

1 **PATTERN OF XYLEM PHENOLOGY IN CONIFERS OF COLD ECOSYSTEMS AT**
2 **THE NORTHERN HEMISPHERE**

3 **Running head:** xylem phenology across cold forest ecosystems

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37 meristem, secondary wall formation

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ABSTRACT

41 The interaction between xylem phenology and climate assesses forest growth and productivity
42 and carbon storage across biomes under changing environmental conditions. We tested the
43 hypothesis that patterns of wood formation are maintained unaltered despite the temperature
44 changes across cold ecosystems. Wood microcores were collected weekly or biweekly
45 throughout the growing season for periods varying between 1-13 years during 1998-2014 and
46 cut in transverse sections for assessing the onset and ending of the phases of xylem
47 differentiation. The dataset represented 1321 trees belonging to 10 conifer species from 39
48 sites in the Northern hemisphere and covering an interval of mean annual temperature
49 exceeding 14 K. The phenological events and mean annual temperature of the sites were
50 related linearly, with spring and autumnal events being separated by constant intervals across
51 the range of temperature analysed. At increasing temperature, first enlarging, wall-thickening
52 and mature tracheids appeared earlier, and last enlarging and wall-thickening tracheids
53 occurred later. Overall, the period of wood formation lengthened linearly with the mean
54 annual temperature, from 83.7 days at -2 °C to 178.1 days at 12 °C, at a rate of 6.5 days °C⁻¹.
55 April-May temperatures produced the best models predicting the dates of wood formation.
56 Our findings demonstrated the uniformity of the process of wood formation and the
57 importance of the environmental conditions occurring at the time of growth resumption.
58 Under warming scenarios, the period of wood formation might lengthen synchronously in the
59 cold biomes of the Northern hemisphere.

60

61

INTRODUCTION

62 Perennial plants synchronize their physiological processes with local climate according to a
63 trade-off between maximization of resource acquisition and damage avoidance due to harsh
64 environmental conditions (Chuine, 2010, Nord & Lynch, 2009). In temperate and cold
65 ecosystems of the Northern hemisphere, the cyclical reactivation and termination of the
66 physiological activity of meristems has a distinct annual pattern that is described by the
67 phenology of buds, flowers and cambium (Delpierre *et al.*, 2015). Their activity is mainly
68 concentrated in a well-defined period of time between spring and autumn, when temperatures
69 are favourable for growth and reproduction, while it stops or slows down during winter.

70 Phenology is a dynamic and sensitive biological process that has been demonstrated to vary at
71 both short and long time scales according to the current weather (Gruber *et al.*, 2010, Swidrak
72 *et al.*, 2011) and increasing temperatures (Chmielewski & Rötzer, 2001, Menzel *et al.*, 2006).
73 The phenological events change according to the clinal variation of environmental factors,
74 with the growing season shortening at higher latitudes and altitudes (Jyske *et al.*, 2014, Moser
75 *et al.*, 2010). Despite the species-specific variations and ecotypic adaptations to local
76 conditions, latitudinal and altitudinal patterns in phenology may also be found across biomes.
77 However, at hemispheric scale, the mere geographical location becomes meaningless because
78 the effects of water bodies and changes in elevation on mesoclimatic conditions can modify
79 the predictable relationships between geographical coordinates and timing of growth.
80 Accordingly, Rossi *et al.* (2011) showed that the milder thermal conditions close to big lakes
81 or the lower temperatures experienced by trees of high altitudes affected xylem phenology
82 irrespective of the latitude of the sites. Temperature is one of the main driving forces for plant
83 growth in terrestrial ecosystems, and interacts with photoperiod in defining the timings of
84 growth resumption (Körner & Basler, 2010). Temperature-based models should therefore be

85 able to provide more intrinsic growth predictions than the models based on latitude or altitude,
86 which merely describe a geographical variation invariable over time.

87 Wood in trees consists of the xylem cells produced by the cambium in order to build the
88 hydraulic transport system, store substances and ensure mechanical support. Cambial cells
89 divide repeatedly and generate cell derivatives that differentiate physiologically and
90 morphologically until final maturity (Rossi *et al.*, 2012). The process of wood formation, or
91 xylogenesis, lasts from a few weeks to several months, according to species and individual
92 growth rate, and, more specifically, to climate (Rathgeber *et al.*, 2011, Schmitt *et al.*, 2004,
93 Treml *et al.*, 2015, Vieira *et al.*, 2015). This wide variation in wood formation demonstrates
94 the high plasticity of trees in adapting their growth processes to local environmental
95 conditions.

96 Trees capture atmospheric carbon dioxide and convert it to structural carbohydrates while
97 building new cells. Wood formation is one of the strongest carbon sinks in trees, making
98 xylogenesis a major process of long-term carbon sequestration and accumulation in terrestrial
99 systems. During the growing season, the outermost wood is composed of a pool of
100 differentiating and mature cells that are interconnected by a sequence of fixed events
101 (Delpierre *et al.*, 2015, Rossi *et al.*, 2012). At mid and high latitudes and altitudes, the annual
102 amount of wood production is determined by the growth rate and length of the period
103 favourable for cell division and maturation (Cuny *et al.*, 2012, Rathgeber *et al.*, 2011, Rossi *et*
104 *al.*, 2014). Although rate and duration of wood formation change with tree species,
105 identifying the timings and climatic conditions driving wood formation is an essential step in
106 understanding the dynamics of tree growth and forest productivity at a hemispheric scale.

107 An extensive dataset from 1321 trees belonging to 10 species was compiled containing intra-
108 annual data on wood formation and temperature collected by the authors in 39 plots with
109 similar methods during 1998-2014. The dataset was collected from temperate to northern
110 boreal sites on three continents, ranging from Southern Italy to Northern Finland and Canada,
111 from low altitudes in France and Slovenia to high ones on the Alps, Bohemian Massif in the
112 Czech Republic and Tibetan Plateau in China (Fig. S1). The sites represented temperate to
113 boreal forests, and treeline ecotones, including the highest treeline of the North hemisphere on
114 the Tibetan Plateau. The aim of the study was to compare wood formation over broad
115 geographical scale by testing the hypothesis that the timings of xylem phenology in conifers
116 change according to the variation of the temperature of the sites across temperate and boreal
117 forest ecosystems.

118

120 Site selection and sampling

121 The air temperature and xylem phenology data were collected in 39 sites located at different
122 altitudes and latitudes of nine countries in North-America, Europe and Asia (Table 1). The
123 sites consisted of permanent plots representing temperate-cold to boreal conifer stands. In
124 each site, from one to 24 adult trees with upright and injury-free stems were selected for
125 sampling. Trees with polycormic stems, partially dead crowns, reaction wood or evident
126 damage were avoided. In each site, 1-3 coniferous species were monitored, for a total of ten
127 species for the whole study.

128 The trees were monitored during 1998-2014, the number of years ranging from one to 13 per
129 site, throughout the growing season from March-April to October-December, according to the
130 climatic conditions of the sites (Table 1). Wood microcores were collected weekly, or
131 occasionally biweekly, at breast height (1.3 ± 0.3 m) on the stem using surgical bone sampling
132 needles or a Trephor (Rossi *et al.*, 2006a). The samples contained mature and developing
133 xylem of the current year, the cambial zone and adjacent phloem, and at least one previous
134 complete tree ring. The microcores were stored in solutions of propionic or acetic acid mixed
135 with formaldehyde or ethanol at 5 °C.

136 The microcores were dehydrated with successive immersions in ethanol and D-limonene, and
137 embedded in paraffin or glycol methacrylate, with the exception of samples from Switzerland,
138 which were not embedded. The microcores were cut with rotary or sledge microtomes in
139 transverse sections of 10-30 μm thick (Gruber *et al.*, 2010, Moser *et al.*, 2010, Rossi *et al.*,
140 2006a). The sections were simple-stained with cresyl violet acetate or double-stained with

141 safranin and astrablue, and examined under bright-field and polarized light (Moser *et al.*,
 142 2010, Rossi *et al.*, 2006b).

143 **Table 1:** Sites, species and years included in the analysis. The name of each site is associated
 144 with the country reported in the form of the alpha-2 code according to the International
 145 Organization for Standardization (ISO 3166). Species are reported with the following
 146 acronyms: ABAL, *Abies alba*; ABBA, *Abies balsamea*; ABGE, *Abies georgei*; LADE, *Larix*
 147 *decidua*; PCAB, *Picea abies*; PCMA, *Picea mariana*; PICE, *Pinus cembra*; PILE, *Pinus*
 148 *leucodermis*; PISY, *Pinus sylvestris*; PIUN, *Pinus uncinata*.

ID	Site	Latitude	Longitude	Altitude		Species	Number of trees
				(m a.s.l.)	Study years		
SYG	CN-Sygera Mountain	29°39'N	94°42'E	3850	2007-2009	ABGE	5
POL	IT-Pollino	39°54'N	16°12'E	2053	2003-2004	PILE	10
SUS	IT-Val di Susa	45°3'N	6°40'E	2030	2003-2004	LADE, PICE, PIUN	15
PAN	SI-Panska reka	46°0'N	14°40'E	400	2009-2012	PCAB	6
MEN	SI-Menina Planina	46°16'N	14°48'E	1200	2009-2012	PCAB	6-8
N22	CH-Lötschental-N22	46°22'N	7°46'E	2150	2007-2008	LADE	4
N08	CH-Lötschental-N08	46°23'N	7°45'E	800	2008	LADE, PCAB	8
N16	CH-Lötschental-N16	46°23'N	7°45'E	1300	2007-2008	LADE, PCAB	8
N13	CH-Lötschental-N13	46°23'N	7°45'E	1350	2007-2008	LADE, PCAB	8
N19	CH-Lötschental-N19	46°23'N	7°46'E	1900	2007-2008	LADE, PCAB	8
S16	CH-Lötschental-S16	46°24'N	7°45'E	1300	2007-2008	LADE, PCAB	8
S19	CH-Lötschental-S19	46°24'N	7°45'E	1900	2007-2008	LADE, PCAB	8
S22	CH-Lötschental-S22	46°24'N	7°44'E	2150	2007-2008	LADE	4
SVT	IT-San Vito di Cadore	46°26'N	12°13'E	1000	2003	PCAB	1
5T1	IT-Cinque Torri 1	46°27'N	12°8'E	2085	2001-2005	LADE, PCAB, PICE	15
5T3	IT-Cinque Torri 3	46°27'N	12°8'E	2085	2004-2005	LADE, PCAB, PICE	15
5T2	IT-Cinque Torri 2	46°28'N	12°8'E	2156	2002-2005	LADE, PCAB, PICE	11

ID	Site	Latitude	Longitude	Altitude		Species	Number of trees
				(m a.s.l.)	Study years		
TIM	AT-Patscherkofel-timberline	47°12'N	11°27'E	1950	2007	PICE	6
TRE	AT-Patscherkofel-treeline	47°12'N	11°27'E	2110	2007	PICE	6
KRU	AT-Patscherkofel-krummholz	47°12'N	11°27'E	2180	2007	PICE	5
DRY	AT-Tschirgant dry-mesic	47°14'N	10°50'E	750	2007-2012	LADE, PCAB, PISY	5-24
SIM	CA-Simoncouche	48°13'N	71°15'W	338	2002-2014	ABBA, PCMA	5-20
ABR	FR-Abreschviller	48°21'N	7°4'E	430	2007-2009	ABAL, PCAB, PISY	13-14
WAL	FR-Walscheid	48°22'N	7°5'E	370	2007-2009	ABAL, PCAB, PISY	14-15
ARV	CA-Arvida	48°26'N	71°9'W	80	1999-2000	ABBA	18
GRD	FR-Grandfontaine	48°29'N	7°9'E	643	2007-2009	ABAL, PCAB, PISY	15
AMN	FR-Amance	48°44'N	6°19'E	270	2006-2007	ABAL	15-20
BER	CA-Bernatchez	48°51'N	70°20'W	611	2002-2014	PCMA	5-10
MIS	CA-Mistassibi	49°43'N	71°56'W	342	2002-2014	PCMA	5-10
L23	CA-Liberal 23	49°58'N	72°30'W	380	1998-2000	ABBA	10
L24	CA-Liberal 24	49°58'N	72°30'W	430	1998-2001	ABBA	5-10
DAN	CA-Camp Daniel	50°41'N	72°11'W	487	2002-2014	PCMA	5-10
LH1	CZ-Lucní Hora-timberline	50°42'N	15°39'E	1310	2010-2012	PCAB	6-10
LH2	CZ-Lucní Hora-treeline	50°43'N	15°40'E	1450	2010-2012	PCAB	6-9
MIR	CA-Mirage	53°47'N	72°52'W	384	2012-2013	PCMA	10
RUO	FI-Ruotsinkylä	60°12'N	25°0'E	60	2008-2010	PCAB, PISY	8-10
HYY	FI-Hyytiälä	61°53'N	24°18'E	181	2008	PCAB, PISY	6
KIV	FI-Kivalo	66°12'N	26°23'E	140	2009	PCAB	5
VAR	FI-Värriö	67°30'N	29°23'E	390	2009	PISY	4

150 **Phenological data**

151 A common protocol of tracheid classification was followed at all sites in order to obtain
152 comparable data. In each sample, the number of tracheids in the radial enlargement,
153 secondary cell-wall thickening, and mature phases were counted along one to three radial
154 tracheid rows. Enlarging tracheids were composed of a thin primary wall but had a radial
155 diameter at least twice that of a cambial cell (Rossi *et al.*, 2006b). Discrimination between
156 enlarging and wall thickening tracheids was based on birefringence of the secondary cell wall
157 under polarized light (Abe *et al.*, 1997). No birefringence was observed in enlarging tracheids,
158 which lack a secondary wall. Colour changes from violet to blue (simple cresyl violet acetate
159 staining) or from blue to red (double safranin astra-blue staining) indicated the termination of
160 lignification. Absence of cytoplasm and a complete colour change over the whole cell wall
161 marked the end of lignification and tracheid maturity (Gričar *et al.*, 2005).

162 The mean number of tracheids in each developmental phase at each sampling date was used to
163 assess the onset and ending of the xylem differentiation phases. In spring, when at least one
164 tangential row of tracheids was observed in the enlarging, wall thickening or mature phase,
165 the phase was considered to have begun. In late summer, when no tracheids were observed in
166 a differentiation phase, wood formation was considered complete. Xylem phenology was
167 represented by the dates corresponding to (1) the first enlarging tracheid, (2) first wall-
168 thickening tracheid, (3) first mature tracheid, (4) last enlarging tracheid, and (5) last wall-
169 thickening tracheid. The timings of xylem phenology, computed as day of the year (DOY),
170 and the total number of tracheids in a radial row produced annually were calculated for each
171 tree, site and year. Tracheid production was assessed by fitting the Gompertz sigmoid curve to
172 the total number of new tracheids produced during the year and estimating the upper
173 asymptote of the function (Deslauriers *et al.*, 2008). Fifty-seven out of 1321 trees (4.3% of all

174 tree-year combinations) were excluded from the analyses because the function produced no
175 asymptote or the tracheid production was anomalous.

176 **Temperature data**

177 Temperature data were collected from sensors fixed at 2-3 m from the soil in shielded weather
178 stations installed in a forest gap beside or close to the sampled trees. Temperature was
179 recorded hourly or sub-hourly by dataloggers, and daily mean, minimum and maximum
180 values were calculated. In Finland, the data from the closest weather station maintained by the
181 Finnish Meteorological Institute were used for each site. The datasets covered the entire year.
182 However, in the case of occasional missing values, temperatures were estimated by using time
183 series available from one or more weather stations located in the proximity of the sites and
184 with similar climatic conditions.

185 **Statistical analyses**

186 A cluster analysis using the average linkage method was used for assessing the distance
187 between the study sites based on the monthly mean temperatures. The distance between two
188 clusters was the arithmetic mean distance between groups, which were represented by the
189 sites.

190 A random-effects analysis of variance was performed to isolate the proportion of variation
191 accounted by the factors site, species, tree and year in the studied variables (phenological
192 events and tracheid production). The analysis included the Restricted Maximum Likelihood
193 (REML) method of the NESTED procedure in SAS (SAS Institute Inc., Cary, NC), which
194 allowed to process data with nested (hierarchical) structure originating from an unbalanced
195 design.

196 The dates of xylem phenology were modelled by Generalized Linear Models (GLM) using the
197 DOY of each event (onset and cessation) as dependent variable, and the mean annual
198 temperature at each site and the five phases of xylem phenology as quantitative and
199 classification variable, respectively. The statistical significance of the differences between the
200 slopes of regressions was tested by orthogonal contrasts. Before and after the fitting, the
201 distributions of the input data and residuals were checked for normality. GLM was performed
202 using the SAS software. The model was also tested with the weekly mean temperatures as
203 independent variables and using a bootstrap procedure with 10,000 replications. Only
204 temperatures occurring before or during the growing period were analysed.

205

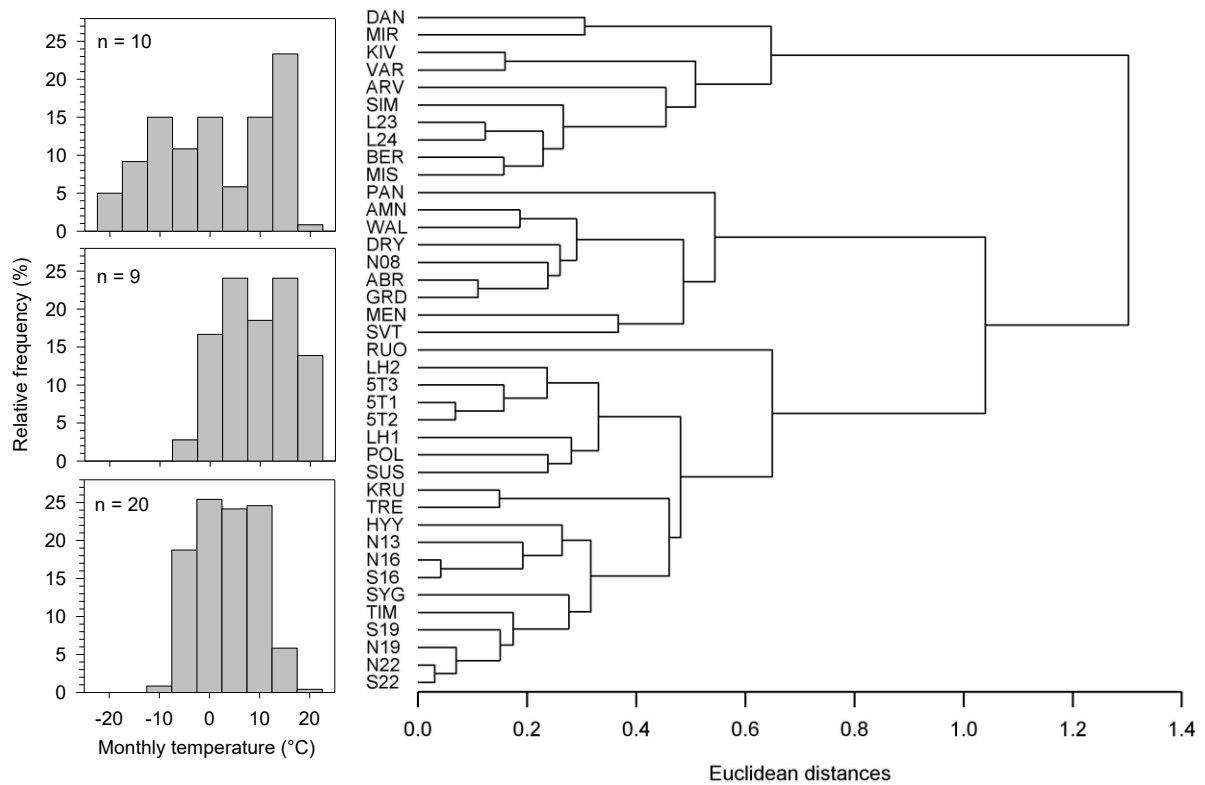
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RESULTS

207 **Temperature across sites**

208 During the study years, the mean annual temperature across the studied sites ranged by more
209 than 14 K, from -2.3 °C in MIR (Canada) to 12.0 °C in PAN (Slovenia). All sites had a cold
210 winter, with the mean daily temperatures below zero for periods varying between 20 days in
211 France (WAL and AMN) and 180 days in Finland (KIV) and Canada (BER). In three sites,
212 VAR (Finland), DAN (Canada), and MIS (Canada), the mean temperature remained below 0
213 °C for more than 190 days. The coldest episodes were observed in Canada (DAN and MIR),
214 with absolute minimum temperatures below -47 °C. The warmest winters occurred in PAN
215 (Slovenia), WAL (France) and N08 (Switzerland), where absolute minimum temperatures
216 reached -7.6 °C. The absolute maximum temperatures ranged between 19.7 °C in Austria
217 (KRU) and 37.1 °C in Slovenia (MEN and PAN).

218 The sites were grouped in three main clusters based on the monthly mean temperatures
219 extracted from the time series available (Fig. 1). The first cluster included 10 sites from
220 Canada and northern Finland. At these sites, the monthly mean temperatures ranged between -
221 20 and 20 °C, representing continental conditions, with short warm summers and long cold
222 winters. The second cluster was represented by nine sites, with monthly mean temperatures
223 varying between -5 and 20 °C. These were the warmest sites of the dataset, and were located
224 in Central Europe and at the lower Alpine altitudes. The European southern boreal sites and
225 treeline and timberline sites were included in the third cluster, representing 20 sites with a
226 narrower range of monthly mean temperature than those observed in the first cluster. The sites
227 of the third cluster were characterized by low winter temperatures and cool summers (Fig. 1).



228

229 **Figure 1:** Dendrogram generated by a cluster analysis based on the monthly temperatures of
 230 the 39 study sites. The left panel shows the frequency distributions of the temperatures of the
 231 three main clusters.

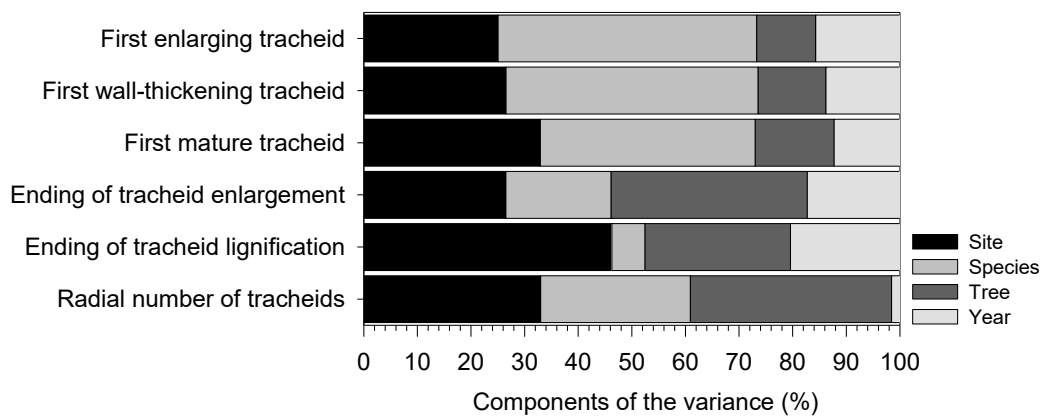
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233 Variance partition

234 The contribution of site, species, tree and year to the total variance in the five phases of xylem
 235 phenology and annual total production of tracheids differed according to two main patterns
 236 (Fig. 2). First, the species accounted for a high proportion of variation (40.0-48.2%) in the
 237 variables related to spring events, i.e. the dates of the first enlarging, wall-thickening and
 238 mature tracheids. The site explained between 25.0 and 32.9% of the variance in the spring
 239 events, with the lowest percentage (25.0%) being estimated for the first enlarging tracheid. In

240 the spring events, the percentage of the variance accounted for by the factors tree and year
241 was clearly lower, and ranged between 10.9 and 15.7% (Fig. 2).

242 In the phenological events occurring in autumn, as well as the total number of tracheids, a
243 lower proportion of the variation was accounted for by species (6.1-27.9%), with a
244 substantially higher variance related to the differences among individual trees (27.1-37.5%).
245 The differences between years in the radial number of tracheids explained only 1.5% of the
246 variance, indicating a small inter-annual variation in tracheid production (Fig. 2).



247

248 **Figure 2:** Variance partition of the studied variables (phenological events and cell
249 production) estimated with the Restricted Maximum Likelihood (REML) method.

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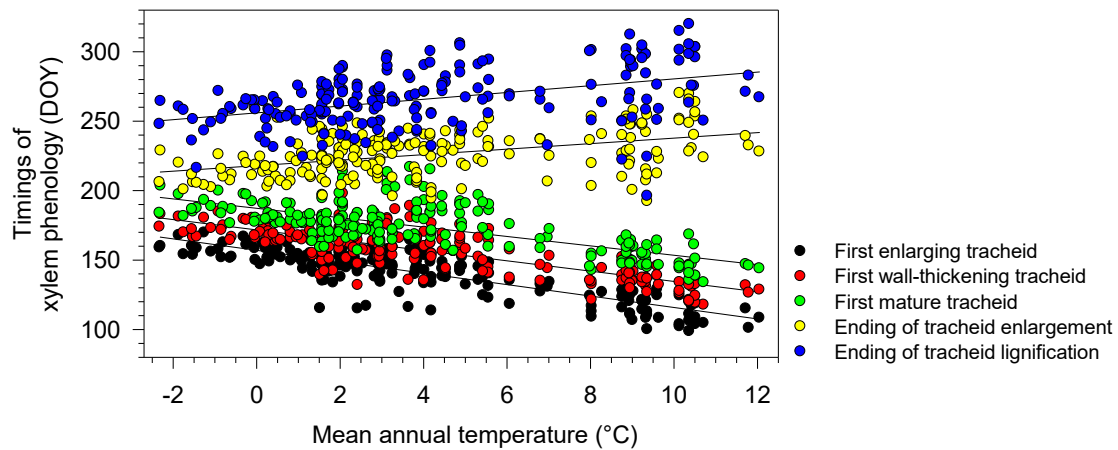
251 **Model definition and application**

252 Two divergent patterns were observed between the phenological events and mean annual
253 temperature of the sites (Fig. 3). With increasing temperature, first enlarging, wall-thickening
254 and mature tracheids appeared earlier, and last enlarging and wall-thickening tracheids
255 occurred later. GLM was able to adequately fit the dataset and produced a highly significant

256 F-value (Table 2). The R^2 indicated that 92% of the variance was taken into account by the
 257 model. A detailed examination of each phenological event demonstrated that all trends were
 258 linear within the thermal range analysed, thus the use of GLM was appropriate. The slopes of
 259 regressions were statistically different from zero, as well as the interaction term phenology \times
 260 temperature, indicating that the slopes of regressions changed according to the phenological
 261 phases (Table 2). Tests for contrasts revealed that the regressions could be separated in two
 262 homogeneous groups. The first one involved the regressions representing spring events: the
 263 dates of first enlarging, wall-thickening and mature cells. Between these three regressions,
 264 there was no difference in slope ($p>0.05$). The second group involved the regressions fitting
 265 autumnal events, the ending of cell enlargement and lignification, with slopes that were not
 266 statistically different ($p>0.05$). Overall, spring and autumnal events had statistically different
 267 slopes ($p<0.0001$).

268 **Table 2:** Results of the Generalized Linear Models (GLM) relating xylem phenology with the
 269 mean annual temperature of the sites, with the phenological events and phases being used as
 270 covariates, respectively.

Source of variation	Regressors			Model		
	Type I SS	F-value	P	F-value	P	R^2
Phenological event (PE)	2223393.5	3059.7	<0.0001			
Mean temperature (MT)	23467.5	129.1	<0.0001	1441.0	<0.0001	0.92
Interaction PE \times MT	109320.9	150.4	<0.0001			



271

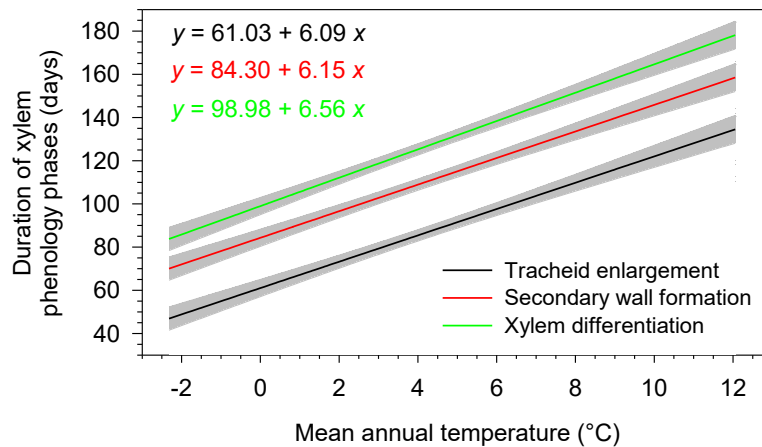
272 **Figure 3:** Variation in xylem phenology according to the mean annual temperature of the
 273 sites. Regression lines represent the predicted values estimated by Generalized Linear Models
 274 (GLM). Each differentiation phase is characterized by 204 observations representing the
 275 different site × year × species combinations.

276

277 The residuals of the model were well distributed around zero, and no clear pattern or tendency
 278 was observed (Fig. S2). Heteroscedasticity was low, with a variance of residuals constant
 279 across the thermal range. The standard deviation of residuals increased gradually during the
 280 growing season, with the higher variability observed for autumnal events (Fig. S2). The
 281 studentized residuals exceeding the 95% confidence interval (the range between -2 and 2)
 282 were less than 5% for spring events, but increased to 5.8 and 11.7% for the ending of cell
 283 enlargement and lignification, respectively.

284 The period of wood formation lengthened linearly with the mean annual temperature of the
 285 site (Fig. 4). Cell enlargement lasted 48 days at -2 °C, and 134 days at 12 °C, increasing by
 286 6.1 days °C⁻¹. Secondary wall formation lasted from 70.0 to 158.5 days, on average 23.5 days
 287 more than cell enlargement. Overall, the period of xylem differentiation (including cell

288 enlargement and secondary wall formation) ranged from 83.7 to 178.1 days, and increased by
289 6.5 days °C⁻¹ (Fig. 4). Along the thermal gradient, the slopes of the three regressions
290 representing cell enlargement, secondary wall formation and xylem differentiation were not
291 statistically different (p>0.05).



292

293 **Figure 4:** Duration of wood formation according to the mean annual temperature of the sites.
294 Regression lines and grey areas represent the predicted values and their 95% confidence
295 interval estimated by Generalized Linear Models (GLM), respectively. Xylem differentiation
296 includes the two phases of cell enlargement and secondary wall formation.

297

298 Xylem phenology and weekly temperature

299 The GLM performed with weekly temperatures produced results similar to the model using
300 annual temperature. However, the variance accounted for changed according to the period of
301 the year considered (Fig. S3). On average, R² ranged between 88 and 92%. The higher values
302 were concentrated in spring, between the end of March and end of May. After this period, R²
303 showed an abrupt reduction that lasted until September. The 95% confidence intervals

304 calculated by the bootstrap replications demonstrated that spring and summer temperatures
305 produced models with statistically different R^2 (Fig. S3). These findings persisted basically
306 unchanged when using averages at daily to monthly scales, although only results with weekly
307 averages are described and reported in this paper.

308

310 Xylem phenology and temperature

311 There is a long-standing assumption that temperature is one of the main driving forces for
312 plant growth in terrestrial ecosystems, but hemispheric comparisons of the dynamics of xylem
313 formation are still scarce. This study quantitatively demonstrated the thermal constraints to
314 xylem phenology by describing the effects of an interval in the mean annual temperature
315 exceeding 14 K on the growth period of trees. Unlike the geographical coordinates, the mean
316 annual temperature of the sites represents one, common, and biologically-meaningful
317 variable, allowing this study to describe the variability in xylem phenology of conifer species
318 over three continents and across a wide range of ecosystems. These findings are in general
319 agreement with previous climate-driven models based on temperature, rather than latitude or
320 altitude, which were able to simulate biological phenomena such as treeline position at global
321 scale (Paulsen & Körner, 2014).

322 Within the range of temperature analyzed, all phases of beginning and ending of wood
323 formation were linearly related to the mean annual temperature. This confirmed the main
324 trends observed along altitudinal (Moser *et al.*, 2010) and latitudinal (Rossi *et al.*, 2014)
325 gradients, although with some differences. In the Swiss Alps, there was a departure from the
326 linear trend for the phases related to the ending of tracheid formation at 1350 m a.s.l., i.e. the
327 lower altitudinal distribution of European larch (*Larix decidua* Mill.) (Moser *et al.*, 2010),
328 which may be associated with a reduced availability of water combined with warm conditions
329 in June-July. When plotted with the temperatures of the growing season, Rossi *et al.* (2014)
330 also found that the relationship between the ending of tracheid enlargement in black spruce
331 [*Picea mariana* (Mill.) BSP] and the growing season temperature was non-linear because of
332 the disproportionate tracheid production of black spruce in the southernmost part of its

333 distribution. Despite the microclimatic effects or local growth dynamics, and regardless of the
334 species considered, this study assessed the linear relationship between temperature and the
335 phenology of xylem growth at wide geographic scale. Thus, it is possible that non-linear
336 trends occur due to multiple environmental effects (i.e. interaction with drought), or under
337 extreme thermal conditions not included in this study. Moreover, the potential changes in
338 sensitivity of trees to the temperature, and long-term adaptation of ecotypes to local climates
339 should be taken into account when interpreting the results of prediction models.

340 **Time window of xylem phenology**

341 Under the coldest conditions, the duration of cell enlargement was estimated at 46 ± 5
342 (mean \pm 95% CI) days. The period length is comparable with the minimum duration of
343 cambium activity of six weeks reported for Scots pine (*Pinus sylvestris* L.) at the Northern
344 treeline (Schmitt *et al.*, 2004). The minimum length of the growing season, defined in
345 literature as the period allowed for the physiological activity of trees, was indicated by
346 measurements and modelling at 90-94 days (Körner & Paulsen, 2004, Paulsen & Körner,
347 2014). In our study, xylem required at least 83 ± 5 (mean \pm 95% CI) days to complete the
348 maturation of all tracheids produced by cambium. Conifers reactivate some physiological
349 processes such as photosynthesis before cell division in the cambium (Goodine *et al.*, 2008),
350 and at high latitudes the gap between onset of photosynthesis and wood formation can exceed
351 two months (Bäck *et al.*, 2013, Jyske *et al.*, 2014). The shorter period of wood formation
352 compared with photosynthesis may support the hypothesis that tree survival in these cold-
353 limited environments is controlled by sink limitation, i.e. limited demand of carbohydrates for
354 growth rather than carbon assimilation in regions with low mean annual temperatures
355 (Guillemot *et al.*, 2015, Körner, 1998, Leuzinger *et al.*, 2013). However, trees also have large
356 reserves of carbohydrates, which could substantially affect the link between photosynthetic

357 production and tracheid formation. Moreover, before the onset of tracheid formation, both the
358 stored and newly assimilated carbon may also be directed to other growing sinks, e.g., shoots,
359 needles and secondary phloem (Gričar *et al.*, 2014, Jyske *et al.*, 2015, Richardson *et al.*, 2015,
360 Schiestl-Aalto *et al.*, 2015).

361 The sites with the warmest annual temperature were located at the lower elevations in
362 Slovenia, France, Switzerland and Austria. These sites are close to the lower distribution limit
363 of larch, Norway spruce [*Picea abies* (L.) Karst.] and silver fir (*Abies alba* Mill.), where cell
364 division lasted up to 134 days and the whole period of wood formation reached 178 days. In
365 the case of reduced summer precipitation, trees in these regions experience water stress during
366 the growing season. The dataset included a dry-mesic site in Austria (called DRY in Table 1),
367 where soil water availability can be a limiting factor for growth during summer (Swidrak *et*
368 *al.*, 2011). Although growth resumption in this site is still strictly related to spring
369 temperatures (Swidrak *et al.*, 2011), the effects of water constraint appeared clearly at the
370 ending of tracheid differentiation, which occurred earlier than in other sites with similar mean
371 annual temperatures. It is thus likely that the observed linear pattern might not be maintained
372 for warmer annual temperatures because other environmental factors may play a role in
373 affecting the dynamics of xylem phenology and should be taken into account if applying the
374 model under warming scenarios (Delpierre *et al.*, 2015, Gruber *et al.*, 2010).

375 **The process of wood formation**

376 In longitudinal data, the sequence of phenological events are represented by the changes in
377 traits or attributes in the same individuals over time (Fitzmaurice *et al.*, 2009). Commonly, the
378 sequence of events is serially correlated (Gourieroux & Jasiak, 2007), as also previously
379 demonstrated for the phases of bud (Rossi & Bousquet, 2014) and cambium (Rossi *et al.*,

380 2012) phenology. A certain phenological phase can only take place after the previous one has
381 occurred. Consequently, delays or advancements of phenological events lead to delay or
382 advancements in the successive events. The correlation between phenological events reveals
383 the interconnection of the differentiation phases and the uniqueness and consistency of the
384 process of wood formation.

385 The constant interval between the phases of wood formation, which are characterized by the
386 parallel regression lines, is maintained across ecosystems and species, and under the entire
387 range of the thermal conditions analysed (Fig. 3). These findings could suggest two possible
388 hypotheses. First, the rate of tracheid differentiation, here represented by the phases of
389 enlargement and wall thickening of the first and last formed tracheids, is insensitive to
390 temperature. But this hypothesis is counterintuitive and conflicting with previous studies,
391 particularly those related to the process of wall formation (Cuny *et al.*, 2015). Second, the
392 gradual advancement of phenological events with annual temperature most likely reflects the
393 dynamics of spring warming and autumnal cooling across ecosystems. As previously
394 demonstrated (Rossi *et al.*, 2008, Swidrak *et al.*, 2011), beginning and ending of growth arise
395 under similar thermal conditions, which however occur in different moments at latitudes and
396 altitudes on a global scale.

397 **Model interpretation and application**

398 The underlying physiological mechanisms that drive the activity of cambium and tracheid
399 differentiation are not considered by the regression analysis presented in this paper. Thus, the
400 resulting statistical model remains a simple, albeit hemispheric, description of the changes in
401 xylem phenology with annual temperature of the site, but lacks deterministic tree functioning
402 (Delpierre *et al.*, 2015). Moreover, the annual temperature was chosen as predictor for the

403 model, although thermal thresholds or heat sums of shorter time windows, or interaction with
404 other factors such as photoperiod may be considered to depict some specific phenological
405 phases rather better (Körner & Basler, 2010, Rossi *et al.*, 2008, Schiestl-Aalto *et al.*, 2015,
406 Swidrak *et al.*, 2011). Nevertheless, from a practical point of view, the model represents a tool
407 for demonstrating the annual time window of wood formation in temperate and cold
408 ecosystems around the Northern hemisphere. The model is based on mean annual temperature
409 that is largely accessible or easily computed by spatial interpolation from nearby weather
410 stations, even in remote areas where daily data are generally unavailable.

411 There is no solid explanation for using the annual mean temperature to describe a biological
412 phenomenon, such as wood formation, that is discontinuous during the year. However, annual
413 mean temperature sufficiently summarized the local climate in cold to temperate regions, and
414 the thermal conditions occurring during wood formation. When reducing the time window of
415 temperatures to shorter periods, April and May resulted in the highest variance accounted for
416 by the model in describing xylem phenology. The models using spring temperatures produced
417 R^2 similar to those with annual temperatures. April and May, the two months with the highest
418 relationship with xylem phenology, match the timing of growth resumption, which is
419 associated with the spring increase in temperature (Deslauriers *et al.*, 2008). The pattern of R^2
420 also suggests that spring warming may have the greatest impacts on xylogenesis at global
421 scale. A lower R^2 with the temperatures occurring in summer and autumn was observed, as
422 well as a marked increase of the contribution of the factor tree to the total variance of the
423 latest phases of xylem phenology and tracheid production. The increasing dispersion of the
424 residuals during late summer and autumn confirmed this pattern (Fig. S2). Although
425 knowledge on the phenological phases of late summer is still insufficient to depict a common
426 rule (Delpierre *et al.*, 2015), there is general agreement that the ending of xylogenesis should

427 also be related to other environmental (Swidrak *et al.*, 2011) and endogenous (Rossi *et al.*,
428 2012, Treml *et al.*, 2015) factors.

429 In temperate and cold regions of the world, trees synchronize the activity of meristems with
430 local climate, concentrating the timings of wood formation within a time window between
431 spring and autumn, when temperature is habitually favorable for growth. This study analyzed
432 an extensive dataset containing intra-annual data on wood formation from sites at mid and
433 high altitudes and latitudes covering an interval of mean annual temperature exceeding 14 K.
434 The timings of wood formation increased linearly according to the local temperature at a rate
435 of 6.5 days °C⁻¹. In warmer sites, spring events (first enlarging, wall-thickening and mature
436 tracheids) appeared earlier, while autumnal events (last enlarging and wall-thickening
437 tracheids) occurred later. Under the range of thermal conditions analysed, the phases of onset
438 and ending of tracheid differentiation were separated by constant intervals, demonstrating the
439 uniformity of the wood formation process in conifer species across ecosystems. The spring
440 temperatures were associated with models showing the higher variance accounted for. This
441 demonstrated the importance of the environmental conditions occurring at the time of growth
442 resumption in defining the dynamics of wood formation in temperate and cold regions of the
443 Northern hemisphere. According to the pattern observed in this study and in absence of water
444 stress during the growing season, under warming scenarios xylem phenology might lengthen
445 synchronously in cold biomes regardless of species and sites.

446

447

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552

554 **Fig. S1:** Location of the study sites.

555 **Fig. S2:** Studentized residuals of the Generalized Linear Models (GLM) relating the dates of
556 xylem phenology with the mean annual temperature of the sites. Residuals are plotted for each
557 level of the covariate in the model. STD indicates the standard deviation of residuals. The
558 range between -2 and 2 represents the 95% confidence interval of the Studentized residuals.
559 Each differentiation phase is characterized by 204 observations representing the different site
560 \times year \times species combinations.

561 **Fig. S3:** R^2 of the Generalized Linear Models (GLM) relating the dates of xylem phenology
562 with the weekly temperature of the sites. The grey area represents the 95% confidence interval
563 estimated by 10,000 bootstrapped replications.