

1 **INTERANNUAL VARIATION IN UV-B AND TEMPERATURE EFFECTS ON BUD**
2 **PHENOLOGY AND GROWTH IN *POPULUS TREMULA***

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21 **ABSTRACT**

22 Warming affects phenological processes such as spring bud break and autumnal bud set, and
23 also growth rates of trees. Recently, it has been shown that these physiological processes also
24 may be influenced by the ultraviolet-B (UV-B) part of the solar spectrum, and there are
25 reasons to expect that the two environmental factors induce interactive effects when acting in
26 concert. In this study, our aim was to elucidate how experimental enhancements in
27 temperature and UV-B, alone and in combination, affect growth and seasonal phenology of
28 Eurasian aspen (*Populus tremula*) over several growing seasons (three years). Moreover, we
29 tested how environmentally induced changes in phenology affect the growth achieved over
30 each season, that is, the importance of a prolonged growing season for growth yield. The
31 plants grew in an outdoor experiment with modulated enhancements of temperature and UV-
32 B during the growing season. Both UV-B and temperature enhancement affected bud set
33 dates, while bud break dates were only affected by temperature enhancement. Temperature
34 delayed bud set in all years, but gradually less over years, while UV-B yielded earlier bud set
35 the first year but showed a delayed response the following years. Bud break was always
36 earlier under temperature enhancement. The experimentally induced extension of the growing
37 season in both ends had a positive effect of growth throughout the three-year period.
38 However, the reduced responsiveness of bud set to both enhancement treatments suggest that
39 the plants gradually acclimated to the modified climate, a finding that should also be
40 investigated for other tree species.

41 INTRODUCTION

42 In environments characterised by seasonal climatic shifts, survival of different tree species
43 requires appropriate timing of growth related processes to the part of the year cycle that has
44 favourable climate. By sensing seasonal shifts in temperature and properties of solar light,
45 trees are provided with a range of environmental cues that control the timing of phenological
46 events (Olsen & Lee 2011). Sensing day length, temperature and light quality allows tree
47 species to couple phenological transitions to seasonal shifts as they occur. As boreal and
48 temperate tree species have wide distributions across latitudes and elevations, the adaptations
49 to climatic seasonal patterns have resulted in different provenances.

50 For several tree species in temperate climates, an increasing amount of evidence points
51 towards an interplay of light and temperature parameters in driving the yearly growth cycle
52 (Hänninen & Tanino 2011). In relation to phenological shifts in autumn, light quality has been
53 shown to affect growth cessation and the formation of winter buds in boreal tree species. In
54 this respect, effects of far-red (FR) light have been shown in *Salix pentandra* (Junttila &
55 Kaurin 1985), hybrid aspen (*Populus tremula* x *Populus tremuloides*) (Olsen *et al.* 1997);
56 Norway spruce (*Picea abies*) (Clapham *et al.* 1998; Mølmann *et al.* 2006) and silver birch
57 (*Betula pendula*) (Tsegay *et al.* 2005). In addition, effects blue light in relation to autumn
58 phenology have been shown in Norway spruce (Mølmann *et al.* 2006; Opseth *et al.* 2015) ,
59 and for bud burst in spring for birch (*Betula pendula*), black alder (*Alnus glutinosa*) and oak
60 (*Quercus robur*) (Brelsford & Robson 2018). Moreover, ultraviolet-B (UV-B) light has been
61 shown to interact with temperature in relation to bud formation (Strømme *et al.* 2015 &
62 2018). Evidence of UV-B as a growth regulator for plants is of recent origin, and a possible
63 role in day-length sensing has been shown in *Arabidopsis thaliana* (Fehér *et al.* 2011).

64 During the last decades, increased growth has been observed for tree species from high
65 latitudes of the northern hemisphere (Jacoby & D'Arrigo 1995; Hember *et al.* 2012; Kauppi *et*
66 *al.* 2014; Schaphoff *et al.* 2016). This growth increase has been related to climatic warming,
67 which has been reported to be particularly strong in northern geographical areas (Serreze *et al.*
68 2000; Hartman *et al.* 2013). A survey of 63 studies investigating temperature effects on tree
69 species suggests that direct effects of warming are generally beneficial to tree growth in non-
70 tropical areas (Way & Oren 2010). In particular, it was shown that photosynthetic rates
71 increased more strongly than respiration. In addition, plant growth is generally considered to
72 occur at temperatures above 5 ° C, possibly due to low-temperature constraints on

73 biochemical processes in the plant cells (Körner 2016). As boreal and temperate tree species
74 are more limited by temperature than tree species in warmer climates (Way & Oren 2010),
75 warming probably results in more days over this critical temperature. Moreover, higher
76 temperatures stimulate tissue growth by shortening the length of the plant cell cycle (Francis
77 & Barlow 1988), and may also favour tree growth by extending the yearly growth period.
78 Widespread observations of advanced spring phenology have been related to global warming
79 (Menzel *et al.* 2006; Bertin 2008). For most temperate and boreal tree species, bud break in
80 spring is driven by accumulating heat sums and is largely, with a few exceptions, a
81 temperature-driven process (Sarvas 1972; Körner & Basler 2010). This process also requires a
82 degree of chilling to occur, which may differ substantially between species (Hänninen &
83 Tanino 2011).

84

85 Experimental warming has been shown to positively affect growth in field conditions
86 (Nybakken *et al.* 2012, Randriamanana *et al.* 2015; Strømme *et al.* 2018). In addition to
87 earlier growth onset in spring, evidence from field studies shows that temperature yields a
88 prolonged growing season, also through delayed bud set in autumn (Rohde *et al.* 2011;
89 Strømme *et al.* 2015; Strømme *et al.* 2018). As for spring phenology, there is concern for
90 insufficient fulfilment of chilling requirement in relation to warming effects on autumn
91 phenology, and that the response may be carried over to bud break in spring (Hänninen &
92 Tanino 2011). As most studies of climatic effects on trees have been performed in controlled
93 or semi-controlled environments, it is not yet clear whether such carry-over effects of
94 warming occur in field conditions.

95

96 To sum up, both from large-scale observation studies and from environmental manipulation
97 with small plants we know that both UV-B and enhanced temperatures affect several
98 physiological processes in trees. However, we know little about to what degree the different
99 processes contribute to the total growth increment through a growing season. Can the same
100 plant make use of both an early start and a late ending of the season, or is there a limitation to
101 growth during one season? As most warming studies have lasted only one growing season,
102 there is also little knowledge on the effect of prolonged exposure of the same individuals.

103

104 In this study, we compiled growth and phenology data of *Populus tremula* subjected to
105 enhanced levels of UV-B and temperature in a modulated field experiment spanning over

106 three years. Our main aim was to detect how climate change influences achieved growth
107 through effects on the length of the growing season. Earlier results on the same plants showed
108 that temperature enhancements increase growth (Randriamanana *et al.* 2015; Nissinen *et al.*
109 2017; Sivadasan *et al.* 2018), and result in earlier bud break in spring and delayed bud set in
110 autumn (Strømme *et al.* 2015; Sivadasan *et al.* 2017). It was also shown that enhanced UV-B
111 forced bud set in the autumn as well as bud break in the spring after the first growing season
112 (Strømme *et al.* 2015). In this study, we hypothesized that 1) a prolonged growing season
113 would be beneficial to plant growth in terms of height and diameter increase across all three
114 study years. We further hypothesized that 2) enhanced levels of UV-B would yield earlier bud
115 set in autumn and earlier bud break in spring. Also, we hypothesized 3) that temperature
116 enhancement would yield delayed bud set in autumn as well as earlier bud break in spring,
117 adding significantly to the positive effect of temperature on growth. Furthermore, we
118 hypothesized 4) that a combined UV-B and temperature treatment would yield dissimilar
119 effects from single treatments on bud set and bud break. We also tested whether any of the
120 tested relationships would differ between plant sexes and across experimental years.

121

122 **MATERIALS AND METHODS**

123 *Plant material*

124 Plants used in the field experiment originated from six female and six male aspens located in
125 Southern and Eastern Finland. For a thorough description of sampling locations,
126 micropropagation of individuals and growth conditions, see Strømme *et al.* (2015). The *in*
127 *vitro* propagated plantlets were potted into 1-litre pots filled with 70% non-fertilised peat and
128 30% vermiculite and kept in a greenhouse between 2 May and 7 June 2012 prior to planting in
129 the field.

130

131 *Experimental set-up*

132 The field experiment was situated in Joensuu, Eastern Finland (62°60' N, 29°75' E). The
133 experimental set-up included 36 plots in a 6×6 matrix with 3 m between the plots in all
134 directions, as explained in details by Nybakken *et al.* (2012), originally containing 60 female
135 and male plants in each (five plants of each clone). Each plot was added a 10 cm layer of
136 0.8% limed mineral soil. A metal net fence of 1.5m was structured around the experimental
137 field to prevent intrusion of large mammals, and a metal sheet shelter was implanted 60cm
138 into the soil and 60cm above the soil level to exclude voles. The plants received a
139 combination of UV-B radiation and temperature enhancement that were obtained through

140 continuous modulation to +30% and +2°C of ambient UV-B and temperature levels,
141 respectively. The achieved levels were $+28.0\pm 0.4\%$ and $+1.35\pm 0.042$ °C of ambient UV-B
142 and temperature levels, respectively. The possible treatment combinations were one of the
143 following six treatments or treatment combinations: enhanced temperature (T), enhanced UV-
144 B (UV-B), UV-B+T, enhanced UV-A (UV-A), UV-A +T, and control with ambient
145 temperature and UV radiation (C). Enhancement treatments involved UV-lamps and IR-
146 heaters mounted above experimental plots and held by adjustable aluminum frames bolted on
147 metallic posts. Each aluminum frame was appended by six 40 W UV fluorescent lamps (1.2m
148 long, UVB-313, Q-Panel Co., Cleveland, OH, USA) following a cosine distribution (Björn;
149 1990). The emission spectrum was measured with an Optronic OL-756 portable UV-VIS
150 spectroradiometer (Optronic Laboratories, Orlando, FL, USA), and cellulose diacetate filters
151 were wrapped around each lamp to screen out radiation below 290nm in the UV-B treatment
152 plots. As the UV-B tubes also emit some UV-A radiation, UV-A controls were also included
153 in the experimental set-up (UV-A and UV-A+T): in twelve plots, the UV tubes were wrapped
154 with polyester film in order to remove UV-B, so that only the enhanced levels of UV-A were
155 achieved. The temperature treatment was provided by two infrared (IR) heaters (CIR 110,
156 FRICO, Partille, Sweden), bolted along the middle axis of the aluminum frames. The frames
157 were lifted every third week to maintain a 60cm distance between the highest shoot tip and
158 the radiators/ UV lamps.

159

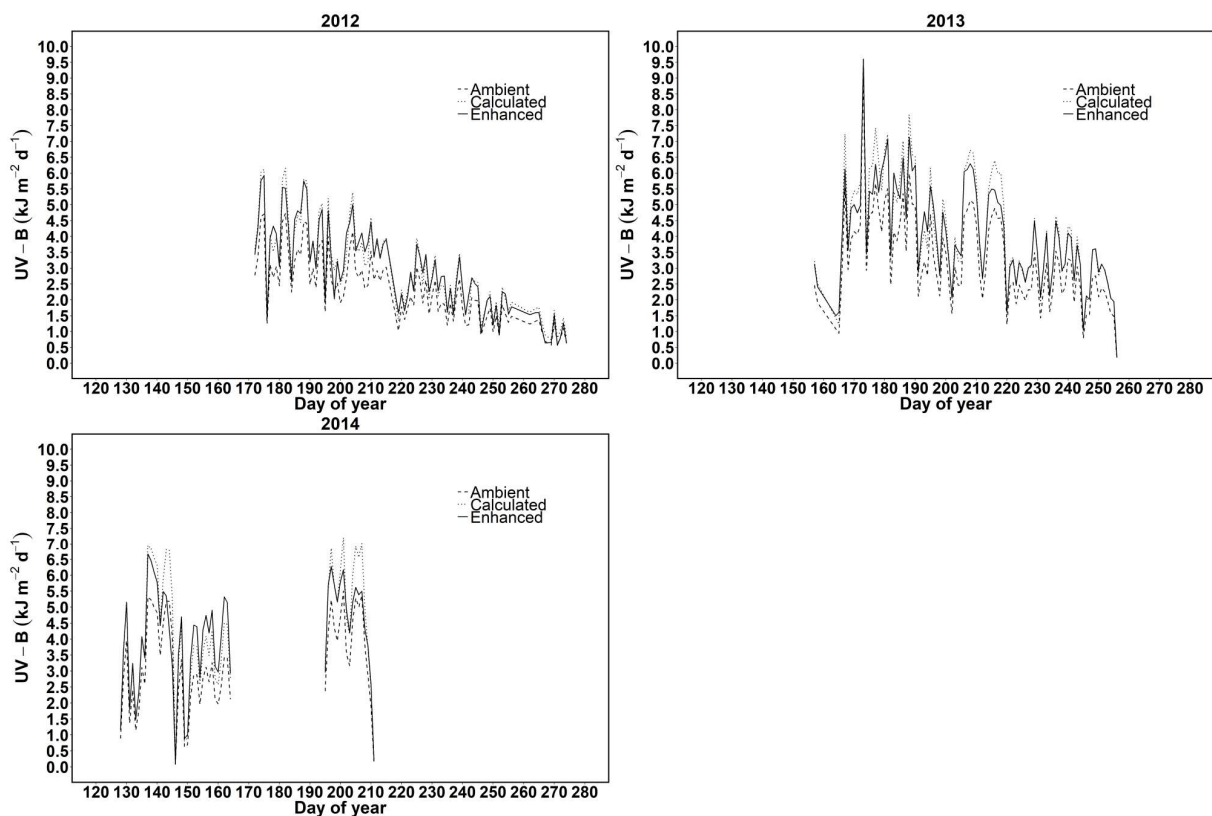
160 The enhancement system was run between 1 June (day 151) and 1 October 2012 (day 275),
161 between 5 June (day 156) and 13 September 2013 (day 256), and between 8 May (day 128)
162 and 28 July (day 209) 2014. In 2013, there was no modulated enhancement of temperature
163 and UV-B between 13 and 24 July due to a thunderstorm. In 2014, there was no climate data
164 recorded between 14 June and 13 July due to a technical error. Furthermore, the system was
165 switched off on July 28th in the same year, since the aluminum frames could be raised no
166 further with increasing plant height.

167

168 *Environmental data*

169 Four ThiesClima sensors (Thies, Göttingen, Germany) were used for measuring the UV-B
170 radiation (250 - 325 nm with a peak of 300nm). Two sensors were placed above the control
171 frames for ambient UV-B levels, and two under the frames of UV-B enhancement plots for set-
172 point values. Temperature enhancement modulation was achieved using self-made linear
173 temperature sensors with four PT1000 probe elements fabricated with four connection cables.

174 The set point values were achieved by placing two probe elements above the control frames
175 and two under the temperature enhancement frames. Calculations of set point values and control
176 of enhancement of UV lamps and IR radiators were implemented by a modulator software
177 (IPC100 configuration program and e-console measuring and data saving program, Gantner
178 Instruments GmbH, Darmstadt, Germany). Both UV-B and T was registered and logged every
179 10 minutes during the periods the system was running (Figure 1; Figure 2). Monthly
180 precipitation measurements were obtained from a meteorological station at Linnunlahti that is
181 situated less than 200 meters away from the experimental site (Figure 3).
182

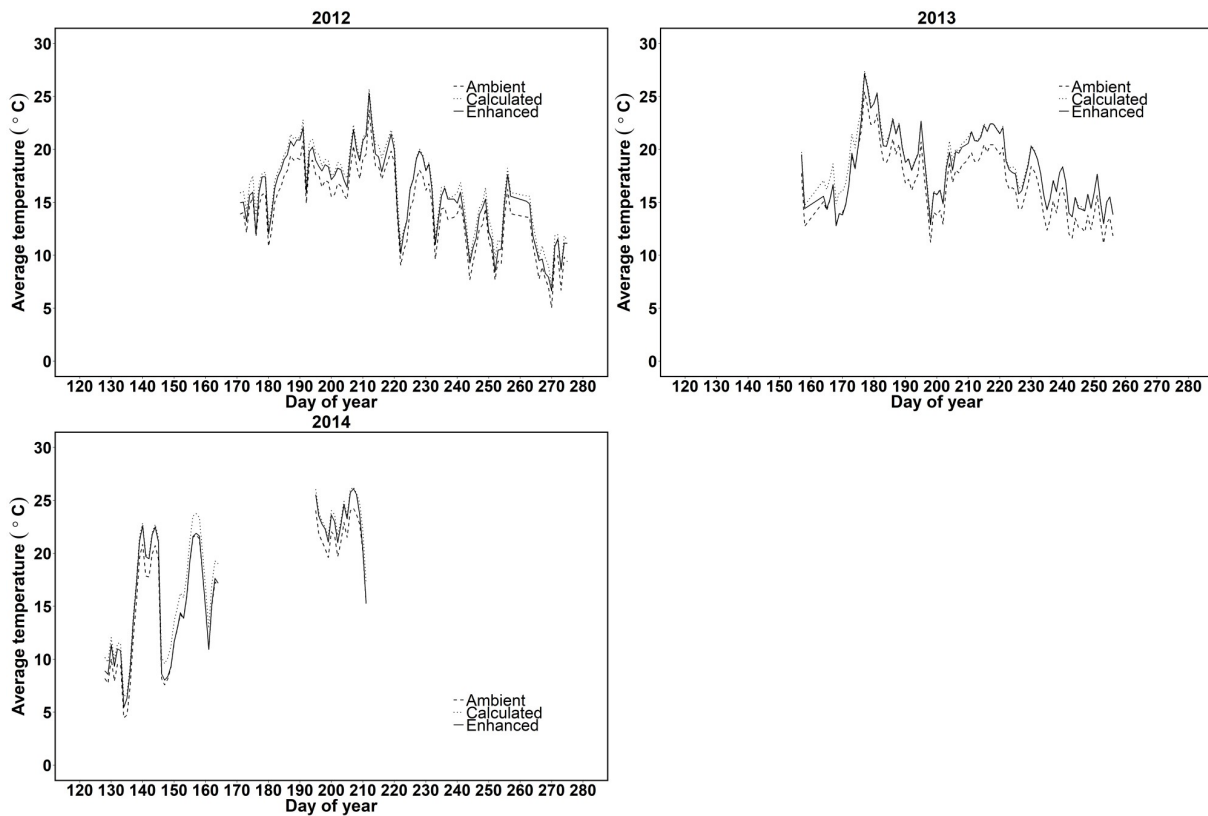


183
184 Figure 1. Performance of ultraviolet (UV)-B enhancement at the experimental site during
185 2012 (top left), 2013 (top right) and 2014 (bottom) measured by four broadband UV-B
186 sensors. Calculated set-point values are 30 % higher than ambient levels, while enhanced
187 values show the performance of the modulated UV-B enhancement.

188 189 *Phenology registrations*

190 We used the scoring system for autumn phenology described in Strømme et al. (2015), which
191 is a simplified version of the scoring system developed by Rohde et al. (2011 b). The three-
192 stage system used for scoring apices during autumnal bud formation discerns between three
193 stages; growing apex (1), green bud having closed bud scales (0.5), and brown/red mature bud

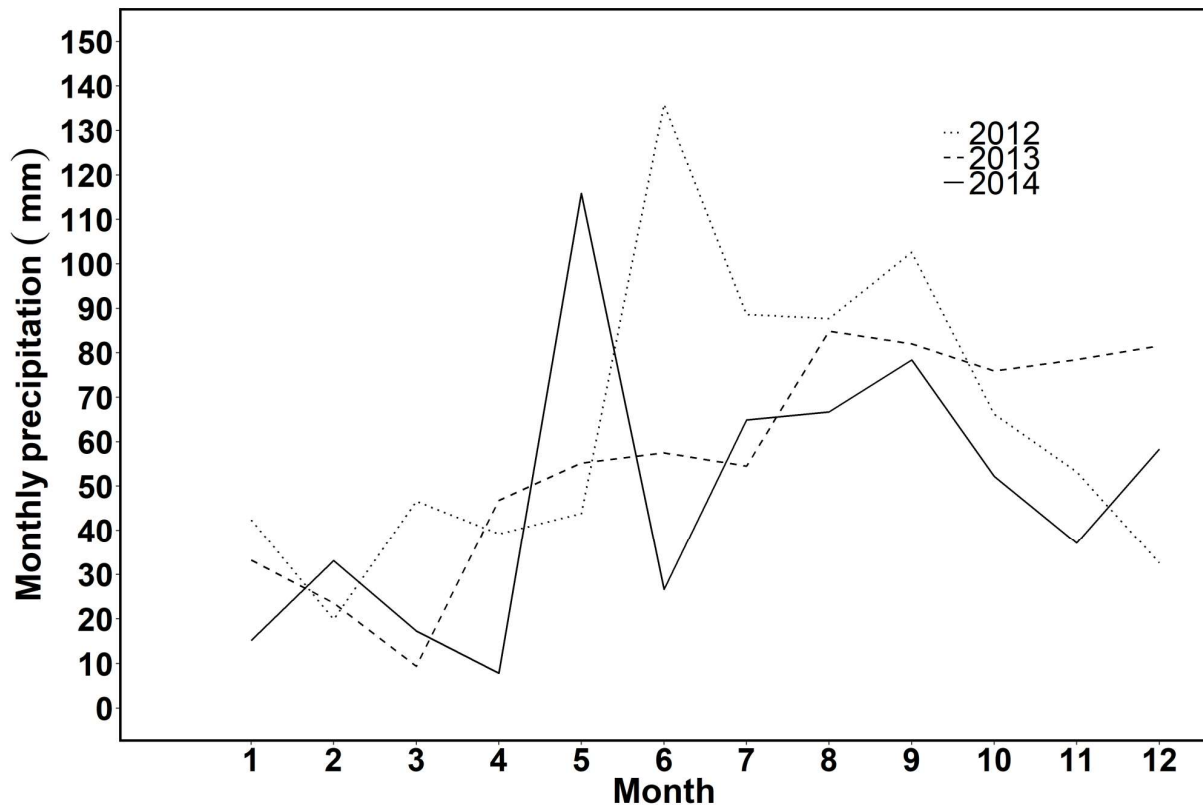
194 (0). The apical stages for each plant were determined throughout the growing season by
 195 observing the terminal end of the primary shoot. As the apices were located on the primary
 196 shoots, branches were not considered. In situations where green closed buds broke in autumn
 197 and apices resumed growth, apices were scored as growing. Some plants were affected by
 198 *Venturia* shoot blight, grazed upon by intruding herbivores or broken by mechanical damage
 199 and therefore not included in the apical scoring. Thus, apical stages were recorded only for
 200 healthy plants introduced during the same growing season, and the number of plants scored
 201 were 672 females and 680 males in 2012, 317 females and 291 males in 2013, and 98 females
 202 and 91 males in 2014. In 2012, the first apical scoring was performed on 15 August, while for
 203 2013 and 2014 the first apical scoring occurred on 20 and 12 August, respectively.
 204



205
 206 Figure 2. Performance of temperature enhancement at the experimental site during 2012 (top
 207 left), 2013 (top right) and 2014 (bottom) measured by four temperature sensors. Calculated
 208 set-point values are 2 °C higher than ambient levels, while enhanced values show the
 209 performance of the modulated temperature enhancement.

210
 211 The scoring system for spring bud-break stages was based on Fu et al. (2012). The registered
 212 stages were as follows: a closed bud (0), a swollen bud or elongated bud with green scales (1),
 213 green leaf tips out of the bud with leaf bases hidden (2), broken bud with at least one petiole

214 (3), and an unfolded leaf with visible leaf blade and stalk (4). In 2013, registrations for spring
215 bud break started as soon as the first stage transitions (from stage 0 to 1) were observed, being
216 on 6 May (day 126), in 2014 on 22 April (day 112), and in 2015 on 4 May (day 124). Spring
217 bud-break stages were recorded every four days for the years 2013-2014 and every second
218 day in 2015.
219



220
221 Figure 3. Total monthly precipitation measured at the Linnunlahti meteorological station in
222 years 2012-2014.

223
224 *Growth registrations*

225 The basal diameter and height of the plants were registered approximately every third week
226 during the growing season. In 2012, the last measurement was done on 25 September (day
227 269), while the first and last day of measurement were 21 May (day 141) and 3 September
228 (day 246) in 2013 and 6 May (day 126) and 9 September (day 252) in 2014. To investigate a
229 possible relationship between bud set dates and plant growth, we only used the total growth
230 achieved from spring to autumn during each growing season. In order to test a possible
231 relationship between bud break dates and growth, we used growth parameters measured when
232 all plants had completed bud break.
233

234 *Statistical analyses*

235 First, we tested the effect of UV-treatment (three levels), temperature treatment (two levels),
236 plantlet sex (two levels) and year (three levels) on bud set dates (day of year) in autumn and
237 date of completed bud break (day of year) in spring using the R software for statistical
238 computing (R Core Team 2015). Using the same covariates, we also tested for effects on plant
239 height and basal diameter measured at the end of the growing season for the years 2012-2014.
240 In order to investigate whether the duration of the growing season affected plant growth, we
241 also tested for possible relationships between bud set date in autumn and final yearly
242 measurements of height and basal diameter. Furthermore, we also tested for possible
243 relationships between spring bud break and growth in terms of height and basal diameter
244 measured on 10 June (day 161) in 2013 and on 17 June (day 168) in 2014 when most plants
245 had fully broken buds. The selection of appropriate statistical tests and models were partly
246 based on procedures described in Zuur *et al.* (2009). For each statistical test, the final model
247 was selected based on a global model that included all relevant covariates and their interactions.
248 We applied the dredge function in the MuMIN-package (Barton 2015) to the global model, and
249 thus obtained a model-selection table where all possible models were ranked based on their
250 respective AIC-values. Thus, each model selection table provided us with the most
251 parsimonious model for each analysis. In the model selection process, we included plant clone
252 (random term) and plot (random term) by using the lmer function in the lme4 package (Bates
253 *et al.* 2015) as their inclusion yielded improved models based on AIC comparison.

254

255 **RESULTS**

256 Plants grown under temperature enhancement finished bud formation later, but the significant
257 interaction with year reveals that plants under this treatment set buds earlier in 2013 by 6.6
258 days ($P < 0.001$) and by 8.3 days in 2014 ($P < 0.001$) (Table 1) than in 2012. Plants grown
259 under UV-B enhancement had earlier bud set dates in 2012, but the significant interaction
260 between UV-B enhancement and year as well as the term coefficients reveals that plants
261 receiving this treatment delayed bud set in 2014 by 3.5 days ($P=0.032$). Male plants grown
262 under UV-B enhancement set buds 1.8 days earlier than females ($P=0.039$), as seen from the
263 significant interaction between plant sex and UV-B enhancement (Table 1). Male plants were
264 also more responsive to temperature enhancement, and finished bud set 1.5 days later than
265 females under this treatment ($P=0.041$), as shown by the significant interaction between plant
266 sex and temperature enhancement (Table 1). Males had in general later bud set 1.8 days later

267 than females in 2013, but not in 2014 ($P=0.026$), as shown by the significant interaction
 268 between plant sex and year (Table 1).

269

270 Table 1. Parameter estimates, SE and t-values for covariates in the linear mixed models used
 271 to investigate the effects of elevated autumn temperature, elevated autumn ultraviolet (UV)-A
 272 and UV-B on bud set dates in females and males of *Populus tremula* in three consecutive years
 273 (2012, 2013, 2014) and bud break dates during the following spring (2013, 2014, 2015).

	Fixed effects terms	Coefficient	SE	t-value
<i>Bud set date</i>	Intercept***	247.0	2.36	104.9
	Male	0.2	3.04	0.05
	Year 2013***	-5.7	0.88	-6.46
	Year 2014***	-11.0	1.41	-7.73
	UVA enhancement	-0.1	1.36	-0.11
	UVB enhancement	-0.5	1.35	-0.37
	Temperature enhancement***	9.5	1.10	8.65
	Male x UVA	-0.7	0.89	-0.82
	Male x UVB*	-1.8	0.88	-2.07
	Male x Temp*	1.5	0.73	2.04
	Year 2013 x UVA enhancement	-1.3	1.00	-1.26
	Year 2014 x UVA enhancement	2.2	1.61	1.40
	Year 2013 x UVB enhancement	1.9	1.01	1.91
	Year 2014 x UVB enhancement*	3.5	1.61	2.15
	Year 2013 x Temperature enhancement***	-6.6	0.83	-8.00
	Year 2014 x Temperature enhancement***	-8.3	1.32	-6.31
	Year 2013 x Male*	1.8	0.82	2.23
Year 2014 x Male	0.6	1.31	0.47	
<i>Bud break date</i>	Intercept***	142.6	0.17	840.0
	Temperature enhancement*	-0.33	0.14	-2.3
	Year 2014***	0.8	0.10	7.8
	Year 2015***	8.9	0.11	83.5

Significance levels: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

274

275

276 Bud break in spring occurred slightly earlier (0.3 days) for plants grown under autumn
 277 temperature enhancement ($P=0.026$) (Table 1). Overall, bud break occurred 0.8 days later in
 278 2014 ($P<0.001$) and 8.9 days later in 2015 ($P<0.001$) than in 2013. For bud break, there were

279 no significant effects of UV-B enhancement, and there were no significant differences
 280 between females and males.

281
 282 Temperature enhancement yielded more pronounced plant growth both in terms of height and
 283 diameter (Table 2). The significant interaction with year shows that this effect was stronger in
 284 2013 ($P < 0.001$) and 2014 ($P < 0.001$) for height increase, and similarly for diameter increase in
 285 2013 ($P < 0.001$) and 2014 ($P < 0.001$) (Table 2).

286
 287 Table 2. Parameter estimates, SE and t-values for covariates in the linear mixed models used
 288 to investigate the effect of elevated autumn temperature on plant size in females and males of
 289 *Populus tremula* in three consecutive years (2012, 2013, 2014).

	Fixed effects terms	Coefficient	SE	t-value
<i>Height</i>	Intercept***	56.9	8.45	6.7
	Temperature enhancement***	42.5	8.08	5.3
	Year 2013***	59.3	2.57	23.1
	Year 2014***	123.5	4.14	29.8
	Temperature enhancement x Year 2013***	44.8	3.91	11.5
	Temperature enhancement x Year 2014***	63.0	6.23	10.1
<i>Basal diameter</i>	Intercept***	6.4	0.45	14.4
	Bud set date***	2.4	0.48	5.0
	Year 2013***	2.9	0.16	18.1
	Year 2014***	6.4	0.26	24.8
	Temperature enhancement x Year 2013***	2.9	0.25	12.0
	Temperature enhancement x Year 2014***	4.7	0.39	12.1

Significance levels: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

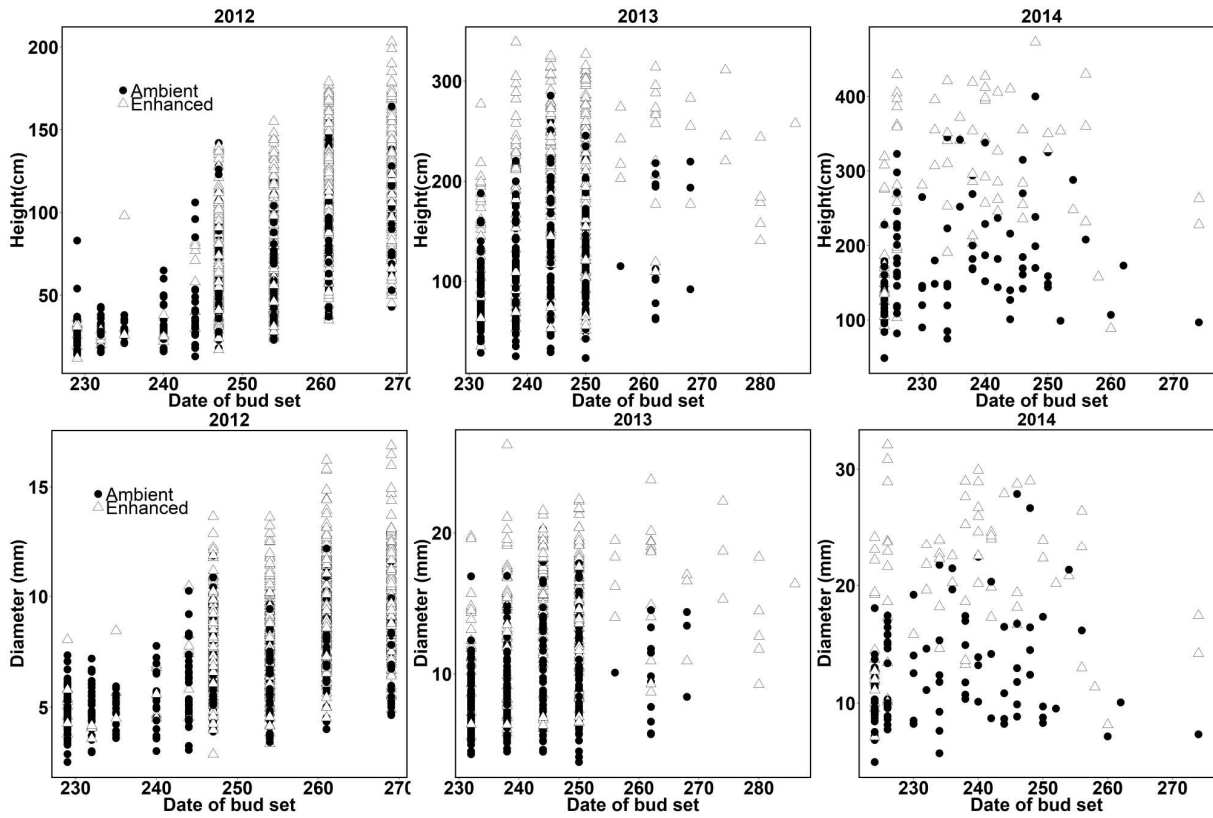
290
 291
 292 There was a significant positive relationship between bud set date and plant growth, both in
 293 terms of height and diameter (Table 3; Figure 4). This means that the prolonged growing
 294 season affected growth positively. In terms of height increase, there was a significant
 295 interaction between bud set date and year, indicating a stronger positive impact of bud set
 296 dates on height growth in 2013 ($P < 0.001$) and 2014 ($P < 0.001$), as compared to 2012. There
 297 was also a similar interaction for diameter growth, being significantly higher in 2013
 298 ($P < 0.001$) and 2014 ($P < 0.001$).

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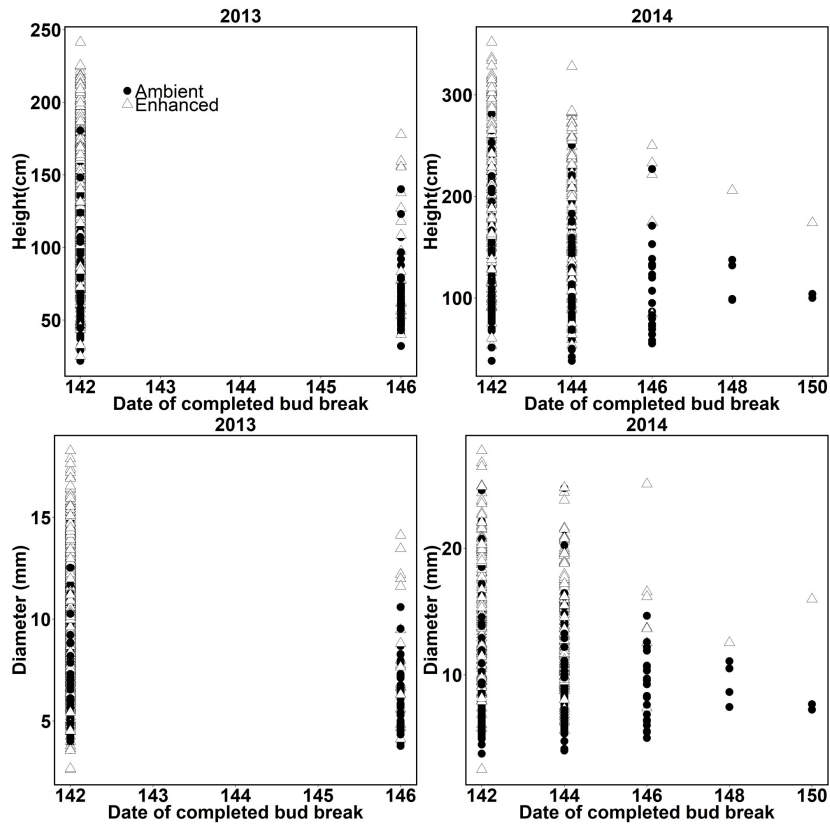
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Figure 4. The relationship between dates of completed bud set and plant growth properties of *Populus tremula* clones grown under ambient temperature and enhanced temperature measured on 25 September (Day 269) for 2012, 3 September (Day 246) for 2013 and 9 September (Day 252) for 2014.



309

310 Figure 5. The relationship between dates of completed bud break and plant growth properties
 311 of *Populus tremula* clones grown under ambient temperature and enhanced temperature
 312 measured on 10 June (Day 161) for 2013 and 17 June (Day 168) for 2014.

313 Table 3. Parameter estimates, SE and t-values for covariates in the linear mixed models used
 314 to investigate the effects of bud set dates on plant size in females and males of *Populus*
 315 *tremula* in three consecutive years (2012, 2013, 2014).

	Fixed effects terms	Coefficient	SE	t-value
<i>Height</i>	Intercept	24.1	30.36	0.79
	Bud set date	0.2	0.12	1.85
	Year 2013***	-225.7	50.04	-4.51
	Year 2014	-85.1	67.48	-1.26
	Bud set date x Year 2013***	1.3	0.21	6.17
	Bud set date x Year 2014***	1.0	0.28	3.60
<i>Basal diameter</i>	Intercept***	9.6	1.92	5.04
	Bud set date	- 8.1*10 ⁻³	7.36*10 ⁻³	-1.11
	Year 2013***	-16.0	3.19	-5.03
	Year 2014*	-9.2	4.30	-2.14
	Bud set date x Year 2013***	8.3*10 ⁻²	1.30*10 ⁻²	6.38
	Bud set date x Year 2014***	7.5*10 ⁻²	1.81*10 ⁻²	4.15

Significance levels: *P < 0.05, **P < 0.01, ***P < 0.001

316
 317 An earlier start of the growing season was also positive for growth , as there was a positive
 318 relationship between early bud break and growth both in terms of height ($P=0.002$) and basal
 319 diameter ($P=0.001$). This effect was stronger in 2014, as shown by the significant interaction
 320 between bud break date and year for both growth responses (Table 4; Figure 5).

321 Table 4. Parameter estimates, SE and t-values for covariates in the linear mixed models used
 322 to investigate the effects of bud break dates on plant size in females and males of *Populus*
 323 *tremula* in two consecutive years (2013, 2014).

	Fixed effects terms	Coefficient	SE	t-value
<i>Height</i>	Intercept*	362.7	175.38	2.07
	Bud break date	-1.8	1.23	-1.43
	Year 2014**	778.3	236.79	3.29
	Bud break date x Year 2014**	-5.1	1.66	-3.05
<i>Basal diameter</i>	Intercept	6.2	12.60	0.50
	Bud break date	0.02	0.09	0.19
	Year 2014***	58.2	17.02	3.42
	Bud break date x Year 2014**	-0.4	0.12	-3.18

Significance levels: *P < 0.05, **P < 0.01, ***P < 0.001

324

325

326 In the model selection process for all statistical tests, the interaction term between UV-B
 327 enhancement and temperature enhancement was included in the global model. Still, the term
 328 was not present in any of the most parsimonious (and thus final) models.

329

330 **DISCUSSION**

331 Our models showed clearly that the climate change mediated lengthening of the growing
 332 season was positive for plant growth, both in terms of later bud set dates in autumn, as well as
 333 earlier bud break dates in spring. In turn, both UV-B and temperature enhancement affected
 334 bud set dates, while bud break dates were only slightly affected by temperature enhancement
 335 in autumn. Still, significant interactions between year and enhancement treatments indicate
 336 that the plants gradually acclimated to the altered climate, resulting in weaker responses in
 337 later years. Considering that most enhancement studies involve exposure lasting a single
 338 growing season, it may be questioned whether results of those studies are representative of
 339 how young trees interact with climate in nature.

340

341 The observed advancement of bud set dates under UV-B enhancement during the first
 342 growing season reflects the positive effect of UV-B on the process of bud formation reported
 343 in Strømme *et al.* (2015), where UV-B was found to interact with temperature enhancement
 344 and plant sex in driving the transitions between phenotypic stages. As UV-B has been shown

345 to inhibit thermomorphogenesis in *A. thaliana* (Hayes *et al.* 2017), it is relevant to further
346 investigate whether similar interactions are found in relation to growth and developmental
347 processes in *P. tremula*. The bud set dates in this study correspond to the final stage of the
348 bud formation process and do not indicate any interactions between UV-B and temperature in
349 this respect. Instead, the data suggest that further studies of autumn phenology in *P. tremula*
350 should consider that responsiveness to higher levels of UV-B or temperature may decrease
351 with plant age.

352

353 Although enhanced temperature delayed bud set dates, also this effect varied between years.
354 Available evidence shows that warming delays the process of bud formation (Rohde *et al.*
355 2011; Strømme *et al.* 2017), and it can be argued that earlier bud set dates in 2013 and 2014
356 may have occurred as a result of colder autumn temperatures. However, temperature data for
357 Joensuu shows that autumn temperatures were similar across years in the period of bud
358 formation (until October 1, day 274) (Figure 4; Appendices Table 1). Moreover, the
359 significant factor Year in the statistical tests accounted for inter-annual differences in bud set
360 timing for all plants. Thus, the significant interaction between temperature enhancement and
361 year clearly show that plants responded progressively less to temperature enhancement in
362 terms of bud set timing. The explanation may be that the *P. tremula* plants gradually change
363 from a free growth pattern to fixed or predetermined growth at later developmental stages. In
364 woody plants with fixed growth, the bud contains all leaf primordia for the annual shoot,
365 while free growth is characterized by a simultaneous formation of leaf initials (nodes) and
366 elongation of internodes (Olsen 2010). Under fixed growth, autumnal bud set in plants are
367 little affected by photoperiod, for example (Junttila 2007). This phenomenon is scarcely
368 described, and the mechanisms behind little studied, but probably deserves more attention in
369 the future if we want to improve tree growth models by including seasonal phenology.

370

371 As most studies of climatic responses in tree species have been conducted on juvenile
372 individuals, it remains unclear whether such effects also occur in adult trees. Phase-dependent
373 responses have been shown in relation to spring phenology for some tree species (Hänninen *et*
374 *al.* 2007; Vitasse 2013), but information related to autumn phenology is scarce. Moreover,
375 there is little evidence of responses to climatic manipulations occurring over several years, but
376 within the same ontogenic phase. Even though our data were obtained using clones
377 originating from adults through micropropagation, the plants grown in the field were at a
378 juvenile stage. Thus, our results indicate that bud set in juvenile trees of *P. tremula* become

379 increasingly less susceptible to temperature modulation in the years following planting. In this
380 respect, there is need for further research that spans more than three years in order to verify
381 whether individuals become progressively less susceptible to such warming effects.

382

383 Insufficient chilling has been suggested to yield delayed bud break the following spring
384 (Hänninen & Tanino 2011), and possibly be a carry-over effect from high temperatures during
385 the previous autumn. Bud break in spring was positively affected by autumn warming, and
386 this effect did not differ across the year (no significant interaction between temperature
387 treatment and Year). Thus, our data does not suggest any such carry-over effect across
388 seasons, as buds most likely received sufficient chilling in winter. However, the possibility of
389 warming yielding insufficient chilling should be tested for *P. tremula* in field conditions
390 where winter climates are substantially warmer in order to verify whether such effects may
391 occur in nature.

392

393 The growth responses across years show that temperature effects on height and basal diameter
394 increase were progressively higher with each year. This could be an indication of cumulative
395 effects of warming on plant growth, where positive effects of warming in one year add to
396 warming effects in the next. Indeed, warming yielded increased specific leaf area and leaf
397 nitrogen content and assimilation rates in 2012 (Randriamanana *et al.* 2015), which may have
398 resulted in higher nutrient storage in stems to be mobilized in spring. Since we did not
399 measure root growth, we cannot account for any warming effects on gross water and nutrient
400 uptake. As root growth occurs above a minimum temperature (Schenker *et al.* 2014), it could
401 be argued that warming had a positive effect in this respect. However, this is contradicted by
402 an analysis based on 63 different studies that shows no increase in root growth under
403 increased temperature, and instead shows decreased biomass allocation to roots (Way & Oren
404 2010).

405

406 In addition, the duration of the growing season was clearly positive for growth in terms of
407 both height and basal diameter. On the one hand, the positive relationship between bud set
408 date and growth shows that delaying bud set was positive for plant growth. On the other hand,
409 the negative relationship between bud break date and growth shows that earlier bud break was
410 beneficial to plant growth. The significant interaction between date and year for both
411 processes shows that plants benefitted more from an extension of the growth period over time
412 both in terms of height and basal diameter growth. This may be due to bud set occurring

413 progressively earlier, and bud break progressively later, with each year, indicating that an
414 extension of the growing season would occur in a warmer period than in 2012. In this respect,
415 our data indicate that *P. tremula* benefits from an extended growing season if temperatures in
416 the extended period are sufficiently warm to sustain growth.

417

418 Winter buds represent a vital physiological adaptation for plants to survive freezing
419 temperature (Welling & Palva 2006; Gusta & Wisniewski 2012), and there is evidence of
420 earlier bud break resulting in frost damage for a number of deciduous species (Augspurger
421 2009). Moreover, a higher susceptibility of juvenile trees to frost damage than adults has been
422 shown for some species (Vitasse *et al.* 2014). We found no evidence of frost damage on shoot
423 tips, neither in late autumn nor in spring, throughout our study.

424

425 In conclusion, our data show that growth in the deciduous tree *P. tremula* benefits from an
426 extended growing season, both due to delayed bud set and earlier bud break. In terms of bud
427 set timing under experimental UV-B and temperature increase, the analysis covering three
428 growing season shows that plants became less responsive to the treatments with increasing
429 age, which is possibly a consequence of acclimation. Moreover, plants showed a gradual shift
430 in timing of bud set and bud break dates with increasing age irrespective of treatments,
431 showing that timing of these processes in plants introduced the first year may differ
432 substantially from observations in the following years. Considering that most tree species
433 have lifespans covering decades and in many cases centuries, their capacity for short- and
434 long term acclimation to shifting climatic conditions is far from understood. Thus, our
435 understanding of tree responses to light and temperature shifts should clearly benefit from
436 further field studies that span over several years.

437

438 **REFERENCES**

439 Augspurger CK (2009) Spring 2007 warmth and frost: phenology, damage and refoliation in a
440 temperate deciduous forest. *Functional Ecology* 23: 1031-1039

441 Barton K (2015) Multi-model inference. R package version 1.15.6.

442 Bates D, Maechler M, Bolker B, Walker S (2015) Fitting Linear Mixed-Effects Models Using
443 lme4. *Journal of Statistical Software* 67, 1-48. DOI: [10.18637/jss.v067.i01](https://doi.org/10.18637/jss.v067.i01)

444 Bertin, R I (2008) Plant phenology and distribution in relation to recent climate change.

445 *Journal of the Torrey Botanical Society* 135, 126-146

446 Brelsford CC, Robson TM (2018) Blue light advances bud burst in branches of three
447 temperate deciduous tree species under short-day conditions. *Trees* doi: 10.1007/s00468-018-
448 1684-1

449 Clapham D, Dormling I, Ekberg I, Eriksson G, Qamaruddin M, Vince-Prue D (1998)
450 Latitudinal cline of requirement for far-red light for the photoperiodic control of bud set and
451 extension growth in *Picea abies* (Norway spruce). *Physiologia Plantarum* 102: 71–78

452 Fehér B, Kozma-Bognár L, Kevei É, Hajdu A, Binkert M, Davis SJ, Schäfer E, Ulm R, Nagy
453 F (2011) Functional interaction of the circadian clock and UV RESISTANCE LOCUS 8-
454 controlled UV-B signaling pathways in *Arabidopsis thaliana*. *The Plant Journal* 67, 37-48

455 Francis D, Barlow PW (1988) Temperature and the cell cycle. *Symposia of the Society for*
456 *Experimental Biology* 42: 181-201

457 Gusta, LV, Wiesniewski M (2012) Understanding plant cold hardiness: an opinion.
458 *Physiologia Plantarum* 147: 4-14

459 Hartman DL, Klein Tank AMG, Rusticucci M, Alexander LV, Broenniman B, et al. (2013)
460 Observations: atmosphere and surface. In: Stocker TF, Qin D, et al., editors. *Climate Change*
461 *2013: The Physical Science Basis*, Cambridge University Press. 159–254

462 Hember RA, Kurz WA, Metsaranta JM, Black TA, Guy RD, Coops NC. Accelerating
463 regrowth of temperate-maritime forests due to environmental change. *Global Change Biology*
464 18: 2026-2040

465 Hänninen H (2007) Dormancy release of Norway spruce under climatic warming: testing
466 ecophysiological models of bud burst with a whole-tree chamber experiment. *Tree Physiology*
467 27: 291-300

468 Hänninen H, Tanino KK (2011) Tree seasonality in a warming climate. *Trends in plant science*
469 16: 412-416

470 Jacoby GC, D'Arrigo RD (1995) Tree ring width and density evidence of climatic and potential
471 forest change in Alaska. *Global Biogeochemical Cycles* 9, 227-234

472 Junttila, O (2007) Regulation of annual shoot growth cycle in northern tree species. In:
473 Taulavuori E, Taulavori K (eds) *Physiology of Northern Plants Under Changing*
474 *Environment*, pp 177–210. Research Signpost, Kerala, India.

475 Junttila O, Kaurin Å (1985) Climatic control of apical growth cessation in latitudinal ecotypes
476 of *Salix pentandra* L. In: Kaurin Å, Junttila O, Nilsen J (eds) *Plant Production in the North.:*
477 *Norwegian University Press, Oslo*, pp 83-91

478 Kauppi PE, Posch M, Pirinen P (2014) Large impacts of climatic warming on growth of boreal
479 forests since 1960. *PLoS One* 9, e111340

480 Körner C (2016) Plant adaptation to cold climates [version 1; referees: 2 approved].
481 *F1000Research* 5(F1000 Faculty Rev):2769 DOI: [10.12688/f1000research.9107.1](https://doi.org/10.12688/f1000research.9107.1)

482 Körner C, Basler D (2010) Phenology under global warming. *Science* 327: 1461-1462.

483 Menzel A, Sparks T, Estrella N, Koch E, Aasa A, Ahas R, Alm-Kübler K, Bissolli P, Braslavská
484 O, Briede A, Chmielewski FM, Crepinsek Z, Curnel Y, Dahl Å, Defila C, Donnelly A, Filella
485 Y, Jatzcak K, Måge F, Mestre A, Nordli Ø, Peñuelas J, Pirinen P, Remisová V, Sheifinger H,
486 Stritz M, Susnik A, van Vliet AJH, Wielgolaski FE, Zach S, Züst A (2006) European
487 phenological response to climate change matches the warming pattern. *Global change biology*
488 12, 1969-1976

489 Mølmann JA, Junttila O, Johnsen Ø, Olsen JE (2006) Effects of red, far-red and blue light in
490 maintaining growth in latitudinal populations of Norway spruce (*Picea abies*). *Plant, Cell and*
491 *Environment* 29: 166-172

492 Nissinen K, Virjamo V, Randriamanana T, Sobuj N, Sivadasan U, Mehtätalo L, Beuker E,
493 Julkunen-Tiitto R, Nybakken L (2017) Responses of growth and leaf phenolics in European
494 aspen (*Populus tremula*) to climate change during juvenile phase change. *Canadian Journal of*
495 *Forest Research* 47: 1350-1363

496 Nybakken L, Hörkkä R, Julkunen-Tiitto R (2012) Combined enhancements of temperature
497 and UVB influence growth and phenolics in clones of the sexually dimorphic *Salix*
498 *myrsinifolia*. *Physiologia Plantarum* 145, 551-564

499 Olsen JE, Junttila O, Nilsen J, Eriksson ME, Martinussen I, Olsson O, Sandberg G, Moritz T
500 (1997) Ectopic expression of oat phytochrome A in hybrid aspen changes critical daylength
501 for growth and prevents cold acclimatization. *The Plant Journal* 12: 1339–1350

502 Olsen JE (2010) Light and temperature sensing and signalling in induction of bud dormancy
503 in woody plants. *Plant Molecular Biology* 73: 37-47

504 Olsen JE, Lee YK (2011) Trees and boreal forests. In: Storey KB, Tanino KK (eds)
505 *Temperature Adaptation in a Changing Climate: Nature at Risk*. CAB International,
506 Wallingford, pp 160–178

507 Opseth L, Holefors A, Rosnes AKR, Lee YK, Olsen JE (2016) *FTL2* expression preceding
508 bud set corresponds with timing of bud set in Norway spruce under different light quality
509 treatments. *Environmental and Experimental Botany* 121, 121-131

510 Randriamanana TR, Lavola A, Julkunen-Tiitto R (2015) Interactive effects of supplemental
511 UV-B and temperature in European aspen seedlings: Implications for growth, leaf traits,
512 phenolic defense and associated organisms. *Plant Physiology and Biochemistry* 93: 84-93

513 Rohde A, Bastien C, Boerjan W (2011) Temperature signals contribute to the timing of
514 photoperiodic growth cessation and bud set in poplar. *Tree Physiology* 31: 472-482

515 Sarvas R (1972) Investigations on the annual cycle of development of forest trees, Active
516 period. *Communicationes Instituti Forestalis Fenniae* 76: 1–110

517 Schenker G, Lenz A, Körner C, Hoch G (2014) Physiological minimum temperatures for root growth in seven common
518 European broad-leaved species. *Tree Physiology* 34: 302-313

519 Schaphoff S, Reyer CPO, Schepaschenko D, Gerten D, Shvidenko A (2016) Tamm Review:
520 Observed and projected climate change impacts on Russia's forests and its carbon balance.
521 *Forest Ecology and Management* 361: 432-434

522 Schenker G, Lenz A, Körner C, Hoch G (2014) Physiological minimum temperatures for root
523 growth in seven common European broad-leaved tree species. *Tree Physiology* 34: 302-313

524 Serreze MC, Walsh JE, Chapin III F S, Osterkamp T, Dyurgerov M, Romanovsky V, Oechel
525 WC, Morison J, Zhang T, Barry RG. 2000. Observational evidence of recent change in the
526 northern high-latitude environment. *Climatic Change* 46, 159-207

527 Sivadasan U, Randriamanana T, Chenhao C, Virjamo V, Nybakken L, Julkunen-Tiitto R
528 (2017) Effect of climate change on bud phenology of young aspen plants (*Populus tremula*.
529 L) *Ecology and Evolution* 7: DOI 10.1002/ece3.3352

530 Sivadasan U, Chenhao C, Nissinen K, Randriamanana T, Nybakken L, Julkunen-Tiitto R
531 (2018) Growth and defence of aspen (*Populus tremula* L.) after three seasons under elevated
532 temperature and UV-B. *Canadian Journal of Forest Research* DOI: [10.1139/cjfr-2017-0380](https://doi.org/10.1139/cjfr-2017-0380)

533 Strømme CB, Julkunen-Tiitto R, Krishna U, Lavola A, Olsen JE, Nybakken L (2015) UV-B
534 and temperature enhancement affect spring and autumn phenology in *Populus tremula*. *Plant,*
535 *Cell and Environment* 38: 867-877

536 Strømme CB, Julkunen-Tiitto R, Olsen JE, Nybakken L (2017) High daytime temperature
537 delays autumnal bud formation in *Populus tremula* under field conditions. *Tree Physiology*
538 37: 71-81

539 Strømme CB, Julkunen-Tiitto R, Olsen JE, Nybakken L (2018) The dioecious *Populus*
540 *tremula* displays interactive effects of temperature and ultraviolet-B along a natural gradient.
541 *Environmental and Experimental Botany* 146: 13-26

542 Tsegay BA, Lund L, Nilsen J, Olsen JE, Mølmann JA, Ernsten A, Junttila O (2005) Growth
543 responses of *Betula pendula* ecotypes to red and far-red light. *Electronic Journal of Molecular*
544 *Biology* 8: 17–23

545 Vitasse Y (2013) Ontogenic changes rather than difference in temperature cause understory
546 trees to leaf out earlier. *New Phytologist* 198: 149-155

- 547 Vitasse Y, Lenz A, Hoch G, Körner C (2014) Earlier leaf-out rather than difference in
548 freezing resistance puts juvenile trees at greater risk of damage than adult trees. *Journal of*
549 *Ecology* 102: 981-988
- 550 Way DA, Oren R (2010) Differential responses to changes in growth temperature between
551 trees from different functional groups and biomes: a review and synthesis of data. *Tree*
552 *Physiology* 30: 669-688
- 553 Welling A, Palva ET (2006) Molecular control of cold acclimation in trees. *Physiologia*
554 *Plantarum* 127: 167-181
- 555 Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009) *Mixed Effects Models and*
556 *Extensions in Ecology with R*. Springer Verlag, Berlin, pp 101-142