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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ABSTRACT

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

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

INTRODUCTION

43

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

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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.

5

109   At each site, five dominant trees were selected in April 2007. These trees had a mean

110   age of 201 ± 24 and 117 ± 14 years, and mean diameters at 1.3-m above ground of 34 ± 4 and

111   44 ± 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°39′ N, 94°42′E, 4390 m a.s.l.) at a linear distance

119   of ≈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

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131   with successive immersions in a graded series of ethanol and *d*-limonene, then embedded in

132   paraffin. Transverse sections (9-12 μm 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 safranine (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 ith phase of the jth sample was calculated as:

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

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                                    $$y = A\exp[- e^{(\beta - \kappa t)}]$$ (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 β 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 Origon 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 χ^2, Wald's χ^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

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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

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**

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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$_{min}$**

274   The critical minimum air temperature (CT$_{min}$) 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 ± 0.4, 3.9 ± 0.5, and 9.1 ±

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

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285   was 6.8 ± 0.4°C.

286

287   **Tree-ring modeling**

288   Applying the Vaganov-Shashkin (VS) model starting with an estimated $CT_{min} = 0.7$ °C, the

289   best estimate of physiological CT_{min} was 0.9 °C (Table 2, Fig. 5). The correlation between

290   observations and predictions varied slightly at CT_{min} of 0.3-0.9°C, while it descreased rapidly

291   for values of $CT_{min} > 1$°C (Fig. 6). Overall, significant, positive correlations were found

292   between the modeled and real chronologies when CT_{min} varied within the range of $0.7 \pm$

293   0.4°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 (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 CT_{min} for xylem formation by direct observations.

305

306   **Effects of climate on xylem formation**

14

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

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

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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 & Helloker 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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366

REFERENCES

368 Anchukaitis K. J., Evans M. N., Kaplan A. , Vaganov E. A., Hughes M. K., Grissino-Mayer

369 H. D. & Cane M. A. (2006) Forward modeling of regional scale tree-ring patterns in the

370 south-eastern United States and the recent influence of summer drought. *Geophysical*

371 *Research Letters* **33,** L04705.

372 Boulouf Lugo J., Deslauriers A. & Rossi S. (2012) Duration of xylogenesis in black spruce

373   lengthened between 1950 and 2010. *Annals of Botany* **110,** 1099-1108.

374   Bräuning, A., & Mantwill B. (2004) Summer temperature and summer monsoon history on

375   the Tibetan plateau during the last 400 years recorded by tree rings. *Geophysical*

376   *Research Letters* **31,** L24205.

377   Camarero, J. J., Olano J. M. & Parras A. (2010) Plastic bimodal xylogenesis in conifers from

378   continental Mediterranean climates. *New Phytologist* **185,** 471-480.

379   Chuine, I. &Beaubien E. G. (2001) Phenology is a major determinant of tree species range.

380   *Ecology Letters* **4,** 500-510.

381   Cui, H., Liu H. & Dai J. (2005) Study on mountain ecology and alpine timberline. Beijing,

382   Science Press.

383   Dünisch, O. (2010) Low night temperatures cause reduced tracheid expansion in *Podocarpus*

384   *latifolius*. *IAWA Journal* **31,** 245-255.

385   Deslauriers, A. &   Morin H. (2005) Intra-annual tracheid production in balsam fir stems and

386   the effect of meteorological variables. *Trees - Structure and Function* **19,** 402-408.

387   Deslauriers, A., Morin, H. & Begin, Y. (2003) Cellular phenology of annual ring formation

388   of *Abies balsamea* in the Quebec boreal forest (Canada). *Canadian Journal of Forest*

389   *Research* **33,** 190-200.

390   Ettinger, A. K.,   Ford K. R. & HilleRisLambers J. (2011) Climate determines upper, but not

391   lower, altitudinal range limits of Pacific Northwest conifers. *Ecology* **92,** 1323-1331.

392   Evans, M. N., Reichert B. K., Kaplan A., Anchukaitis K. J., Vaganov E. A., Hughes M. K. &

393   Cane M. A. (2006) A forward modeling approach to paleoclimatic interpretation of

394   tree-ring data. *Journal of Geophysical Research-Biogeosciences* **111,** G03008

395   Fajardo, A.,Piper F. I. & Hoch G. (2013) Similar variation in carbon storage between

396   deciduous and evergreen treeline species across elevational gradients. *Annals of Botany*

397   **112,** 623-631.

398   Frank, D. & Esper J. (2005) Characterization and climate response patterns of a

399   high-elevation, multi-species tree-ring network in the European Alps.

400   *Dendrochronologia* **22,** 107-121.

401   Fritts, H. (1976) Tree rings and climate. Academic Press, New York.

402   George, S. S., Meko D. M. & Evans M. N. (2008) Regional tree growth and inferred summer

403   climate in the Winnipeg River basin, Canada, since AD 1783. *Quaternary Research* **70,**

404   158-172.

405   Gou, X., Zhou F., Zhang Y., Chen Q. & Zhang J. (2013) Forward modeling analysis of

406   regional scale tree-ring patterns around the northeastern Tibetan Plateau, Northwest

407   China. *Biogeosciences Discuss*. **2013,** 9969-9988.

408   Grace, J., Berninger F. & Nagy L. (2002) Impacts of climate change on the tree line. *Annals*

409   *of Botany* **90,** 537-544.

410   Gričar, J., Zupančič, M., Čufar, K., Koch, G., Schmitt, U. & Oven, P. (2006) Effect of local

411   heating and cooling on cambial activity and cell differentiation in the stem of Norway

412   spruce (*Picea abies*). *Annals of Botany* **97,** 943-951.

413   Gruber, A., Baumgartner, D., Zimmermann, J. & Oberhuber, W. (2009) Temporal dynamic

414   of wood formation in *Pinus cembra* along the alpine treeline ecotone and the effect of

415   climate variables. *Trees - Structure and Function* **23,** 623-635.

416   Hoch, G. & Körner, C. (2003) The carbon charging of pines at the climatic treeline: a global

417   comparison. *Oecologia* **135,** 10-21.

418   Holtmeier, F. K. (2003) Mountain timberlines: ecology, patchiness and dynamics. Dordrecht,

419   Kluwe.

420   Hosoo, Y., Yoshida, M., Imai, T. & Okuyama, T. (2002) Diurnal difference in the amount of

421   immunogold-labeled glucomannans detected with field emission scanning electron

422   microscopy at the innermost surface of developing secondary walls of differentiating

423   conifer tracheids. *Planta* **215,** 1006-1012.

424   Huang, J.-G., Deslauriers A. & Rossi S. (2014) Xylem formation can be modeled statistically

425   as a function of primary growth and cambium activity. *New Phytologist* **203,** 831-841.

426   Körner, C. (1998) A re-assessment of high elevation treeline positions and their explanation.

427   *Oecologia* **115,** 445-459.

428   Körner, C. (2003) Alpine plant life: functional plant ecology of high mountain ecosystems,

429   2nd edn. Springer, Berlin Heidelberg New York.

430   Körner, C. (2012) Alpine treelines: functional plant ecology of the global high elevation tree

431   limits. Springer, Berlin Heidelberg New York.

432   Körner, C. &Paulsen J. (2004) A world-wide study of high altitude treeline temperatures.

433   *Journal of Biogeography* **31,** 713-732.

434   Kollas, C., Körner, C. & Randin, C.F. (2014). Spring frost and growing season length

435   co-control the cold range limits of broad-leaved trees. *Journal of Biogeography* **41,**

436   773-783.

437   Lenz, A., Hoch, G. & Körner, C. (2012) Early season temperature controls cambial activity

438   and total tree ring width at the alpine treeline. *Plant Ecology & Diversity* **6,** 365-375.

439   Li, M., Xiao, W., Wang, S., Cheng, G., Cherubini, P., Cai, X., Liu, X., Wang, X. & Zhu, W.

440   (2008) Mobile carbohydrates in Himalayan treeline trees I. Evidence for carbon gain

441   limitation but not for growth limitation. *Tree Physiology* **28,** 1287-1296.

442   Li, X., Liang, E., Gričar, J., Prislan, P., Rossi, S. & Čufar, K. (2013) Age dependence of

443   xylogenesis and its climatic sensitivity in Smith fir on the south-eastern Tibetan Plateau.

444   *Tree Physiology* **33,** 48-56.

445   Liang, E.,Wang Y., Eckstein D. & Luo T. (2011) Little change in the fir tree-line position on

446   the south-eastern Tibetan Plateau after 200 years of warming. *New Phytologist* **190,**

447   760-769.

448   Liang, E., Shao, X. & Xu, Y. (2009) Tree-ring evidence of recent abnormal warming on the

449   southeast Tibetan Plateau. *Theoretical and Applied Climatology* **98,** 9-18.

450   Lupi, C., Morin, H., Deslauriers, A. & Rossi, S. (2012) Xylogenesis in black spruce: does soil

451   temperature matter? *Tree Physiology* **32,** 74-82.

452   Makinen, H., Nojd P. & Mielikainen K. (2000) Climatic signal in annual growth variation of

453   Norway spruce (*Picea abies*) along a transect from central Finland to the Arctic

454   timberline. *Canadian Journal of Forest Research* **30,** 769-777.

455   Miehe, G., Miehe, S., Vogel, J., Co, S. & Duo, L. (2007) Highest treeline in the northern

456   hemisphere found in southern Tibet. *Mountain Research and Development* **27,** 169-173.

457   Moser, L., Fonti, P., Büntgen, U., Franzen, J., Esper, J., Luterbacher, J. & Frank, D. (2010)

458   Timing and duration of European larch growing season along altitudinal gradients in the

459   Swiss Alps. *Tree Physiology* **30,** 225-233.

460   Oberhuber, W. (2004) Influence of climate on radial growth of *Pinus cembra* within the

461   alpine timberline ecotone. *Tree Physiology* **24,** 291-301.

462   Opgenoorth, L., Vendramin, G.G., Mao, K.S., Miehe, G., Miehe, S., Liepelt, S., Liu, J.Q. &

463   Ziegenhagen, B. (2010) Tree endurance on the Tibetan Plateau marks the world's

464   highest known tree line of the Last Glacial Maximum. *New Phytologist* **185,** 332-342.

465   Oribe, Y., Funada, R., Shibagaki, M. & Kubo, T. (2001) Cambial reactivation in locally

466   heated stems of the evergreen conifer *Abies sachalinensis* (Schmidt) Masters. *Planta*

467   **212,** 684-691.

468   Rathgeber, C.B.K., Rossi, S. & Bontemps, J.-D. (2011) Cambial activity related to tree size in

469   a mature silver-fir plantation. *Annals of Botany* **108,** 429-438.

470   Rossi, S., Deslauriers, A., Anfodillo, T. & Carraro, V. (2007) Evidence of threshold

471   temperatures for xylogenesis in conifers at high altitudes. *Oecologia* **152,** 1-12.

472   Rossi, S., Deslauriers, A., Griçar, J., Seo, J.-W., Rathgeber, C. B. K., Anfodillo, T., Morin, H.,

473   Levanic, T., Oven, P. & Jalkanen, R. (2008) Critical temperatures for xylogenesis in

474   conifers of cold climates. *Global Ecology and Biogeography* **17,** 696-707.

475   Rossi, S., Morin, H., Deslauriers, A. & Plourde, P.-Y. (2011) Predicting xylem phenology in

476   black spruce under climate warming. *Global Change Biology* **17,** 614-625.

477   Seo, J.-W., Eckstein, D., Jalkanen, R., Rickebusch, S. & Schmitt, U. (2008) Estimating the

478   onset of cambial activity in Scots pine in northern Finland by means of the heat-sum

479   approach. *Tree Physiology* **28,** 105-112.

480   Shi, J., Liu Y.,Vaganov E.., Li J. & Cai Q. (2008a). Statistical and process-based modeling

481   analyses of tree growth response to climate in semi-arid area of north central China: A

482   case study of *Pinus tabulaeformis*. *Journal of Geophysical Research-Biogeosciences*

483   **113,** G01026**.**

484   Shi, P., Körner, C. & Hoch, G. (2008b) A test of the growth-limitation theory for alpine tree

485   line formation in evergreen and deciduous taxa of the eastern Himalayas. *Functional*

486   *Ecology* **22,** 213-220.

487   Smith, W. K., Germino M. J., Hancock T. E. &Johnson D. M. (2003) Another perspective on

488   altitudinal limits of alpine timberlines. *Tree Physiology* **23,** 1101-1112.

489   Steppe K., Sterck F. & Deslauriers A. (2015) Diel growth dynamics in tree stems: linking

490   anatomy and ecophysiology. *Trends in Plant Science* **20:** 335-343

491   Stevens, G.C. & Fox, J.F. (1991) The Causes of Treeline. *Annual Review of Ecology and*

492   *Systematics* **22,** 177-191.

493   Susiluoto, S., Hilasvuori, E. & Berninger, F. (2010) Testing the growth limitation hypothesis

494   for subarctic Scots pine. *Journal of Ecology* **98,** 1186-1195.

495   Tranquillini, W. (1979) Physiological ecology of the alpine timberline. Springer, Berlin

496   Heidelberg New York.

497   Vaganov, E.A., Hughes, M.K., Kirdyanov, A.V., Schweingruber, F.H. & Silkin, P.P. (1999)

498   Influence of snowfall and melt timing on tree growth in subarctic Eurasia. *Nature* **400,**

499   149-151.

500   Vaganov, E. A., M. K. Hughes, and A. V. Shashkin. (2006) Introduction and factors

501   influencing the seasonal growth of trees. Springer, New York.

502   Wang, Y., Li, X., Dawadi, B., Eckstein, D. & Liang, E. (2013) Phenological variation in

503   height growth and needle unfolding of Smith fir along an altitudinal gradient on the

504   southeastern Tibetan Plateau. *Trees - Structure and Function* **27,** 401-407.

505   Wiley, E. & Helliker B. (2012) A re-evaluation of carbon storage in trees lends greater

506   support for carbon limitation to growth. *New Phytologist* **195,** 285-289.

507   Zhai, L.,Bergeron Y., Huang J. & Berninger F. (2012) Variation in intra-annual wood

508   formation, and foliage and shoot development of three major Canadian boreal tree

509   species. *American Journal of Botany* **99,** 827-837.

510   Zhang, Y. X., Shao X. M., Xu Y. & Wilmking M. (2011) Process-based modeling analyses of

511   Sabina przewalskii growth response to climate factors around the northeastern Qaidam

512   Basin. *Chinese Science Bulletin* **56,** 1518-1525.

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527 **Table 1** Mean±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±0.2	4.3±0.1	9.7±0.4	0.8±0.3	3.9±0.3	9.0±0.4
	2008	0.8±0.2	3.7±0.3	8.9±0.4	0.6±0.2	3.5±0.2	8.7±0.1
	2009	0.8±0.3	4.4±0.1	9.8±0.2	1.1±0.1	4.7±0.1	9.9±0.1
	2010	0.3±0.1	3.4±0.2	9.1±0.3	0.4±0.1	2.9±0.3	8.0±0.2
2	2007	0.5±0.3	4.3±0.2	9.4±0.5	0.9±0.4	4.1±0.5	9.3±0.6
	2008	1.0±0.3	3.9±0.3	8.7±0.4	0.7±0.3	3.6±0.2	8.7±0.1
	2009	0.8±0.3	4.4±0.4	9.8±0.2	1.1±0.3	4.5±0.4	9.8±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 (°C)	0.9
T_{opt1}	Lower end of range of optimal temperatures (°C)	5.9
T_{opt2}	Upper end of range of optimal temperatures	9.3
T_{max}	Maximum temperature for tree growth (°C)	19.9
W_{min}	Minimum soil moisture for tree growth (v/v)	0.06
W_{opt1}	Lower end of range of optimal soil moisture (v/v)	0.18
W_{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_{beg}	Temperature sum for initiation of growth (°C)	30
D_{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/ °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).

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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.

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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.

558

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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567   Figure 1

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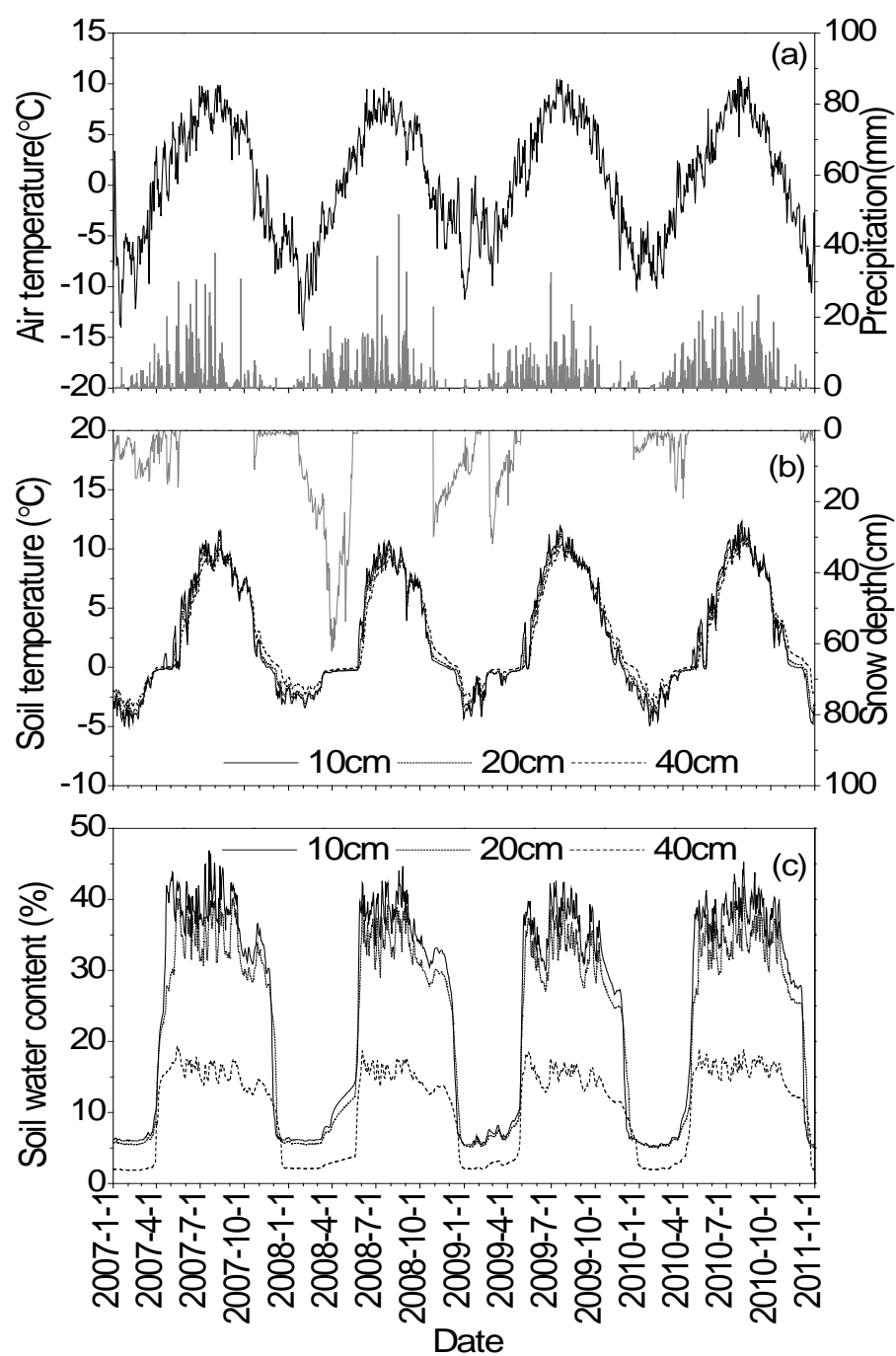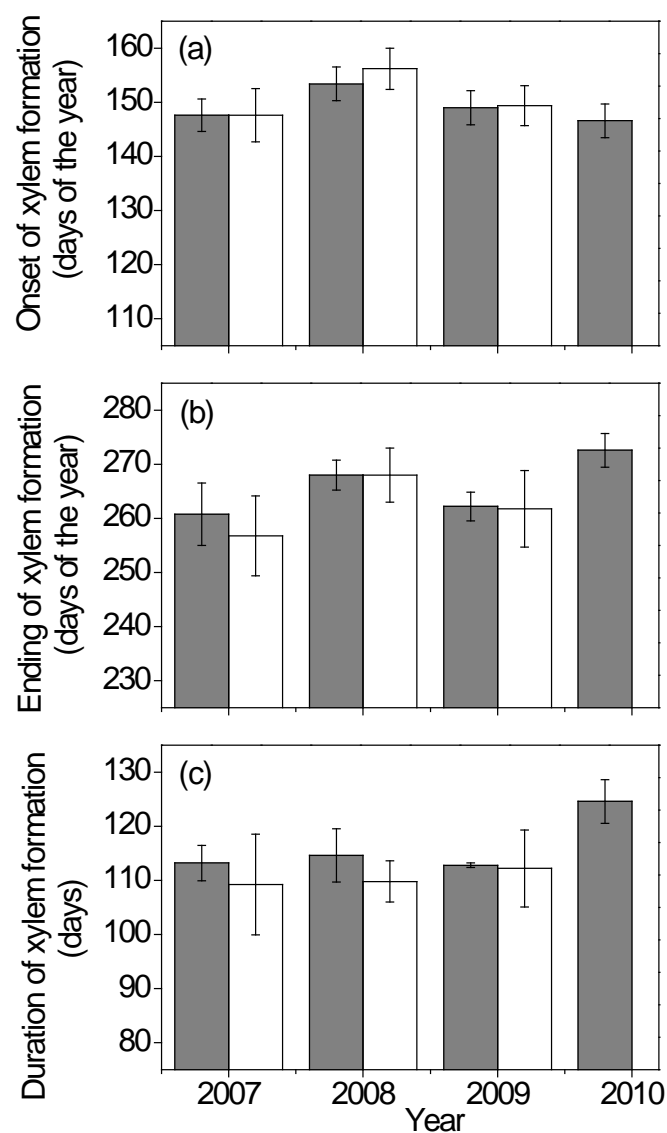
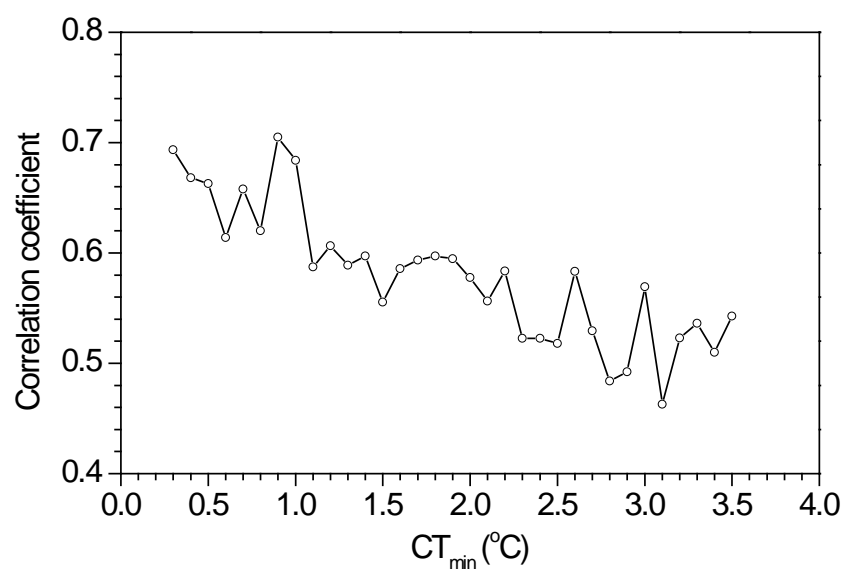570   Figure 2
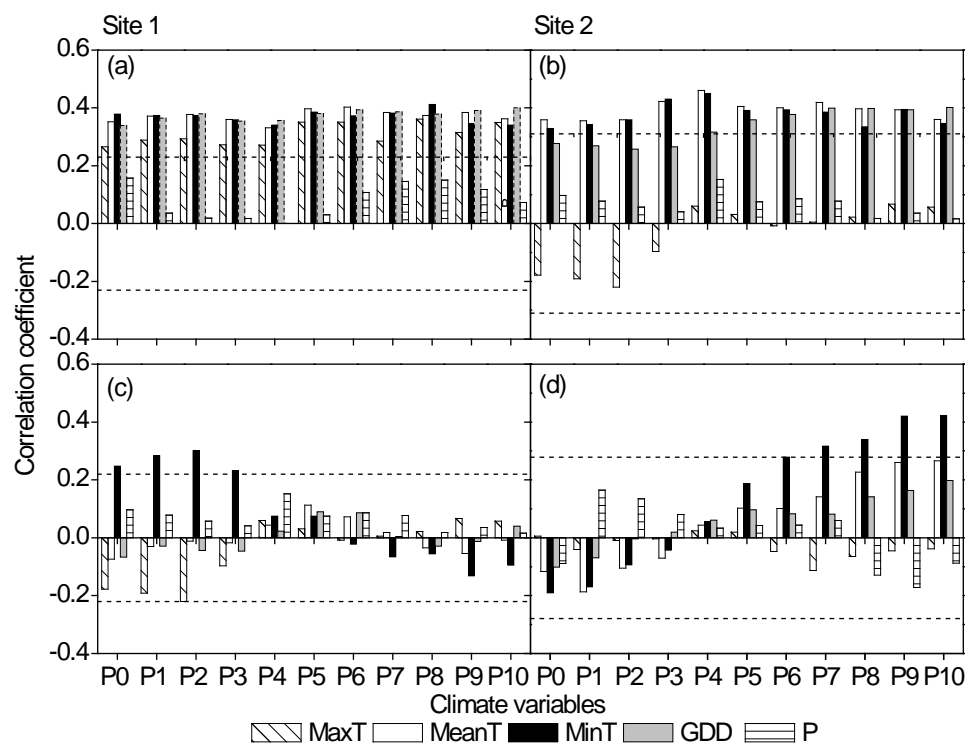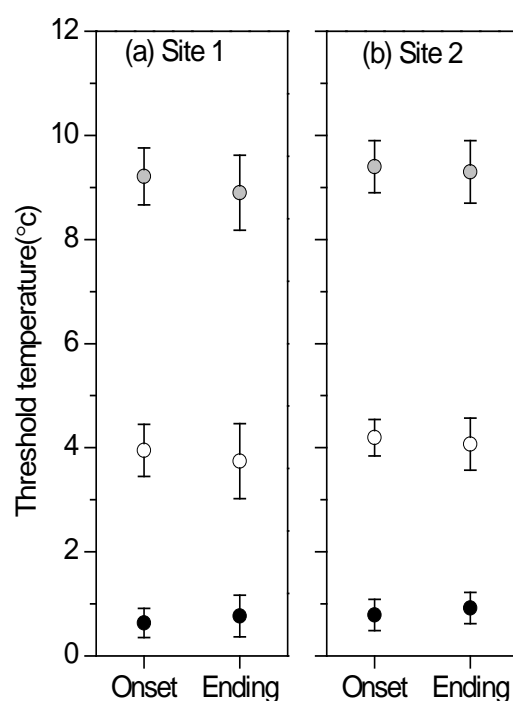
Figure 3

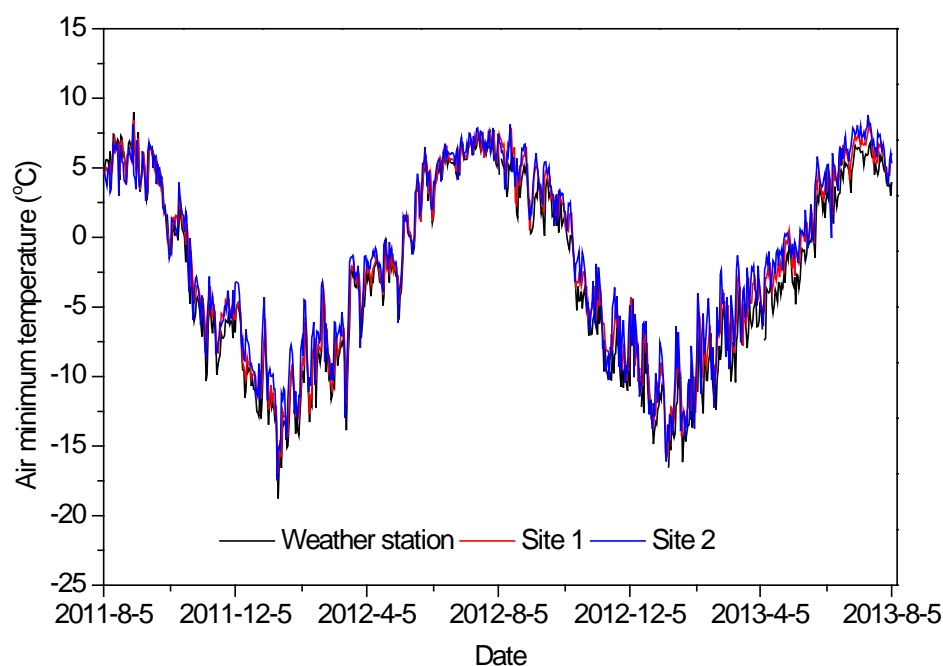Figure 4

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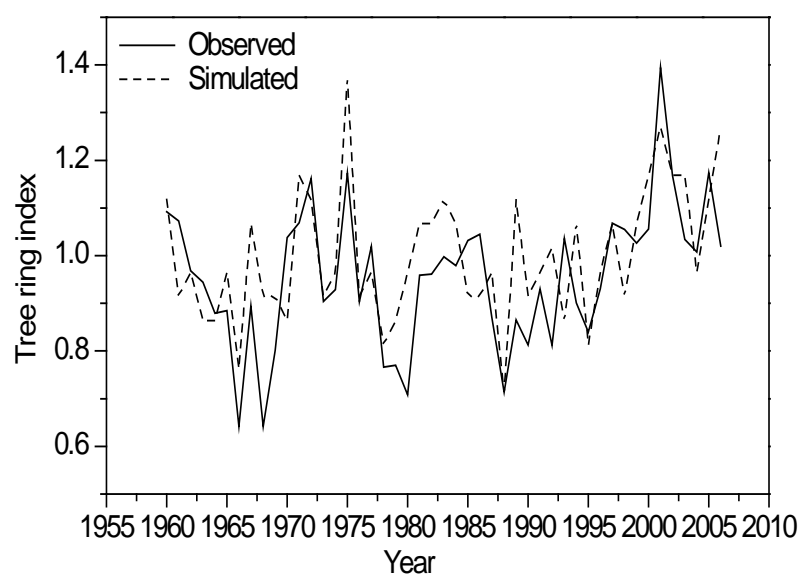
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579   Figure 5

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582   Figure 6

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584 **SUPPORTING INFORMATION**

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

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

	Year	A	ß	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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Figure S1. Minimum air temperatures recorded by the automatic weather station (black line) and temperature data logger at Site 1 (red line) and Site 2 (blue line) from August 5, 2011 to August 5, 2013.
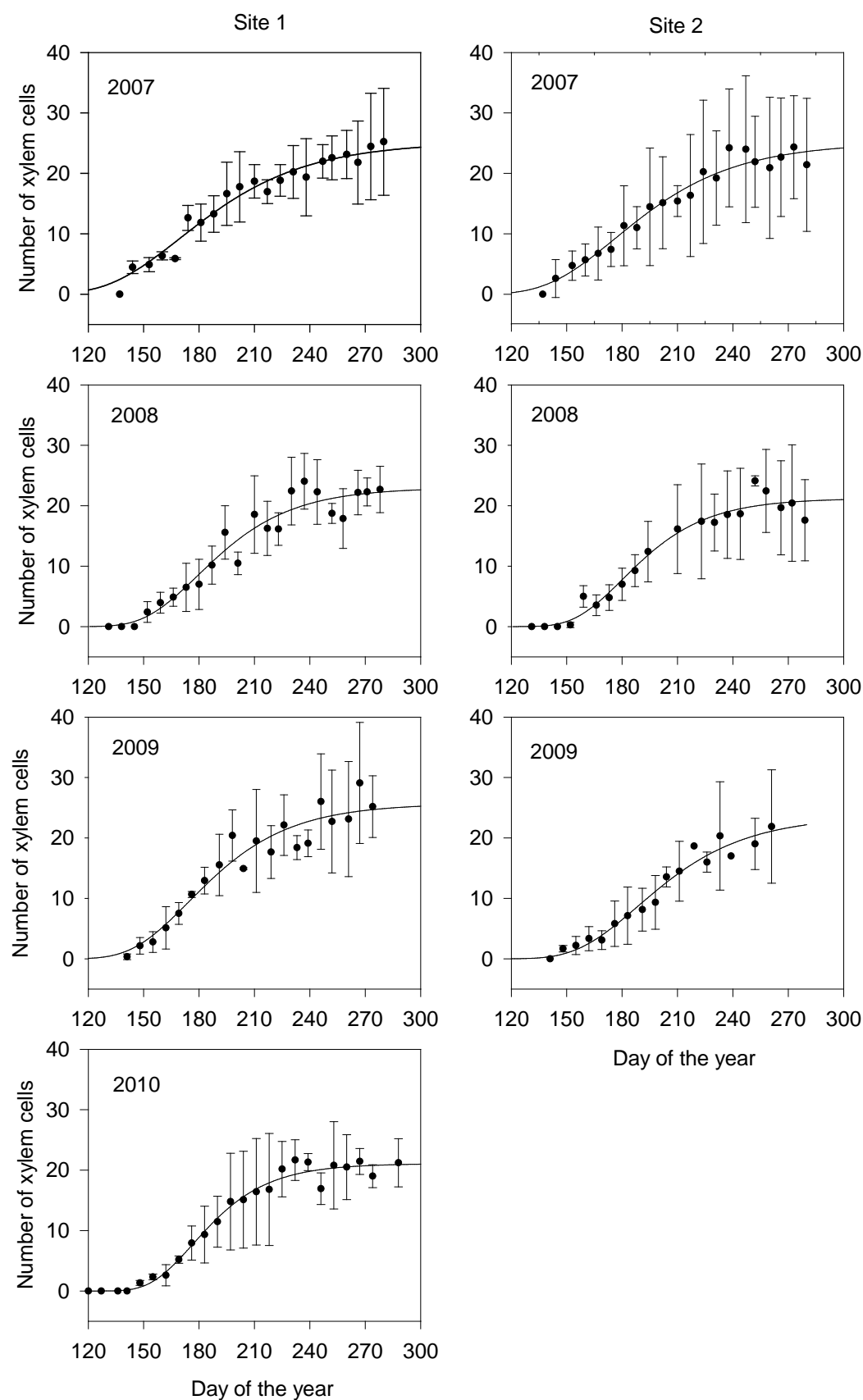
Figure S2. Dynamics of xylem growth (including enlarging, wall thickening and mature xylem cells) at two Smith fir treelines as modeled using a Gompertz function.

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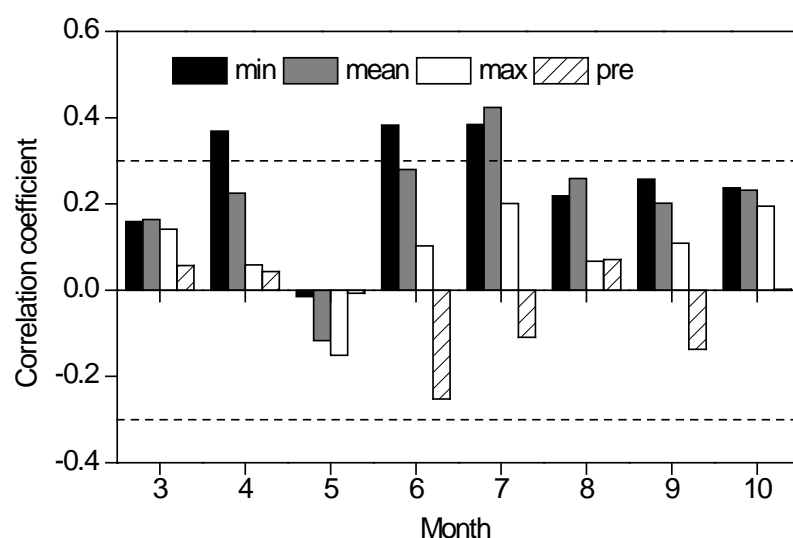
601 **Figure S3**. Correlations between the simulated tree ring chronology and monthly temperature
602 and precipitation at Smith fir treeline in the Sygera Mountains on the south-eastern Tibetan
603 Plateau. Dotted horizontal lines show the 95% confidence limits. *Abbreviations*: MaxT =
604 maximum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

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