

1 Running title: CRITICAL TEMPERATURE LIMITS XYLOGENESIS

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3 Critical minimum temperature limits xylogenesis and maintains  
4 treelines on the Tibetan Plateau

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25 **ABSTRACT**

26 **Physiological and ecological mechanisms that define treelines are still debated. It is**  
27 **suggested that the absence of trees above the treeline is caused by the low temperature**  
28 **that limits growth. Thus, we raise the hypothesis that there is a critical minimum**  
29 **temperature (CT<sub>min</sub>) preventing xylogenesis at treeline. We tested this hypothesis by**  
30 **examining weekly xylogenesis across three and four growing seasons in two natural**  
31 **Smith fir (*Abies georgei* var. *smithii*) treeline sites on the south-eastern Tibetan Plateau.**  
32 **Despite differences in the timing of cell differentiation among years, minimum air**  
33 **temperature was the dominant climatic variable associated with xylem growth; the**  
34 **critical minimum temperature (CT<sub>min</sub>) for the onset and end of xylogenesis occurred at**  
35 **0.7±0.4 °C. A process-based-modeled chronology of tree-ring formation using this CT<sub>min</sub>**  
36 **was consistent with actual tree-ring data. This extremely low CT<sub>min</sub> permits Smith fir**  
37 **growing at treeline to complete annual xylem production and maturation and provides**  
38 **both support and a mechanism for treeline formation.**

39  
40 *Key- words:* cambial activity; climate; growing season; growth limitation hypothesis;  
41 threshold temperatures; xylem; treeline formation

42

## 43 INTRODUCTION

44 The explanations for treeline formation focus on limitations of available resources (Stevens &  
45 Fox 1991; Susiluoto *et al.* 2010), establishment sites (e.g., Smith *et al.* 2003), or time  
46 available for growth (e.g., Körner 1998), although this ecophysiological causes still remain  
47 debated (Tranquillini 1979; Holtmeier 2003; Körner 2003; Cui *et al.* 2005). Based on notable  
48 similarities in temperatures at treelines (Körner & Paulsen 2004), Körner (1998) proposed  
49 that low temperatures limit the time available for meristematic growth and cell division  
50 (Körner 1998). This hypothesis has been supported by phenomenological data. For example,  
51 treeline trees tend to have higher amounts of non-structural carbohydrates than trees growing  
52 at lower elevation, suggesting that treeline are limited more by growth processes than by  
53 photosynthesis and carbon assimilation (e.g. Hoch & Körner 2003; Shi *et al.* 2008; Fajardo *et*  
54 *al.* 2012). In parallel, dendroclimatic studies have identified a signal of reduced growth  
55 during periods with low temperatures at treelines in cold and humid areas (e.g., Mäkinen *et al.*  
56 2000; Oberhuber 2004; Frank & Esper 2005; Ettinger *et al.* 2011).

57 Physiological manifestations of the growth limitation hypothesis include a constraint on  
58 the production of new cells by meristems below a critical minimum temperature ( $CT_{min}$ )  
59 (Körner 1998) and a trade-off between taking maximal advantage of the length of the  
60 growing season while avoiding cellular damage due to early (fall, winter) or late (winter,  
61 spring) freezing events (Chuine & Beaubien 2001; Kollas *et al.* 2014). Such a trade-off would  
62 suggest a narrow thermal window for the onset and cessation of xylem formation at treeline.  
63 Indeed, recent studies focused on temporal dynamics of xylem formation in various tree  
64 species at treeline (Rossi *et al.* 2007; Seo *et al.* 2008; Gruber *et al.* 2009; Moser *et al.* 2010;

65 Lenz *et al.* 2012). One study reported that a gradual increase in temperature (heat sum) was  
66 associated with the onset of cambial activity (Seo *et al.* 2008), whereas another estimated a  
67  $CT_{\min}$  of 5.6 –8.5 °C for xylogenesis at the altitudinal treeline in the Eastern Alps (Rossi *et al.*  
68 2007). Separating gradual (heat-sum) and threshold ( $CT_{\min}$ ) effects on xylogenesis at treeline  
69 has not yet been accomplished.

70 A mechanistic model can provide a deeper understanding on a climatic control on tree  
71 growth dynamics. The process-based forward model, such as, Vaganov-Shashkin (VS) model,  
72 was used to simulate the climatic control on conifer tree-ring growth (Vaganov *et al.* 1999;  
73 Anchukaitis *et al.* 2006; Evans *et al.* 2006). The critical temperature for cambial activity is a  
74 key parameter to model tree growth despite that its observed data are less available as a  
75 model input.

76 Our observations at the upper Smith fir (*Abies georgei* var. *smithii*) treeline of the  
77 south-eastern Tibetan Plateau, including a decade of uninterrupted *in situ*  
78 micrometeorological measurements and weekly collection of microcores containing cambium  
79 and forming wood during three consecutive growing seasons provides an opportunity to  
80 examine both gradual and threshold effects of temperature on xylogenesis at a natural alpine  
81 treeline. Specifically, we tested the potential for thermal control of xylogenesis to be a  
82 mechanism underlying the growth limitation hypothesis by (1) identifying the timing and  
83 dynamics of xylem formation in Smith fir growing at treeline as a function of climatic factors;  
84 and (2) detecting a plausible  $CT_{\min}$  for xylogenesis. Previous studies have found that its  
85 growth near treeline is constrained by the minimum temperature in summer (Bräuning &  
86 Mantwill 2004; Liang *et al.* 2009). The onset of bud swelling and needle unfolding in Smith

87 fir is delayed by 3.5 days per 100 m increase in elevation (Wang *et al.* 2013), indicating a  
88 thermal limitation of tree phenology. Therefore, we hypothesized that minimum temperature  
89 limits xylem formation and that a threshold minimum temperature controls the timing of the  
90 onset and ending of xylem formation. If this potential  $CT_{min}$  is reasonable, as a primary  
91 temperature parameter, it will be expected to be used in Vaganov-Shashkin (VS) model of  
92 tree-ring formation to simulate regional tree growth at Smith fir treeline on the south-eastern  
93 Tibetan Plateau.

94

## 95 **MATERIALS AND METHODS**

### 96 **Study sites and tree selection**

97 The study focused on the natural alpine treeline of Smith fir (*Abies georgei* var. *smithii*)  
98 growing on the eastern side of the Sygera Mountains (29° 10' – 30° 15' N, 93° 12' – 95° 35' E)  
99 on the south-eastern Tibetan Plateau (Liang *et al.* 2011). The south-eastern Tibetan Plateau is  
100 characterized by a cold and humid climate, and has the highest natural treeline (up to 4900 m  
101 a.s.l.) in the Northern Hemisphere (Miehe *et al.* 2007). Smith fir (*Abies georgei* var. *smithii*)  
102 is one of the dominant treeline species in this region. This tree is a shade-requiring conifer  
103 and the upper treeline depends on topographic aspect and ranges from 4,250 to 4,400 m a.s.l.  
104 We studied two sites at open-canopy treelines: Site 1, was at 4360 m a.s.l. on an east-facing  
105 slope, and Site 2, was at 4250 m a.s.l. on a south-east-facing slope. The sites were 200 m  
106 apart, on slopes < 15° *Rhododendron aganniphum* var. *schizopeplum* dominated the  
107 understory. The coverage of Smith fir was < 20% and the podzolic soils had an average pH  
108 value of 4.5.

109           At each site, five dominant trees were selected in April 2007. These trees had a mean  
110 age of  $201 \pm 24$  and  $117 \pm 14$  years, and mean diameters at 1.3-m above ground of  $34 \pm 4$  and  
111  $44 \pm 7$  cm in Sites 1 and 2, respectively. Because repeated sampling could cause severe  
112 wounding that could modify xylem formation, another five trees per site with homogeneous  
113 diameters were chosen for the samplings in 2009 and 2010. Trees with polycormic stems,  
114 partially dead crowns, reaction wood, or other evident damage were avoided.

115

### 116 **Meteorological data**

117 An automatic weather station (Campbell Scientific, CR1000) was installed in November  
118 2006 in an open area above the treeline ( $29^{\circ}39'$  N,  $94^{\circ}42'$  E, 4390 m a.s.l.) at a linear distance  
119 of  $\approx 150$  m and 200 m from Sites 1 and 2, respectively. Measurements of air (3-m above  
120 ground) and soil temperature (at 10-, 20- and 40-cm depths), precipitation, snow fall, and soil  
121 water content (at 10-, 20-, and 40-cm depths) were collected at 30-minute intervals. These  
122 data were used to compute daily averages, minima, and maxima of each variable.

123

### 124 **Microcoring and histological analyses**

125 Xylem formation was studied from 2007 until 2010 at Site 1 and from 2007 to 2009 at Site 2.  
126 One microcore (15-mm long, 2-mm diameter) was collected from each tree weekly from May  
127 until October around the stem at breast height using a Trephor tool. Immediately after  
128 removal from the trees, the microcores were fixed in a formalin-ethanol-acetic acid (FAA)  
129 solution. The microcores contained innermost phloem, cambium, developing xylem, and at  
130 least three previous xylem growth rings. In the laboratory, the microcores were dehydrated

131 with successive immersions in a graded series of ethanol and *d*-limonene, then embedded in  
132 paraffin. Transverse sections (9-12  $\mu\text{m}$  in thickness) were cut from the samples with a Leica  
133 RM 2245 rotary microtome using Feather N35H knives (Osaka, Japan). Sections were stained  
134 with a mixture of safranin (0.5 % in 95 % ethanol) and astra blue (0.5 % in 95% ethanol)  
135 and observed with a Nikon Eclipse 800 light microscope under bright field and polarized  
136 light to identify the phases of differentiation of the developing xylem cells. In cross-section,  
137 cambial cells were characterized by thin cell walls and small radial diameters (Deslauriers *et*  
138 *al.* 2003). Newly-formed xylem cells in the phase of cell enlargement contained protoplasts,  
139 had thin primary walls, and a radial diameter at least twice the size of the cambial cells. The  
140 onset of cell-wall thickening was determined by birefringence in the cell walls under  
141 polarized light. Mature cells had completely red-stained walls and empty lumen. For each  
142 sample, the total current xylem cell number was determined by counting the number of cells  
143 undergoing enlargement, cell-wall thickening, and the number of mature cells along three  
144 radial files (Deslauriers *et al.* 2003).

145

#### 146 **Data standardization and estimating the rate of xylem formation**

147 The data were standardized to compensate the variation in the number of xylem cells along  
148 the tree circumference. The total cell number of the previous years was counted on three  
149 radial files per sample and used for standardization. The standardized number of cells  $nc_{ij}$   
150 number in the  $i^{\text{th}}$  phase of the  $j^{\text{th}}$  sample was calculated as:

$$151 \quad nc_{ij} = n_{ij} (a_m / a_j) \quad (1)$$

152 where  $n_{ij}$  is the number of cells in the current year,  $a_m$  is the mean number of cells of the

153 previous ring of all  $j$ -samples and  $a_j$  is the mean number of cells of the previous ring in each  
154  $j$ -sample .

155 We modelled the dynamics of xylem formation by fitting a Gompertz function to the  
156 number of xylem cells that were produced through time:

$$157 \quad y = A \exp[-e^{(\beta - kt)}] \quad (2)$$

158 where  $y$  is the weekly cumulative sum of tracheids,  $t$  is the time of the year computed as day  
159 of the year,  $A$  is an asymptote (constant) and  $\beta$  and  $k$  are constants reflecting the  $x$ -intercept  
160 placement and rate of change, respectively (Deslauriers *et al.* 2003). Model parameters were  
161 estimated using the Origin software package (Version 8.5, OriginLab Corporation,  
162 Northampton, MA, USA).

163

#### 164 **Estimation of the onset and ending of xylem formation**

165 We used directly observations of cell differentiation to identify the onset, ending, and

166 duration of xylem formation from counts of the number of cells in three radial files per tree.

167 In spring, xylem formation was considered to have started when at least one tangential row of

168 cells was observed in the enlarging phase. Because of the weekly resolution of the monitoring,

169 we used the occurrence of 1-2 enlarging xylem cells along any of the checked three radial

170 files as an indicator the xylem formation had begun (Li *et al.* 2013). In late summer, when

171 cells were no longer observed in the wall thickening and lignification phase, xylem formation

172 was considered to have ended. The duration of xylogenesis was estimated as the number of

173 days between the dates of onset and ending of xylem formation; we report the average among

174 trees for each studied site and year.

175 Comparisons between sites in onset, duration, and ending of differentiation in the  
176 developing xylem ring, were done with generalized linear models (GLM). Homoscedasticity  
177 was checked using Shapiro-Wilk and Levene tests.

178

### 179 **Identifying $CT_{\min}$**

180 Logistic regression (LOGISTIC procedure in SPSS 16.0) was used to model the probability  
181 of xylogenesis as a function of air temperature. Xylem cell production was coded zero (not  
182 occurring) or one (occurring).  $CT_{\min}$  was estimated as that temperature when the probability  
183 of ongoing xylem growth equalled 0.5 (Rossi et al. 2008).  $CT_{\min}$ s also were calculated for 5%  
184 and 95% of the maximum number of xylem cells. For each tree and year, the model was  
185 fitted with three respective daily temperature series (mean, absolute minimum, and absolute  
186 maximum). Model verification included the likelihood-ratio  $\chi^2$ , Wald's  $\chi^2$  for regression  
187 parameter and goodness of fit, and Hosmer-Lemeshow  $\hat{C}$  for possible lack of fit. None of the  
188 models were excluded because of a lack of fit.  $CT_{\min}$ s were compared between sites and years  
189 using analysis of variance (ANOVA) models. Model validation was performed by comparing  
190 the observations with the predicted values when using estimated  $CT_{\min}$ s.

191

### 192 **Climate- growth relationships**

193 We used two approaches to identify relationships between intra-annual xylem growth and  
194 climatic variables during four growing seasons. One approach consisted of computation of  
195 Pearson's correlation coefficients between xylem cell production and weather data for weekly  
196 intervals. Weather data here include daily mean, daily absolute minimum, daily absolute

197 maximum temperatures, growing degree-days (GDD) > 5°C, and sums of precipitation.

198 Intra-annual xylem growth may be controlled both by endogenous (e.g. hormonal  
199 regulation) and exogenous factors (e.g., climate). To analyse the climatic effect, a common  
200 approach was used to remove the endogenous growth trend by fitting a growth curve, and to  
201 estimate the growth departure, calculated as the dimensionless ratio between observed and  
202 expected growth (Fritts 1976). This ratio (hereafter called ‘growth index’) was calculated as  
203 the number of tracheids produced during the week divided by the expected values estimated  
204 using the Gompertz function (Zhai *et al.* 2012). To account for possible effects of time-lags,  
205 daily weather data were averaged (temperature) or summed (precipitation) weekly from 1 to  
206 10 d prior to each sampling date (referred to as P1 to P10). To minimize the effects of  
207 temporal autocorrelation, correlation coefficients were calculated on first-order differences  
208 for both datasets.

209

## 210 **Tree-ring modeling**

211 We used the Vaganov-Shashkin (VS) model to simulate tree-ring growth at the Smith fir  
212 treelines in the Sygera Mountains. The VS model estimates xylem formation and its internal  
213 characteristics based on equations relating daily temperature, precipitation, and sunlight to  
214 the kinetics of xylem development (Vaganov *et al.* 2006). It assumes that climatic influences  
215 are directly but nonlinearly related to tree-ring characteristics through controls on the rates of  
216 cambial activity processes. To date, it has been successfully used to simulate and evaluate the  
217 relationships between climate and tree-ring formation under a variety of environmental  
218 conditions in many different regions (Anchukaitis *et al.* 2006; Evans *et al.* 2006; George *et al.*

219 2008; Shi *et al.* 2008; Zhang *et al.* 2011; Gou *et al.* 2013). Variables used as input for the VS  
220 model included soil moisture, depth of root system, temperature sum for initiation of growth,  
221 soil water drainage rate, and maximum daily precipitation falling into soil were taken from  
222 field observations. For the parameter of minimum temperature, we used the abovementioned  
223  $CT_{min}$ . Model fit was evaluated against an actual high-quality tree-ring width chronology  
224 from Smith fir treeline in the Sygera Mts., which was developed and used for paleoclimatic  
225 reconstructions in this region (Liang *et al.* 2009). The best estimate of physiological  $CT_{min}$   
226 was found by iteration and comparison between simulated and observed chronologies (1960 –  
227 2006).

228 Finally, a single simulated tree-ring width chronology was created for the Smith fir  
229 treeline in the Sygera Mts. based on daily climate data from Nyingchi meteorological stations  
230 (3,000 m a.s.l.). To account for the altitude differences between Nyingchi and the study sites,  
231 we extended the time series of daily temperatures at the treeline back to 1960 based on a  
232 linear regression of the Nyingchi data and our own micrometeorological data ( $r^2 \geq 0.89$ ,  
233 2007-2010).

234

## 235 **RESULTS**

### 236 **Micrometeorological conditions at the upper treeline**

237 The sampling sites at the upper treeline were cold and humid. In spite of a difference of 110  
238 m in elevation and different topographical aspects of the two treeline sites, they had fairly  
239 similar temperatures (Supporting Information [Fig. S1](#)). Annual temperatures (2007 – 2010)  
240 ranged from 0.1 to 0.9 °C, while growing-season (June-September) temperatures ranged from

241 6.4 to 7.1 °C (Fig. 1). On average, the annual precipitation was 951 mm, of which 62 % fell  
242 during the monsoon season (June to September). Snowfall mainly occurred from November  
243 to May. Due to snowmelt and increased precipitation, soil moisture content increased rapidly  
244 from the beginning of April and remained above 30 % from early May until November, and  
245 finally decreased to near 0 in late November and early December. The year 2008 was  
246 characterized by heavy spring snowfall and had the latest snowmelt and soil thawing during  
247 the four studied years (Fig. 1).

248

### 249 **Xylem formation**

250 The onset of xylem formation occurred from late May to early June and differed significantly  
251 among years ( $F = 15.73$ ,  $P < 0.001$ ). The onset of xylem formation was observed 4 - 9 days  
252 later in 2008 than in the other years, at both sites (Fig. 2 a). No difference was found in onset  
253 of xylogenesis between sites ( $F = 2.31$ ,  $P > 0.05$ ). Xylem formation ended between the  
254 beginning and the end of September and differed significantly among years ( $F = 10.42$ ,  $P <$   
255  $0.005$ ), and 1-2 weeks later in 2010 at site 1 (Fig. 2 b).

256 Overall, the duration of xylem formation lasted from 109 to 125 days (Fig. 2 c), with no  
257 significant differences detected between sites ( $F = 3.80$ ,  $P > 0.05$ ). Conversely, there were  
258 significant variations among years ( $F = 4.71$ ,  $P < 0.05$ ). From 2007 to 2009, the average  
259 period between the onset and ending of xylem formation was 107 days, whereas an average  
260 of 118 days was required to complete xylem formation in 2010.

261

### 262 **Relationship between climate and xylem formation**

263 Weekly cumulative xylem production was fit well by the Gompertz function ( $0.96 \leq r^2 \leq 0.98$ ;  
264 Supporting Information [Table S1](#) and [Fig. S2](#)). Intra-annual xylem cell production was  
265 significantly and positively correlated with daily minimum and mean air temperatures and  
266 GDD > 5 °C at both sites (Fig. 3 a, b). However, only minimum temperature was  
267 significantly correlated with growth indices after removing the growth trends (Fig.3 c, d). At  
268 site 1, positive correlations between growth indices and minimum temperature were found for  
269 time lags of 0 – 3 days ( $r = 0.34$ ,  $P < 0.05$ ), whereas the corresponding time lags were 7 – 10  
270 days at site 2 ( $r = 0.42$ ,  $P < 0.05$ ). No significant correlations were found between xylem cell  
271 production or growth index and precipitation from P0 to P10.

272

### 273 **CT<sub>min</sub>**

274 The critical minimum air temperature (CT<sub>min</sub>) at which there was a 0.5 probability that xylem  
275 formation was ongoing is shown in [Fig. 4](#) and [Table 1](#) for Site 1 (2007 – 2010) and Site 2  
276 (2007 – 2009). Based on direct observations of xylogenesis, the values respectively for  
277 minimum, mean, and maximum temperatures of 0.6, 4.0, and 9.3°C were estimated for the  
278 onset of xylogenesis, while the corresponding values for the ending of xylem differentiation  
279 were 0.7, 3.9, and 9.0 °C. There were no differences among critical temperatures for the onset  
280 and ending of xylogenesis (ANOVA,  $P > 0.05$ ), with values of  $0.7 \pm 0.4$ ,  $3.9 \pm 0.5$ , and  $9.1 \pm$   
281  $0.6$  °C for the minimum, mean and maximum temperatures, respectively. No significant  
282 differences were found between the two sites in terms of the estimated air temperature  
283 thresholds for the onset and ending of xylogenesis (ANOVA,  $P > 0.05$ ). The mean air  
284 temperature during the period of xylem formation based on weekly observations at both sites

285 was  $6.8 \pm 0.4^\circ\text{C}$ .

286

## 287 **Tree-ring modeling**

288 Applying the Vaganov-Shashkin (VS) model starting with an estimated  $\text{CT}_{\min} = 0.7^\circ\text{C}$ , the  
289 best estimate of physiological  $\text{CT}_{\min}$  was  $0.9^\circ\text{C}$  (Table 2, Fig. 5). The correlation between  
290 observations and predictions varied slightly at  $\text{CT}_{\min}$  of  $0.3\text{-}0.9^\circ\text{C}$ , while it decreased rapidly  
291 for values of  $\text{CT}_{\min} > 1^\circ\text{C}$  (Fig. 6). Overall, significant, positive correlations were found  
292 between the modeled and real chronologies when  $\text{CT}_{\min}$  varied within the range of  $0.7 \pm$   
293  $0.4^\circ\text{C}$  ( $r = 0.62$ ,  $P < 0.01$ ).

294

## 295 **DISCUSSION**

296 The importance of temperature for xylem formation during and after its onset has been  
297 demonstrated repeatedly (e.g., Oribe *et al.* 2001; Gričar *et al.* 2006; Rossi *et al.* 2008). These  
298 and other data suggest that air temperature, not soil temperature, directly limits xylem  
299 formation at high latitudes and altitudes (Rossi *et al.* 2007; Lenz *et al.* 2012; Lupi *et al.* 2012).  
300 Minimum temperature is assumed to be an important driver of tree species range limits  
301 (Körner 2003; Kollas *et al.* 2014), and so a critical minimum temperature ( $\text{CT}_{\min}$ ) with fairly  
302 narrow bounds should exist for the onset and ending of xylogenesis. However, long-term  
303 monitoring of xylem formation at natural treelines is limited, which has precluded assessment  
304 of  $\text{CT}_{\min}$  for xylem formation by direct observations.

305

## 306 **Effects of climate on xylem formation**

307 As predicted, minimum air temperature strongly limited xylem formation of Smith fir at the  
308 upper treeline on the south-eastern Tibetan Plateau. This finding accords with those from  
309 dendroclimatological analysis in the same study area (Liang *et al.* 2009) and xylem formation  
310 studies at high latitudes and altitudes (e.g., Rossi *et al.* 2008; Gruber *et al.* 2009). The  
311 importance of minimum air temperature may be related to the timing of cell differentiation,  
312 which perhaps occurs mainly during the night, when the temperature is lower (Hosoo *et al.*  
313 2002, Steppe *et al.* 2015). Controlled experiments in Hamburg, Germany also showed that  
314 night temperatures could directly influence xylem cell expansion of *Podocarpus latifolius*  
315 (Dünisch 2010). According to Körner (2003), cell doubling time, which is highest and fairly  
316 constant at temperatures of 10-25°C, approaches infinity at 1-2°C, suggesting a minimum  
317 temperature limit on cell division. The simulated ring-width chronologies produced by VS  
318 model of tree-ring formation also exhibit similar positive correlations with the minimum  
319 temperature during summer (Supporting Information Fig. S3,  $P < 0.01$ ).  $CT_{\min}$  is thus expected  
320 to limit xylogenesis of Smith fir at the treeline.

321

### 322 **Critical temperatures for xylem formation**

323 Despite the variance in timing and duration of xylem formation during our four years of  
324 observations, minimum, average, and maximum temperatures for the onset and ending of  
325 xylogenesis were narrowly bounded with average values of 0.7, 3.9, and 9.1 °C, respectively.  
326 Most studies to date have indicated that xylogenesis in conifers growing in cold climates can  
327 take place when the daily minimum temperatures  $\geq 4 - 5$  °C (e.g. Rossi *et al.* 2008; Boulouf  
328 Lugo *et al.* 2012). However, based on the presented 4-year observations of xylogenesis and

329 uninterrupted *in situ* micrometeorological measurements directly at the treeline, we found  
330 that the  $CT_{min}$  for xylogenesis in Smith fir is as low as 0.7 °C. In particular, based on this  
331  $CT_{min}$ , the modeled chronology produced by VS model is consistent with actual tree-ring  
332 data, suggesting that minimum temperature could be considered as a significant driver of  
333 xylem growth. Such a low  $CT_{min}$  may have evolved to provide sufficient time to complete  
334 xylogenesis at alpine treelines. The length of the growing season for stem growth diminishes  
335 with altitude and reaches a minimum at the alpine treeline. According to some authors (Rossi  
336 *et al.* 2008; Körner 2012), a tree can only survive when the length of growing seasons is at  
337 least 3 months and the mean growing season air temperature is 6.4.°C, since each of them  
338 critically constrains the growth and development of trees. At Smith fir treelines in  
339 south-eastern Tibet, the duration of xylem growth of 115 days provided by a  $CT_{min} < 1$  °C,  
340 together with a mean air temperature of 6.8 °C during the growing season extended by this  
341 low  $CT_{min}$  meets these prerequisites for tree growth and development.

342 The dates of snow melting and soil thawing also are thought to be critical for the onset of  
343 xylogenesis and could therefore determine the annual xylem production (Vaganov *et al.*  
344 1999). At our treeline sites, the onset of xylem growth occurred 7 - 30 days after soil thawing  
345 in spring, which coincided with the surpassing of  $CT_{min}$ . This temporal lag might be related to  
346 a strategy of avoiding the risk of freezing damage during early cell development.

347 The growth limitation hypothesis predicts that the absence of trees above the treeline is  
348 attributable to critical minimum temperature for growth (Körner 1998). Treeline trees often  
349 have slower growth rates and higher non-structural carbohydrate levels than trees at lower  
350 altitudes (e.g. Hoch & Körner 2003; Shi *et al.* 2008; Fajardo *et al.* 2012), suggesting a carbon

351 sink rather than carbon limitation. However, some authors have argued that tree populations  
352 with the highest non-structural carbohydrate concentrations may be the most carbon limited  
353 in terms of growth (Li *et al.* 2008; Wiley & Helleker 2012). Although our observations of  
354 xylogenesis cannot differentiate between carbon limitation and a carbon sink in Smith fir, the  
355 significant effect of a narrowly bounded  $CT_{min}$  on xylem cell production provides a  
356 physiological, rather than an ecological, mechanism for the growth limitation hypothesis.

357

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527 **Table 1** Mean  $\pm$  standard deviation of the threshold daily maximum (MaxT), mean (MeanT)

528 and minimum (MinT) temperatures for the onset and ending of xylogenesis.

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Site	Year	Onset of xylogenesis			Ending of xylogenesis		
		MinT(°C)	MeanT(°C)	MaxT(°C)	MinT(°C)	MeanT(°C)	MaxT(°C)
1	2007	0.6 $\pm$ 0.2	4.3 $\pm$ 0.1	9.7 $\pm$ 0.4	0.8 $\pm$ 0.3	3.9 $\pm$ 0.3	9.0 $\pm$ 0.4
	2008	0.8 $\pm$ 0.2	3.7 $\pm$ 0.3	8.9 $\pm$ 0.4	0.6 $\pm$ 0.2	3.5 $\pm$ 0.2	8.7 $\pm$ 0.1
	2009	0.8 $\pm$ 0.3	4.4 $\pm$ 0.1	9.8 $\pm$ 0.2	1.1 $\pm$ 0.1	4.7 $\pm$ 0.1	9.9 $\pm$ 0.1
	2010	0.3 $\pm$ 0.1	3.4 $\pm$ 0.2	9.1 $\pm$ 0.3	0.4 $\pm$ 0.1	2.9 $\pm$ 0.3	8.0 $\pm$ 0.2
2	2007	0.5 $\pm$ 0.3	4.3 $\pm$ 0.2	9.4 $\pm$ 0.5	0.9 $\pm$ 0.4	4.1 $\pm$ 0.5	9.3 $\pm$ 0.6
	2008	1.0 $\pm$ 0.3	3.9 $\pm$ 0.3	8.7 $\pm$ 0.4	0.7 $\pm$ 0.3	3.6 $\pm$ 0.2	8.7 $\pm$ 0.1
	2009	0.8 $\pm$ 0.3	4.4 $\pm$ 0.4	9.8 $\pm$ 0.2	1.1 $\pm$ 0.3	4.5 $\pm$ 0.4	9.8 $\pm$ 0.3

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532 **Table 2** The best estimate of parameters of VS model used in this study.

Model parameter	Description(unit)	Value
$CT_{\min}$	Minimum temperature for tree growth ( $^{\circ}C$ )	0.9
$T_{\text{opt1}}$	Lower end of range of optimal temperatures ( $^{\circ}C$ )	5.9
$T_{\text{opt2}}$	Upper end of range of optimal temperatures	9.3
$T_{\max}$	Maximum temperature for tree growth ( $^{\circ}C$ )	19.9
$W_{\min}$	Minimum soil moisture for tree growth (v/v)	0.06
$W_{\text{opt1}}$	Lower end of range of optimal soil moisture (v/v)	0.18
$W_{\text{opt2}}$	Upper end of range of optimal soil moisture (v/v)	0.22
$W_{\max}$	Maximum soil moisture for tree growth (v/v)	0.50
$T_{\text{beg}}$	Temperature sum for initiation of growth ( $^{\circ}C$ )	30
$D_{\text{root}}$	Depth of root system (mm)	50
$P_{\max}$	Maximum daily precipitation for saturated soil (mm)	20
$K_1$	Fraction of precipitation penetrating soil (dimensionless)	0.86
$K_2$	First coefficient for calculation of transpiration (mm/day)	0.12
$K_3$	Second coefficient for calculation of transpiration ( $1/^{\circ}C$ )	0.175
$K_r$	Coefficient for water infiltration from soil (dimensionless)	0.006

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535 **Figure legends:**

536 **Figure 1.** Micrometeorological conditions (2007 – 2010) at the upper treeline in the Sygera  
537 Mts., SE Tibetan Plateau, showing (a) daily mean air temperature and daily total precipitation,  
538 (b) daily soil temperature (at depths of 10, 20 and 40 cm) and snow depth, and (c) daily mean  
539 soil volumetric moisture contents (at depths of 10, 20 and 40 cm).

540

541 **Figure 2.** Onset (a), ending (b) and duration (c) of xylem formation of Smith fir (*Abies*  
542 *georgei* var. *smithii*) based on weekly xylogenesis observations at Site 1 (4360 m a.s.l.) (grey  
543 columns) and Site 2 (4250 m a.s.l.) (white columns). Error bars indicate standard deviation  
544 among trees.

545

546 **Figure 3.** Lagged (0 - 10 days) Pearson correlation coefficients between xylem cell  
547 production (a, b), growth index (c, d) and corresponding climatic variables. P0 represents the  
548 weekly climatic mean for the exact period between two sampling dates. P1 to P10 represent  
549 the weekly means lagged 1 - 10 days before the sampling date. Dotted horizontal lines show  
550 the 95% confidence limits. *Abbreviations:* MaxT = maximum temperature, MeanT= mean  
551 temperature, MinT= minimum temperature, P = precipitation and GGD = growing degree  
552 days above 5°C.

553

554 **Figure 4.** Critical minimum (black dots), mean (white dots) and maximum (grey dots) air  
555 temperatures at Sites 1 and 2, corresponding with the 0.5-probability of the onset and ending

556 of xylem formation according to xylogenesis observations in Smith fir. Error bars indicate the  
557 standard deviation among trees.

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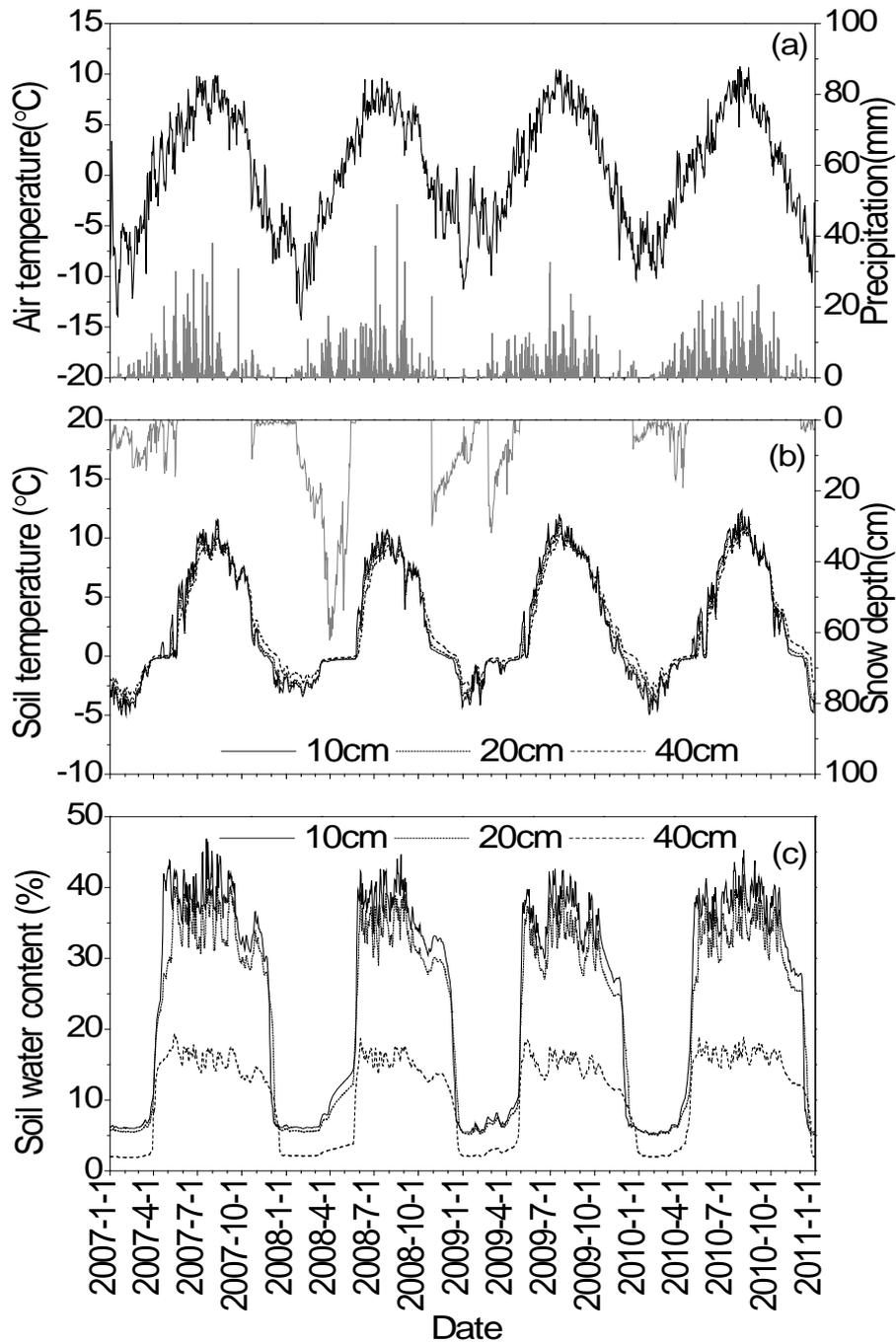
559 **Figure 5.** Observed (solid line) and simulated (dashed line) tree-ring width indices at Smith  
560 fir treeline in the Sygera Mts. on the south-eastern Tibetan Plateau, 1960 - 2006.

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562 **Figure 6.** Pearson correlation coefficients between the observed and calculated values of tree  
563 ring width when the parameter of  $CT_{\min}$  varied.

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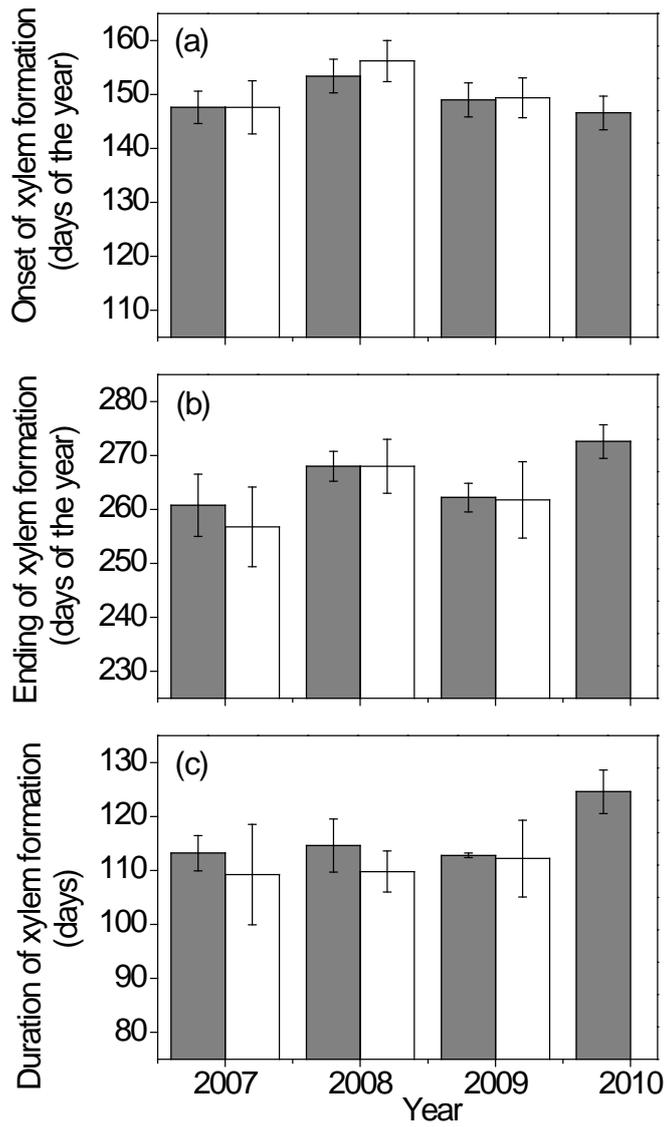


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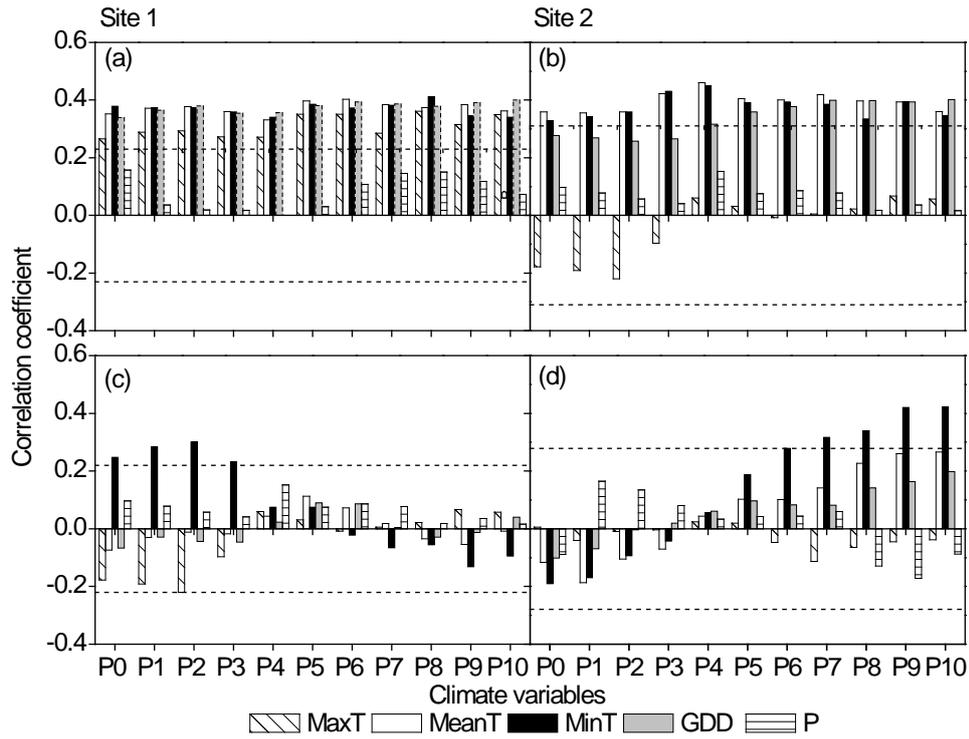
Figure 1

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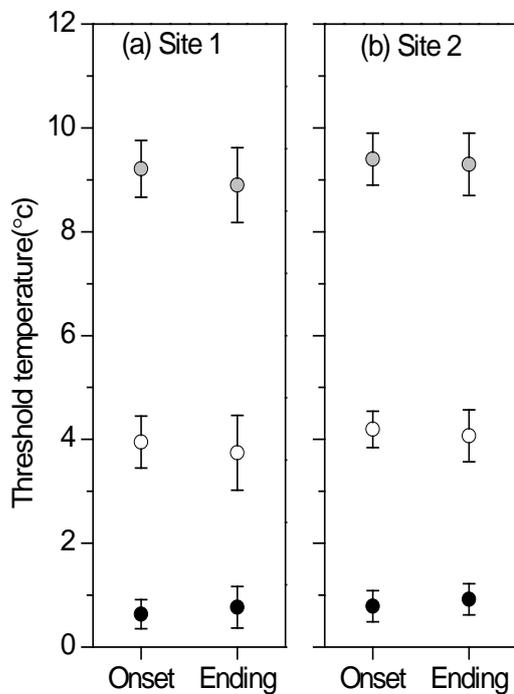
570 Figure 2



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573 Figure 3

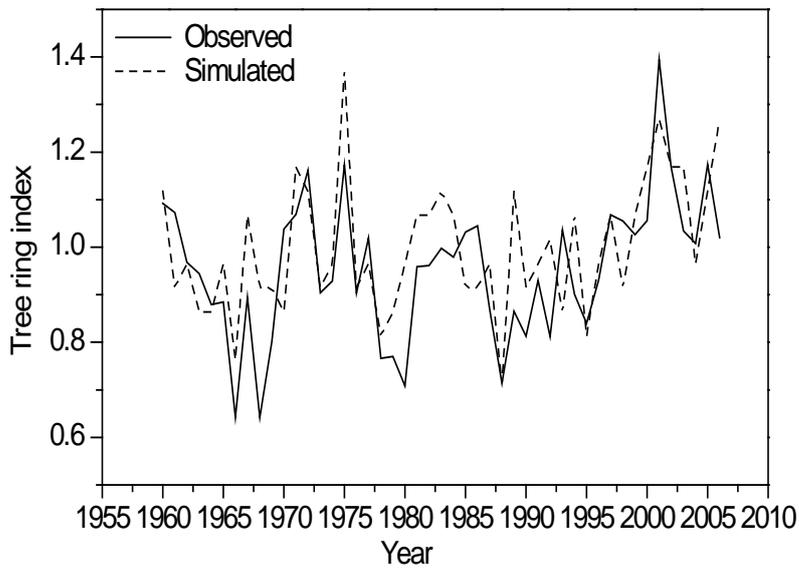


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575 Figure 4

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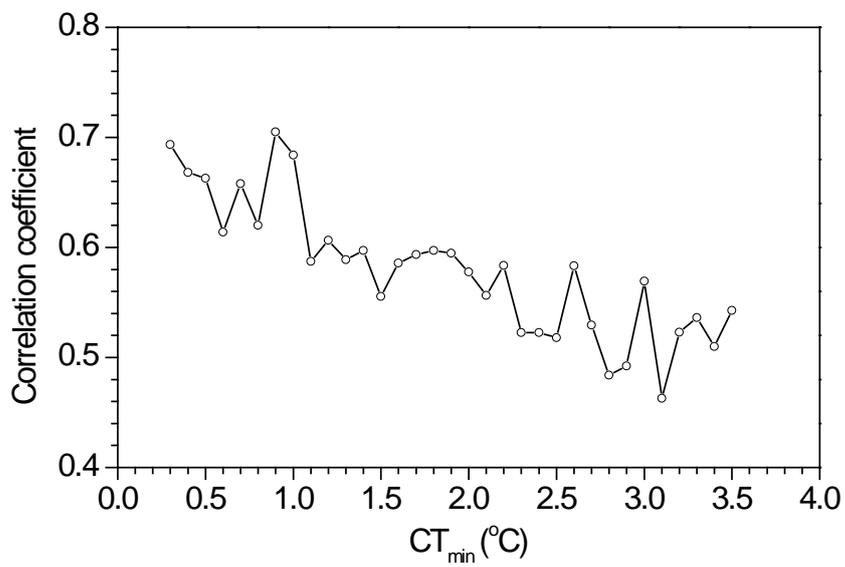
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582 Figure 6

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584 **SUPPORTING INFORMATION**

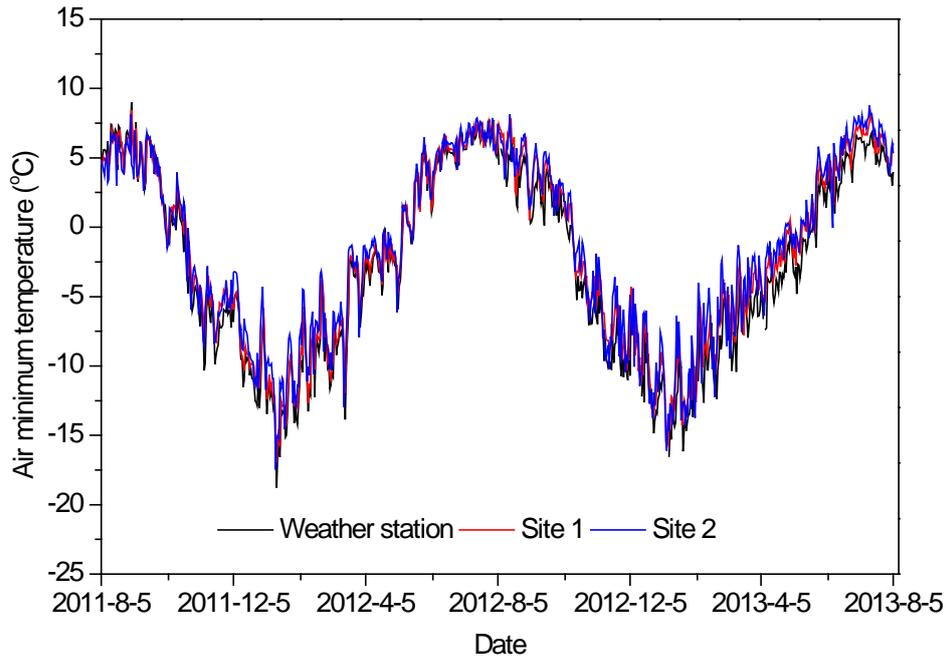
585 **Table S1** Parameters of the Gompertz function ( $A$ ,  $\beta$ ,  $K$ ),  $R^2$  and day of the inflection

586 point (tp) for Smith fir growing at two treeline sites, 2007 – 2010.

	Year	A	$\beta$	$K(10^{-2})$	tp	$R^2$
Site 1	2007	23.95	5.48	3.25	170.59	0.98
	2008	22.97	5.97	3.32	179.32	0.96
	2009	25.68	7.07	4.08	174.50	0.96
	2010	21.08	5.67	3.25	176.67	0.99
Site 2	2007	24.79	5.03	2.85	176.56	0.98
	2008	21.22	6.97	3.87	178.13	0.98
	2009	23.62	5.70	3.02	188.53	0.98

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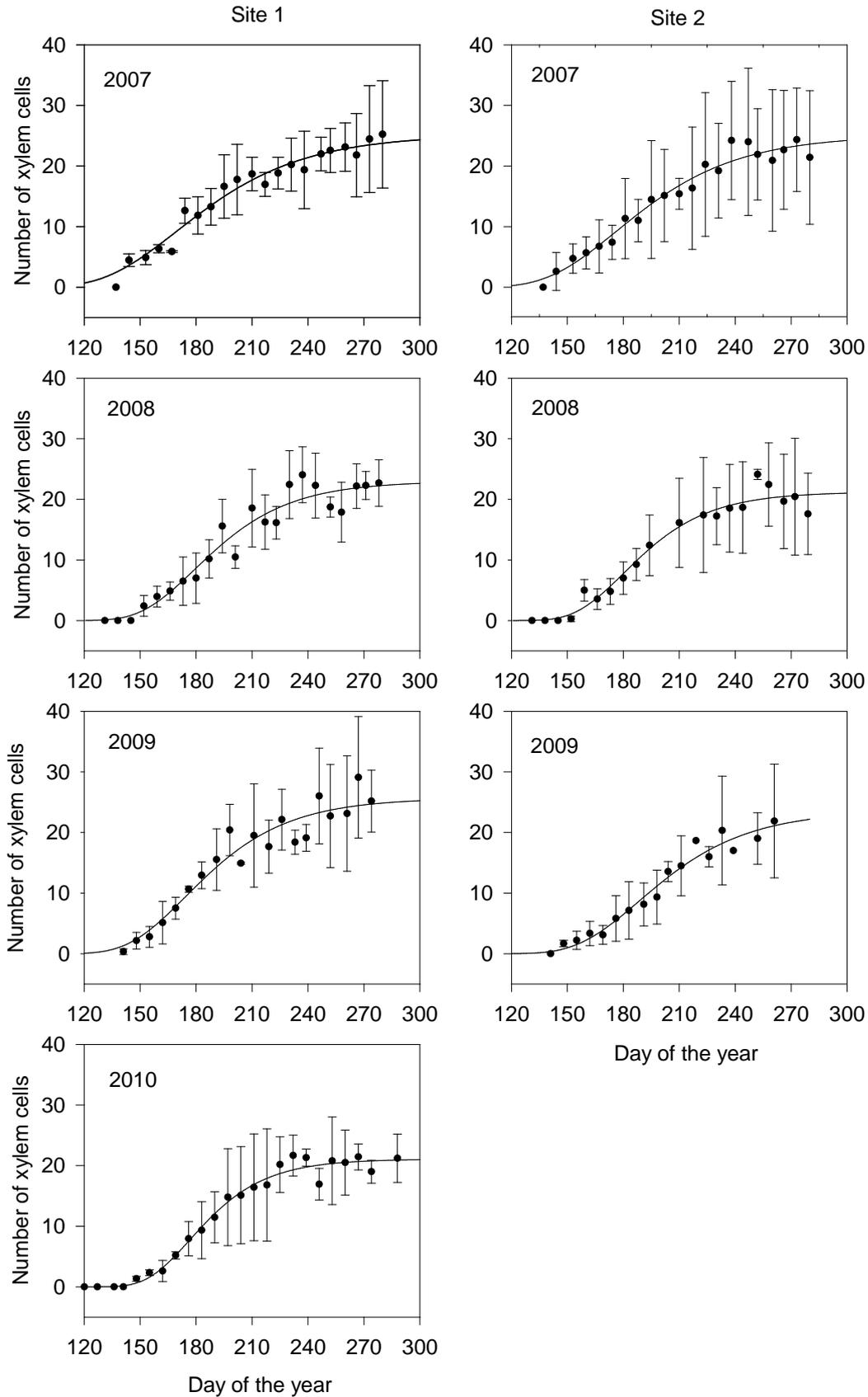
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590 **Figure S1.** Minimum air temperatures recorded by the automatic weather station (black line)

591 and temperature data logger at Site 1 (red line) and Site 2 (blue line) from August 5, 2011 to

592 August 5, 2013.

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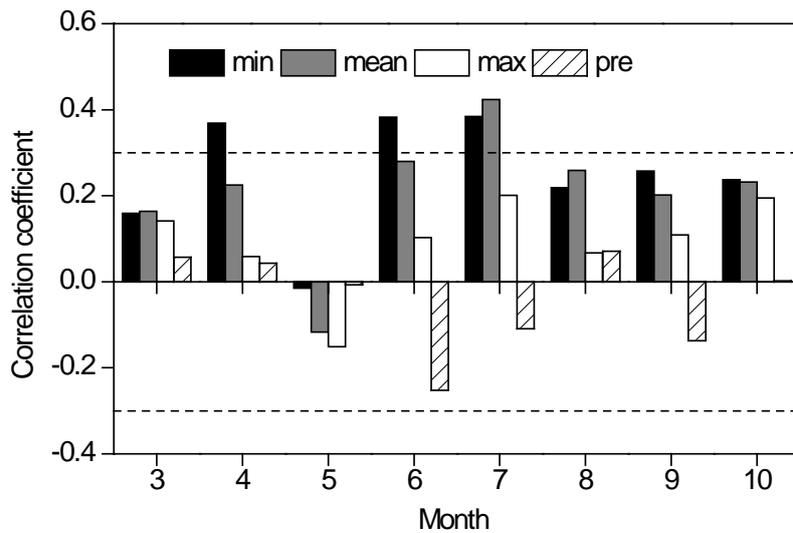
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**Figure S2.** Dynamics of xylem growth (including enlarging, wall thickening and mature xylem cells) at two Smith fir treelines as modeled using a Gompertz function.

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601 **Figure S3.** Correlations between the simulated tree ring chronology and monthly temperature  
602 and precipitation at Smith fir treeline in the Sygera Mountains on the south-eastern Tibetan  
603 Plateau. Dotted horizontal lines show the 95% confidence limits. *Abbreviations:* MaxT =  
604 maximum temperature, MeanT= mean temperature, MinT= minimum temperature and P =  
605 precipitation.

606