & T. Larson), chapter 2, 13 pp. Arctic

533   Monitoring and Assessment Program, Oslo.

534   Yoshikawa, K., Bolton, W.R., Romanovsky, V.E., Fukuda, M. & Hinzman, L.D. (2002)

535   Impacts of wildfire on the permafrost in the boreal forests of Interior Alaska. *Journal of*

536   *Geophysical Research-Atmospheres*, **108,** 8148. 14 pp.

537

538

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: $F = 7.77$, $P < 0.001$,

564   treatment × time: $F = 2.30$, $P = 0.052$). (c) Length-to-width ratio (treatment: $F = 0.51$, $P =$

565   0.485, time: $F = 8.69$, $P < 0.001$, treatment × time: $F = 1.67$, $P = 0.151$). $n = 4$–6 per

566   treatment and time combination (some plots with low abundance and hence not sampled in all

567   occasions). Treatment years are left and recovery years right of the dashed vertical line. Error

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

569   0.001. Letters placed on bars reflect time differences within the control (black letters) and

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

571   significantly different.

572

573   **Fig. 3.** Relationship between length and width of second-year shoot segments. Shoots from

574   (a) control plots ($r^2 = 0.38$, $P < 0.001$), and (b) winter warming simulation plots ($r^2 = 0.37$, P

575   < 0.001).

576

577   **Fig. 4.** Fluctuating asymmetry of second-year shoot segments of *Hylocomium splendens*

578   measured on dried samples from the growing seasons following the winter warming events

579   (2007-2009) and from the growing season 2 years after the last winter warming event (2011)

580   (treatment $F = 2.20$, $P = 0.150$, time: $F = 1.27$, $P = 0.297$, time × treatment: $F = 2.59$, $P =$

581   0.033). $n = 6$ for each treatment. Treatment years are left and recovery years right of the

582   dashed vertical line. Error bars are ± 1 SE. Different letters indicate significant differences (P

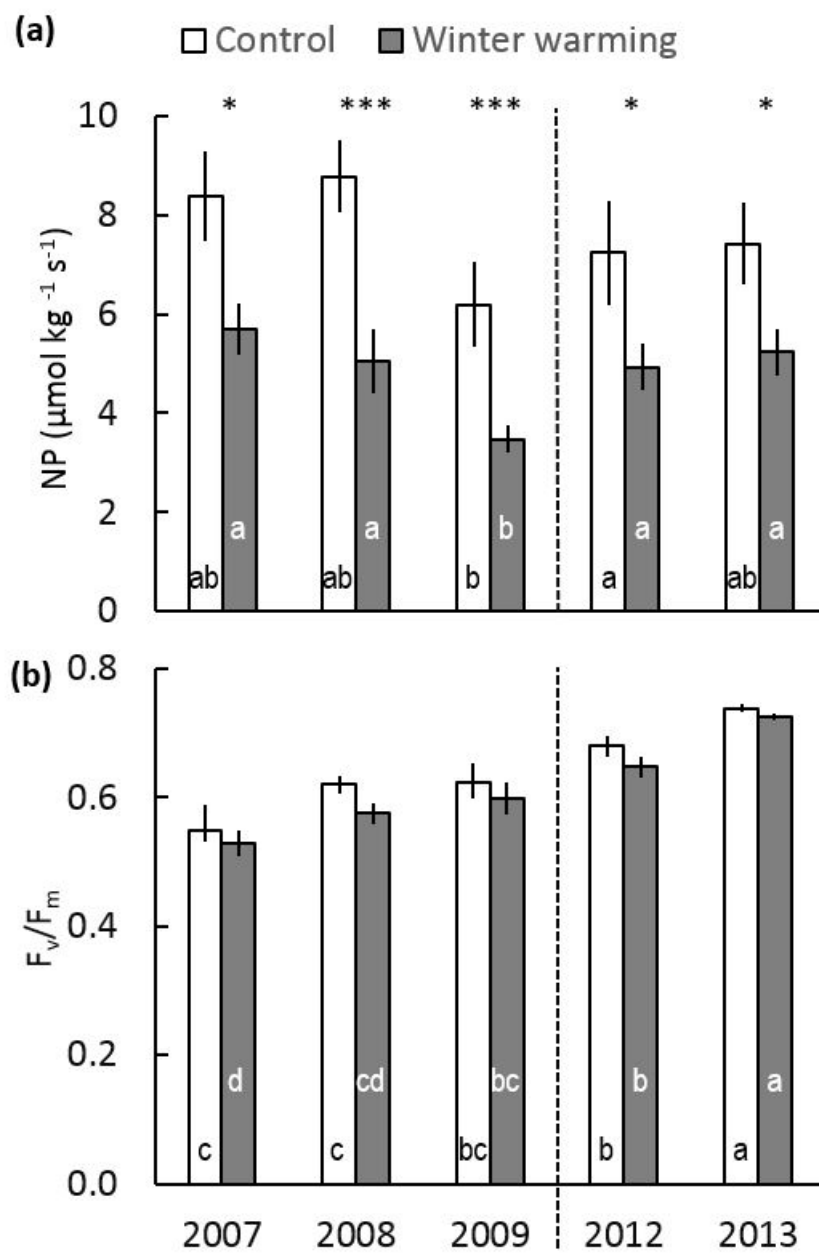583 < 0.05) between treatments. n.s. = not significant.

584

585 **Table 1.**

586

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**

587

588   **Fig. 1.**

589

590

591

Fig. 2

596 **Fig. 3**

597

598

599   **Fig. 4**