



Phenology of wood formation in larch (*Larix decidua* Mill.) trees growing along a 1000-m elevation gradient in the French Southern Alps

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Abstract

• **Key message** Spring temperature increase is the main driver of larch tree wood formation onset along a 1000-m elevation gradient in the Southern Alps, while its cessation is more probably controlled by water stress at the lowest elevation and photoperiod at higher ones.

• **Context** The survival of perennial plants depends on their adaptation to changing environment and specially temperature, which in trees is notably implemented through wood formation process.

• **Aims** Our main objective is to understand how the phenology of wood formation is related to environmental factors and to temperature in particular.

• **Methods** We monitored the xylogenesis of 60 larch trees, distributed in four stands along an elevation gradient of 1000 m in the French Southern Alps.

• **Results** Cambial activity started around mid-May at the lowest site (1350 m) and around mid-June at the highest one (2300 m), showing a delay of 5.4 days per °C. The onset of wall-thickening and mature phenophases followed the same linear trend with a delay of 5.2 and 3 days per °C, respectively. Phenophase cessations followed a parabolic trend with trees from the lowest site finishing their growth the first, while those from 1700 m finished the last. Our results show that the onset of xylem formation is mainly driven by spring temperature increase, while its cessation is more related to photoperiod, with water shortage being able to hasten it.

• **Conclusion** Future climatic changes will most probably increase growing season length (but not necessarily wood production) and shift upwards the optimal elevation for larch growth in the Southern Alps.

Keywords Cambial activity · Wood anatomy · Tree growth · Species adaptation · Climate change · *Larix decidua*

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This article is part of the topical collection on *Wood formation and tree adaptation to climate*

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1 Introduction

Wood (i.e. xylem) is the tissue allowing trees to transport sap and stand upright. Wood formation (i.e. xylogenesis) is a key process determining tree functioning, adaptation, and survival. Trees, as long-lived fixed organisms, are exposed to varying environmental conditions and meet extreme climatic events during their lifetime. Phenology (i.e. the timing of organism or tissue seasonal development) is an important means trees use to adapt and acclimate to their ever-changing environments. Phenology of tree-ring formation, even if less studied than leaf phenology, is a crucial component of tree adaptation to environmental changes (Delpierre et al. 2016). Indeed, the length of the growing season—resulting from minimizing the risk of frost damage to plant organs and maximizing the capture and assimilation of carbon by photosynthetic tissues—has a crucial importance for plant growth, fitness, and survival (Chuine 2010).

Xylogenesis is a complex process, which can be divided into four sub-processes: (1) the periclinal division of a cambial mother cell that creates a new daughter cell; (2) the enlargement of the newly formed xylem cell; (3) the differentiation and deposition of cellulose and hemi-cellulose to build the secondary cell wall and the impregnation of the cell walls with lignin; and finally, (4) the programmed cell death. These processes are under the control of endogenous and exogenous factors (Larson 1969; Wilson 1971). Temperature is one of the main environmental factors controlling the phenology of tree-ring formation (Begum et al. 2018). In the boreal and temperate zones, for example, the cambial activity, and specially its onset, was found to be mainly under the control of temperature (Oribe et al. 2001, 2003; Gricar et al. 2006; Begum et al. 2007; Rossi et al. 2007, 2008b, 2013, 2016). The effect of temperature on the resumption of cambial activity was also experimentally demonstrated through artificial heating of tree stems (Oribe et al. 2001; Gricar et al. 2007; Begum et al. 2013, 2015).

Water availability is also an important factor for cambial activity. Low soil water content can hasten growth termination (Ren et al. 2015; Zhang et al. 2018). Decreasing soil water availability under high temperature can induce water stress in trees, when stem and leaf water potential fall down beyond the boundaries of hydraulic security, causing xylem to cavitate and threatening tree survival (McDowell et al. 2008). Ziaco et al. (2018) pointed the paramount role of soil water content as the main driver of cambial phenology for *Pinus ponderosa* in the Mojave Desert. Meta-analysis of wood formation monitoring studies suggested that while the control of temperature on wood formation phenology is general for cold and temperate forests, the effect of water stress is mainly restricted to the drought-prone areas (Rossi et al. 2008b, 2013, 2016).

The effect of other environmental factors such as photoperiod was also commonly reported on leaf phenology but much

less frequently on wood formation (Delpierre et al. 2016). In boreal and temperate regions, photoperiod was found to be one of the most important factors triggering phenological phases in plants (Vitasse et al. 2014). Photoperiod was reported to have an effect on growth cessation as species display a shorter growing season in higher than in lower latitudes (Jackson 2009).

Alternatively, we also know that phenology of wood formation can be affected by endogenous factors and tree characteristics such as tree age and size. Rossi et al. (2006) observed shorter periods of wood formation in older trees. Additionally, Rathgeber et al. (2011b) reported that cambial activity started earlier, stopped later, and lasted longer in dominant silver fir trees than in intermediate and suppressed ones. So, investigation on wood formation duration provides useful information about the specific life strategies of species. The genetic background also influences tree phenology and growth (Vitasse et al. 2009). Even if we still lack direct evidences in the case of cambium phenology, Nardin et al. (2015) reported the absence of clear genetic structure of larch (*Larix decidua* M.) in the forest stands of our elevation gradient. As a consequence of the lack of genetic structure, any differences in the phenology of wood formation are mostly due to phenotypic acclimation rather than genetic adaptation (King et al. 2013).

Elevation gradients provide unique “natural experiments” to investigate species response to environmental factors that change consistently with elevation, such as temperature (Korner 2007). The general trend for temperature variation with elevation (i.e. the adiabatic temperature decrease) was reported as $-0.54\text{ }^{\circ}\text{C}/100\text{ m}$ (Korner 2007). Thus, monitoring wood formation along an elevation gradient is a smart way to investigate tree response to very contrasting temperature conditions across very short distances. Indeed, in a previous study on larch trees grown along an 800-m elevation gradient in the Swiss Northern Alps, Moser et al. (2010) investigated the influence of temperature on leaf and tree-ring phenology to find a delay of about 7 days per $^{\circ}\text{C}$ for the onset of cambial activity and duration of tree-ring formation.

The main objective of our study was to unravel the relationships between environmental factors and the phenology of wood formation along an elevation gradient. We assumed that temperature was the main driver of larch tree wood formation phenology in the Southern Alps. Then, we tested the following hypothesis: (1) at the beginning of the growing season, we expect a delay in the onset of wood formation phenophases, with critical dates increasing linearly from the bottom to the top of the gradient because of decreasing temperatures; (2) at the end of the growing season, we expect a delay in the cessation of wood formation phenophases, with critical dates increasing linearly from the top to the bottom, because of increasing temperature; (3) if hypotheses 1 and 2 hold, wood formation durations should increase linearly from the top to

the bottom of the elevation gradient because of increasing temperature. Moreover, we will also explore the potential effects of predicted climatic changes on tree-ring formation phenology and resulting wood production.

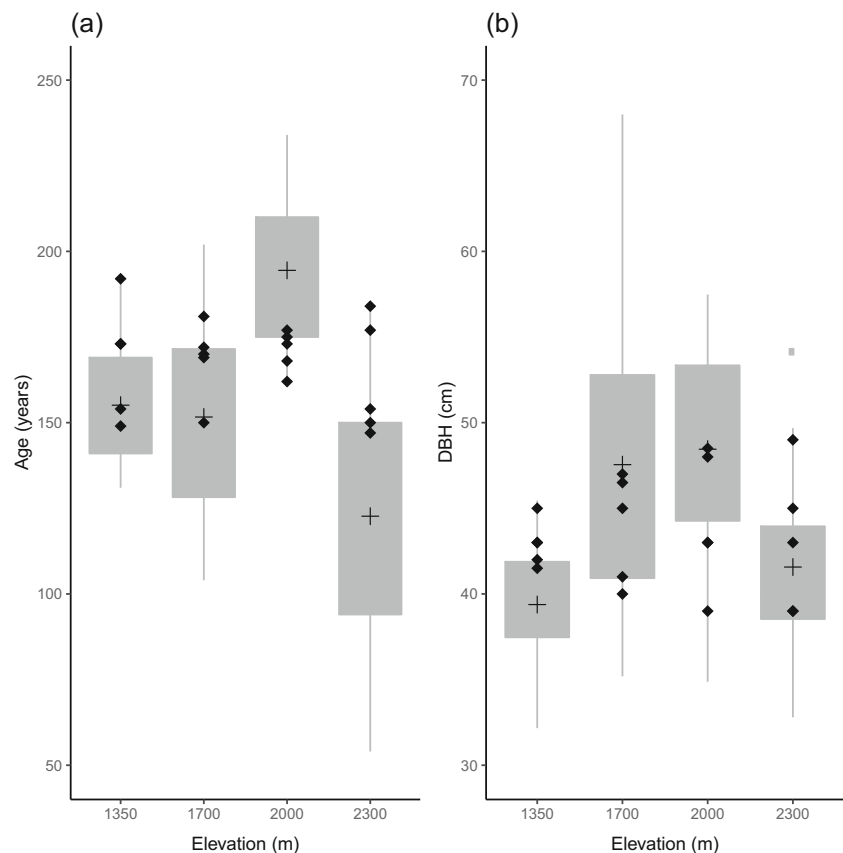
In order to test these hypotheses, we monitored weekly the wood formation of 60 larch trees, distributed in four forest stands (4×15 trees) and spread along an elevation gradient of about 1000 m (at 1350, 1700, 2000, and 2300 m) in the French Southern Alps.

2 Material and methods

2.1 Site description

The study was conducted on a north-facing slope located in the village of Villard St. Pancrace ($44^{\circ} 53' 47''$ N, $6^{\circ} 38' 08''$ E), close to the city of Briançon, in the Southern French Alps. The hillside is covered mostly by a pure, uneven-aged larch forest (*Larix decidua* Mill.) containing some patches of silver firs (*Abies alba* Mill.), Scots pines (*Pinus sylvestris* L.), stone pines (*Pinus cembra* L.), and mountain pines (*Pinus uncinata* Ramond). We installed four instrumented plots along the slope at 1350, 1700, 2000, and 2300 m a.s.l., covering an elevation range of about 1000 m.

Fig. 1 Characteristics of the selected trees along the elevation gradient. **a** Age. **b** Diameter at breast height (DBH). The points show the five sub-sampled trees presenting comparable ages and DBHs



2.2 Tree selection

In total, 60 dominant European larch trees were selected and their heights and diameter at breast height (DBH) were measured (Tables 6 and 7 in the Appendix). The mean height of the 60 trees was about 23 ± 0.6 m, with trees at the top and bottom sites being significantly smaller than the intermediate sites (Fig. 1, Table 1 and 6). The mean DBH of the selected trees was about 44 ± 0.9 cm (mean \pm SE), again with the bottom and top sites being significantly thinner than the intermediate ones. Standard 5-mm increment cores were taken at breast height to estimate tree age. The mean age of the selected trees was about 155 ± 5 years, but trees were significantly older at 2000 m and younger at 2300 m.

2.3 Tree sampling and sample preparation

For each selected tree, microcores were taken at breast height, every week from May to November 2013. Microcoring was done using a Trephor® tool (Vitzani, Belluno, Italy) respecting a distance of 2 cm between two sampling points (Rossi et al. 2006). The collected microcores (2 mm in diameter and 15 to 20 mm in length) were placed in Eppendorf vials in a diluted solution of ethanol 50% and stored at 5 °C to prevent the deterioration of the living tissues. In the laboratory, the microcores were impregnated by successive dipping in eight

Table 1 Means \pm standard errors for age, diameter at breast height (DBH), and height of selected trees for each site along the elevation gradient

Site elevation (m)	Age	DBH (cm)	Height (m)
2300	122 \pm 10	41 \pm 1	17.2 \pm 1
2000	194 \pm 6	48 \pm 1	27 \pm 2
1700	151 \pm 8	47 \pm 2	27.3 \pm 2
1350	155 \pm 6	39 \pm 0.9	22.3 \pm 1

treatment baths using an impregnation machine (STP 120, Microm MM France, Francheville, France). The microcores were first cleaned by immersion in two successive baths of histoclear; then, they were immersed in four successive baths of ethanol of increasing concentration from 50 to 100%; finally, they were immersed in two hot baths (65°C) of liquid paraffin, which penetrates the wood and fills the cell lumens. The impregnated microcores were then embedded in paraffin blocks. Finally, 5–10- μ m-thick transverse sections were cut using a rotary microtome (HM 355 S, Microm MM France, Francheville, France), stained using cresyl-violet acetate dye and fixed for anatomical observations under a microscope (Harroue et al. 2011).

2.4 Microscopic observations

The anatomical sections were observed using an optical microscope (ZEISS, Germany) at a magnification of \times 100–400. Visible and polarized lights were used for distinguishing different stages of cell differentiation. Xylogenesis starts with the production of new xylem mother cells by the cambium (Rathgeber et al. 2016). The cambial cells were recognized thanks to their thin primary walls, small radial size, and rectangular shape compared with phloem cells. The newly divided xylem cells then undergo profound transformations passing through the successive differentiation stages of cell enlargement, secondary cell wall deposition and lignification, and programmed cell death. The enlarging cells also consist of primary cell walls but have wider radial diameter than cambial cells. Polarized light was used to distinguish the cells in the wall-thickening stage because these cells were shining as a result of secondary wall formation. Wall-thickening cells also exhibit a gradient of pink-violet to blue color from inside to outside their cell walls, while the walls of fully mature cells are totally stained in blue. The successive stages of cell differentiation create a zonation pattern at the xylem tissue level, which makes it possible to define critical dates for the onset and cessation of the different phenophases (Rathgeber et al. 2011a). Phenology of wood formation can be characterized at the tree level by five critical dates, which are the onset and cessation of the enlarging and thickening phenophases and the onset of the mature phenophase. In each sample, three

radial files were chosen and the number of cambial (nC), enlarging (nE), wall-thickening (nW), and fully mature (nM) cells were counted in the developing xylem.

2.5 Timing of wood formation

Wood formation calendar was assessed using the R package CAVIAR which is specially made for wood formation analysis (Rathgeber et al. 2011a, 2018). CAVIAR computes the critical dates of wood formation based on logistic regressions. These dates include (1) the beginning of the enlarging period (bE); (2) the beginning of the wall-thickening period (bW); (3) the beginning of the mature period (bM); (4) the end of the enlarging period (cE); and (5) the end of the wall-thickening period (cW). The critical dates correspond to the 50% probability of a phenophase being started or stopped. As proposed by Rathgeber et al. (2011a), the onset and cessation of xylem enlargement are used as the best proxy for the onset and cessation of cambial activity.

Using these critical dates makes it possible to compute critical durations describing wood and cambium phenology: (1) the duration of the enlarging period ($dE = cE - bE$); (2) the duration of the wall-thickening period ($dW = cW - bW$); (3) the total duration of the wood formation period ($dX = cW - bE$).

Since using the number of cambial cells does not allow to define the onset and cessation of cambial activity correctly, we used bE and cE instead, as suggested by Rathgeber et al. (2011a, 2016). Although bE and cE have been proven to be a reliable proxy for the onset and cessation of cambial activity, the time between the onset of cell division (bD) and bE may increase with elevation, resulting in the slight underestimation of the observed trends.

2.6 Meteorological data

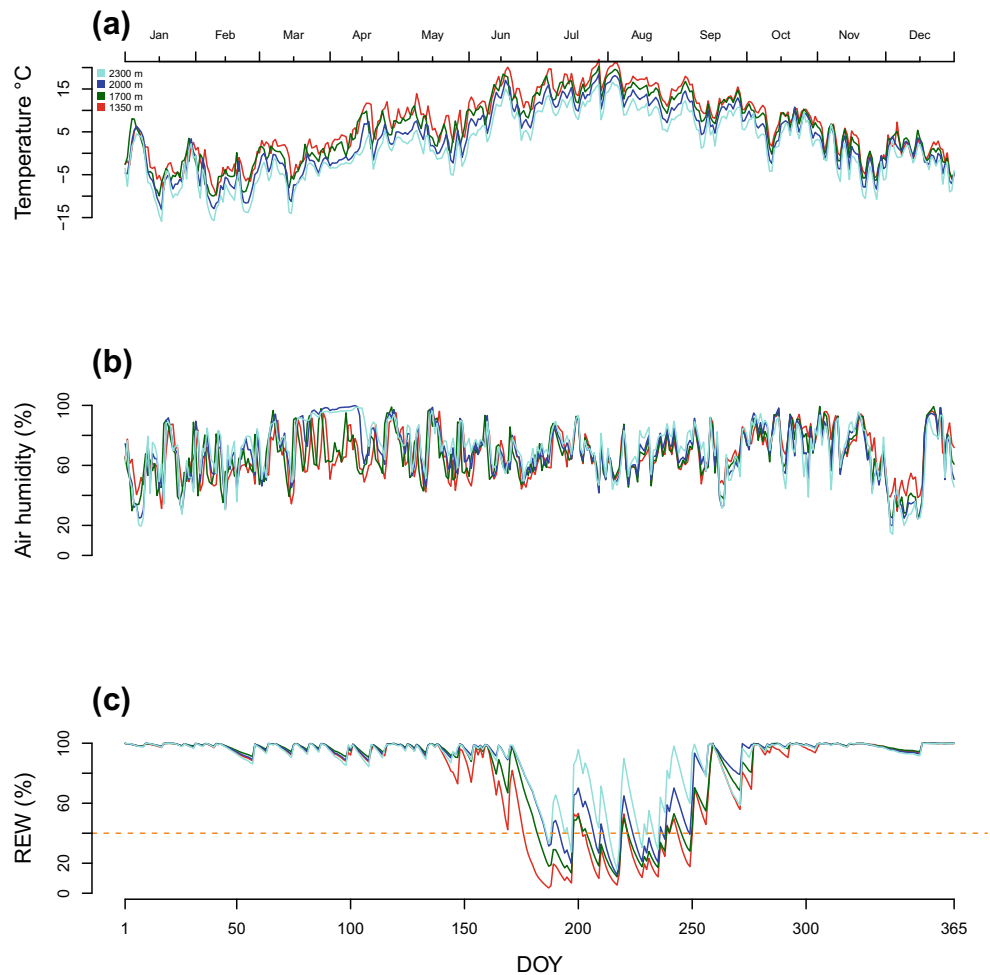
2.6.1 In situ weather stations

Weather stations were installed along the elevation gradient in small clearings in the monitored stands to record meteorological data. The daily minimum, maximum, and mean temperatures along with air humidity, vapor pressure deficit, global radiation, and wind speed were directly obtained from these local weather stations (Fig. 2a, b). Meteorological data from our own weather stations are available upon request on a dedicated website (https://w3.avignon.inra.fr/storm/Accueil_GRAAL.php).

Additionally, the minimum and maximum temperatures were used to compute the sum of growing degree days, i.e. the amount of heat accumulated above a threshold temperature (McMaster and Wilhelm 1997), which is classically fixed at 5 °C (GDD5) (Grigorieva et al. 2010).

$$GDD = \sum \left(\frac{T_{\min} + T_{\max}}{2} \right) - T_{\text{base}} \quad (1)$$

Fig. 2 Seasonal climatic course for the year of 2013 and the four plots of the elevation gradient. **a** Daily mean temperature. **b** Daily air humidity. **c** Relative extractable water (REW) (the yellow line shows the critical REW). Daily temperature and air humidity are recorded directly on the plot using in situ weather stations; REW is simulated using a water balance model



Vapor pressure deficit (VPD), i.e. the difference between the saturated air and the actual air vapor pressure, was computed using the air humidity data (Jones 1992).

$$\text{VPD(kPa)} = ((100 - \text{Air Humidity})/100) \quad (2)$$

$$* (6.11 * \exp(17.27 * T / (T + 273)))$$

2.6.2 Local meteorological stations

Daily precipitations (and temperatures) from Villard St. Pancrace meteorological station (1300 m a.s.l.) were provided by Météo-France. In addition, we used the Worldclim global data base (<http://www.worldclim.org>) to compute, based on linear regressions, monthly augmentation rates of precipitation with elevation in the study area. Then, using Villard St. Pancrace daily data and monthly augmentation rates from Worldclim, we extrapolated daily precipitations for the four studied plots (1350, 1700, 2000, and 2300 m a.s.l.).

The photoperiod for the study site was computed using the “geosphere” R package (Karney 2013). This package computes the annual course of day length of a site based on its geographical position.

2.6.3 Soil water balance assessment

In order to assess the soil water balance at each site, we used the Biljou model (Granier et al. 1999), which is designed to quantify drought intensity and duration in forest stands (<https://appgeodb.nancy.inra.fr/biljou/>). The inputs of this model are daily meteorological data such as the mean air temperature, total precipitation, global radiation, relative humidity, and wind speed. The model also needs stand characteristics (such as forest type or maximum leaf area index) and soil characteristics (such as the gravimetric water content, proportion of roots, depth of the organic layer (cm), water reserve (mm), and

Table 2 Soil characteristics of the studied sites

Site elevation (m)	Soil water capacity (mm)	Soil type
2300 m	38.7	Calisol eutric
2000 m	53.2	Brunisol/calisol
1700 m	66.7	Colluviosol
1350 m	46.5	Regosol/colluviosol

bulk density). The maximum leaf area index was estimated here using the data base of the model for different species. The soil type and maximal soil water capacity were measured directly for each stand (Table 2). The depth of soil layers and the proportion of fine roots per layer, water reserve, and soil bulk density were estimated for two soil layers using soil water capacity and soil type data based on the reference books of the model.

In this model, water stress is assumed to occur when the amount of relative extractable (soil) water (REW) drops below a critical threshold of 0.4 (REW_c, unitless), under this threshold stomata are assumed to close progressively, leading to a gradual reduction of tree transpiration. The outputs of the model are the number of days of water stress (WD_{dur}) and a water stress index (*I_s*), which cumulates the area between the daily course of REW and REW_c (Fig. 2c).

2.7 Data analysis

In order to investigate the link between elevation and wood formation phenology, we compared the medians of critical dates and durations from each plot using bootstrap tests, thanks to a dedicated function of the CAVIAR package (Rathgeber et al. 2011a). To assess the effects of environmental factors (temperature, water availability, and photoperiod) on the onset or cessation of xylem formation, we compared the daily parameters averaged over spring (from March to July) or autumn (from August to November) between sites, also using bootstrap tests on the medians. Although the seasonal course of the photoperiod is exactly the same along the elevation gradient, computing the photoperiod occurring at the very time of the critical dates results in different values for each individual tree. Comparing then these values together indicates if photoperiod is an important factor (trees should then exhibit similar values) or not (trees should then exhibit significantly different values).

To more specifically assess the effect of temperature on the onset and cessation of xylem formation, we developed two different approaches. First, we averaged the mean daily temperatures over 1 week before bE and cE for each tree and compared their medians between the plots using bootstrap tests. Second, we computed the GDD5 at the onset and cessation of cambial activity for each tree and compared again their medians between the plots using bootstrap tests. Moreover, in order to check that the changes observed in the phenology of wood formation along the elevation gradient were not due to the effect of age or diameter but to the environment, we sub-sampled a group of five trees per site presenting similar age and DBH values (Fig. 1, Table 7), and we repeated all the analyses again.

3 Results

3.1 Climatic factors along the elevation gradient

The total annual precipitations and the mean annual temperatures of the year of 2013 obtained from Villard St. Pancrace meteorological station were compared with the other years of the period 2004–2015. The 2013 mean annual temperature ($8.1\text{ °C} \pm 1$) was not significantly different from the mean of the other years of the period ($8.5\text{ °C} \pm 0.5$). The total precipitation of 2013 (702 mm) did not differ from the mean of the other years of the period ($634\text{ mm} \pm 40$). Thus, we concluded that the year 2013 represents well the normal climate of the studied site.

3.1.1 Temperature changes along the elevation gradient

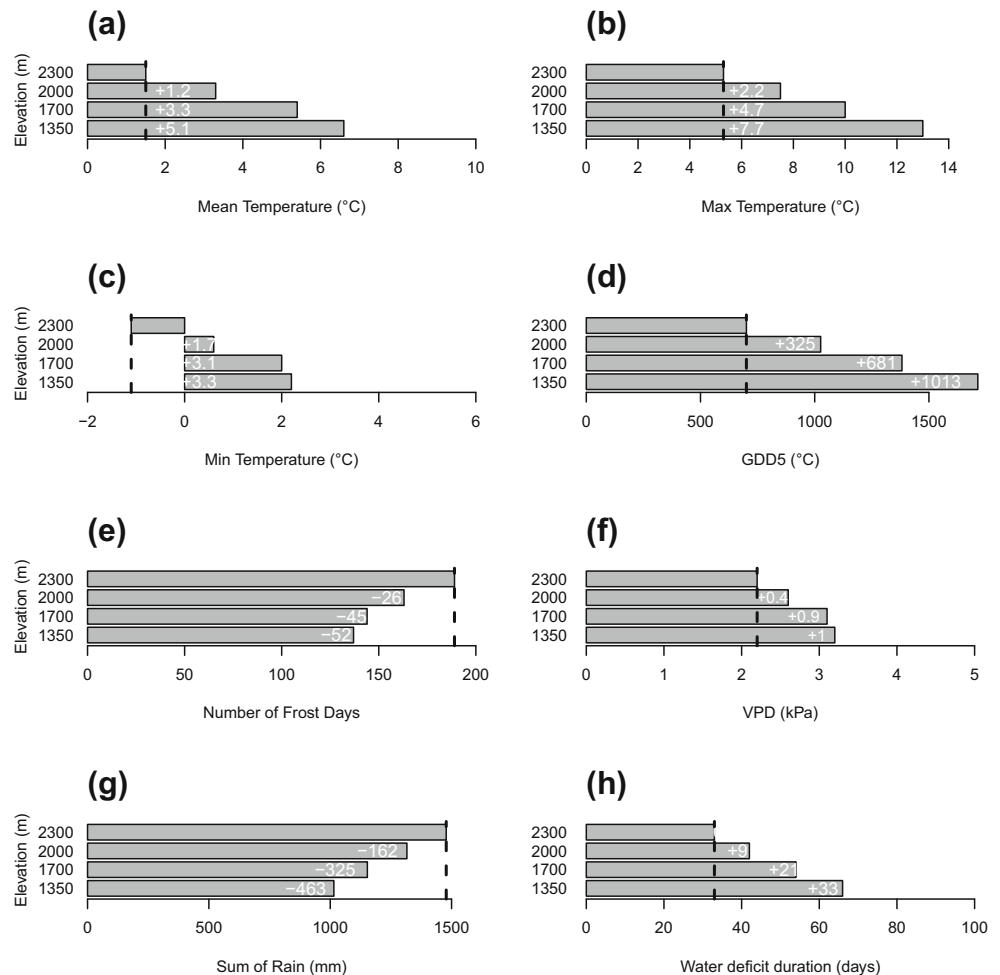
The comparison of the mean annual temperatures recorded on the four plots along the elevation gradient revealed a significant linear decreasing trend of about $-0.5\text{ °C}/100\text{ m}$ (Fig. 3a, Table 3). The minimal and maximal annual temperature confirmed this linear trend (Fig. 3b, c, Table 3).

The comparison of GDD5 along the elevation gradient also showed a decreasing trend with increasing elevation. The GDD5 value decreased by more than half from the bottom to the top of the gradient (Fig. 3d, Table 3). Interestingly, the number of frost days increased by 50 days from the lowest toward the highest stand (Fig. 3e, Table 3).

3.1.2 Water balance changes along the elevation gradient

Vapor pressure deficit (VPD) exhibited high values (indicating high evaporative demand) and a strong linear trend with values increasing from 2.2 ± 0.09 (kPa) at the top stand to 3.2 ± 0.1 (kPa) at the bottom one (Fig. 3f, Table 3). The total annual precipitation was about 1015, 1153, 1316, and 1478 mm for the plots located at 1350, 1700, 2000, and 2300 m, respectively. It showed an increase of about 50% from the lowest elevation toward the highest (Fig. 3g). The duration of water deficit (WD_{dur}) was the longest at the bottom site (66 days) and decreased toward the higher elevations (54, 42, 33 days at 1700, 2000, and 2300 m, respectively) (Fig. 3h). The bottom site also presented the highest value of water stress intensity (*I_s* index of 33.3). The value of *I_s* decreased with moving to higher elevation (*I_s* of 20.3, 15.5 and 12.7 at 1700, 2000, and 2300 m respectively). These indicators suggest that water stress is a serious constraint at the bottom site, but it gradually decreased when moving upwards.

Fig. 3 Annual climatic data comparison at the four study plots along the elevation gradient. **a** Annual mean of daily mean temperature. **b** Annual mean of daily maximum temperature. **c** Annual mean of daily minimum temperature. **d** Annual growing degree days (GDD5). **e** Number of frost days. **f** Annual mean vapor pressure deficit (VPD). **g** Annual total amount of rain. **h** Total number of water deficit days. The white numbers show the differences of a plot relative to the value recorded at the highest plot



3.2 Phenology of wood formation

3.2.1 Onset of the xylogenesis phenophases

The onset of the enlargement phenophase (bE) occurred around May 22 at the lowest elevation and progressed then toward the higher elevations (Table 4). The regression analysis of these dates revealed a linear trend ($P < 0.05$), which can be translated into a delay of 2.7 days per 100 m (Fig. 4a). The

Table 3 Linear regression analysis between elevation and the mean, minimum, and maximum annual temperatures (MAT, NAT, XAT), vapor pressure deficit (VPD), and growing degree days of base 5 °C (GDD5) for the 2013 studied year along the elevation gradient

	Equation	P	R^2
NAT	$-0.0030\text{Alt} + 7.8$	< 0.001	0.63
XAT	$-0.0080\text{Alt} + 23.0$	< 0.001	0.45
MAT	$-0.0054\text{Alt} + 14.3$	< 0.001	0.97
VPD	$-0.001\text{Alt} + 4.9$	< 0.001	0.41
GDD5	$-0.48\text{Alt} + 13$	< 0.001	0.82

bootstrap tests on the medians confirmed statistically significant differences between bE from all the plots except between the 1350- and 1700-m plots (Table 5).

The wall-thickening phenophase (bW) started about 3 weeks after bE (Table 4). The regression analysis also revealed a linear trend parallel to bE and of a similar slope with 2.6 days per 100 m ($P < 0.001$) (Fig. 4b). The bootstrap tests on the medians confirmed that the differences between the onsets of the wall-thickening phenophase were statistically significant between all the elevations (Table 5).

The appearance of the first fully mature cells (bM) was also observed about 3 weeks after bW (Fig. 4c, Table 4). The regression analysis of these dates revealed a less steep linear trend of 1.5 days per 100 m ($P < 0.001$). The bootstrap tests also confirmed that the differences between sites were statistically significant, except again for the 1350- and 1700-m plots (Table 5).

3.2.2 Cessation of the xylogenesis phenophases

The cessation of the enlargement phenophase (cE) exhibited a parabolic trend along the elevation gradient (Table 4, Fig. 4d).

Table 4 Means \pm standard errors for the critical dates of wood formation (bE, bW, bM, cE, cW) and the duration of enlargement, thickening, and xylogenesis periods (dE, dW, dX) for each site along the elevation gradient

Site elevation (m)	bE (DOY)	bW (DOY)	bM (DOY)	cE (DOY)	cW (DOY)	dE (Days)	dW (Days)	dX (Days)
2300	169 \pm 1	194 \pm 1	213 \pm 3	259 \pm 3	294 \pm 2	91 \pm 3	101 \pm 2	126 \pm 2
2000	158 \pm 1	187 \pm 1	206 \pm 2	260 \pm 2	295 \pm 1	102 \pm 3	108 \pm 2	138 \pm 2
1700	148 \pm 3	179 \pm 2	201 \pm 2	263 \pm 3	296 \pm 2	116 \pm 4	118 \pm 3	149 \pm 4
1350	143 \pm 2	169 \pm 1	198 \pm 1	253 \pm 3	289 \pm 3	110 \pm 3	120 \pm 3	146 \pm 3

The regression analysis of these dates did not show any significant linear trend. The bootstrap tests did not find any significant differences between the plots except for the 1350- and 1700-m plots (Table 5).

The cessation of wall-thickening phenophase (cW) also exhibited a parabolic trend along the elevation gradient (Table 4, Fig. 4e). Both the regression analysis and the bootstrap tests on these dates did not show any significant trend (Table 5).

3.2.3 Duration of enlargement, thickening, and xylogenesis periods

The observed duration of the enlargement period (dE) was about 3 months and 3 weeks at 1350 m, 3 months and 4 weeks at 1700 m, about 3 months and 1 week in 2000 m, and

3 months at 2300 m (Table 4). The regression analysis did not show any linear trend. However, the bootstrap tests showed that the highest plot exhibited shorter dE than the other ones (Table 5). The observed duration of the thickening period (dW) presented pretty much the same pattern as dE (Table 5).

The total duration of wood formation (dX) also showed a parabolic trend along the elevation gradient (Fig. 4f). The shortest period of wood formation was observed at the top site and lasted about 4 months, while the longest duration occurred at 1700 m and lasted about 5 months. dX was less than 5 months for the two remaining sites (Table 4). The regression analysis of these dates did not show any linear trend. However, the bootstrap tests showed that the differences between the plots were statistically significant, except for the 1350- and 1700-m elevations (Table 5).

Fig. 4 Phenology of wood formation along the elevation gradient. **a** Onset of enlargement period (bE). **b** Onset of wall-thickening period (bW). **c** Appearance of the first mature cells (bM). **d** Cessation of enlargement period (cE). **e** Cessation of wall-thickening period (cW). **f** Wood formation duration (dX)

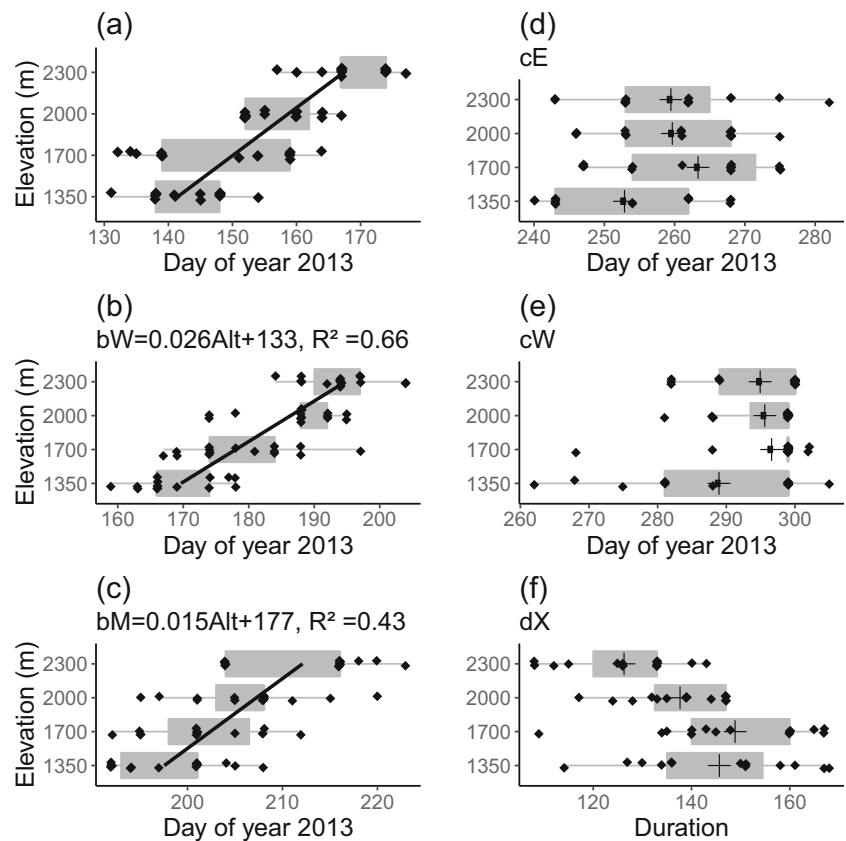


Table 5 The results of bootstrap tests (i.e. P values for 1000 iterations) between each pair of plots along the elevation gradient for the beginning of the enlarging period (bE), the beginning of the wall-thickening period (bW), the beginning of the mature period (bM), the end of the enlarging period (cE), the end of the wall-thickening period (cW), the duration of

the enlarging period (dE), the duration of the wall-thickening period (dW), and the duration of the total xylogenesis period (dX). The significant P values are indicated using * (with $***P < 0.01$, $**P < 0.05$, $*P < 0.1$)

Elevations	bE	bW	bM	cE	cW	dE	dW	dX
1350 vs 1700	0.17	0.005***	0.48	0.046**	0.50	0.15	0.35	0.46
1350 vs 2000	0***	0.002***	0.029**	0.20	0.46	0.31	0.028**	0.02**
1350 vs 2300	0.008***	0***	0.005***	0.12	0.38	0.002***	0***	0.005***
1700 vs 2000	0.017**	0.043**	0.007***	0.16	0.49	0.10	0.035**	0.01**
1700 vs 2300	0.014**	0.003***	0***	0.20	0.23	0.003***	0.005***	0.005***
2000 vs 2300	0.02**	0.018**	0.043**	0.41	0.27	0.02**	0.006***	0.13

3.3 Effect of environmental factors on the onset and cessation of the enlargement phenophase

3.3.1 Comparison of the temperature at the onset and cessation of the enlargement along the elevation gradient

The steep increase in temperature observed in April 2013 did not trigger the onset of the enlargement phenophase in the

studied stands (Fig. 5a–d). Surprisingly, at the lowest elevations, bE occurred in May (early May at 1350 m and late May at 1700 m), when temperatures were decreasing. At the highest elevations, bE occurred in June (beginning of June at 2000 m and mid-June at 2300 m) when temperature were rising again. The cessation of the enlargement period occurred late June (at 1350 m) or early September (at 1700, 2000, and 2300 m) when air was warming up again after a period of cooling (Fig. 6a–d).

Fig. 5 Course of the mean daily temperatures from March to July at the four studied stands. The dark green lines show the average date for the onset of the enlargement phenophase (bE) for each stand, while the light green areas show bE full range of variation for all the trees in a stand

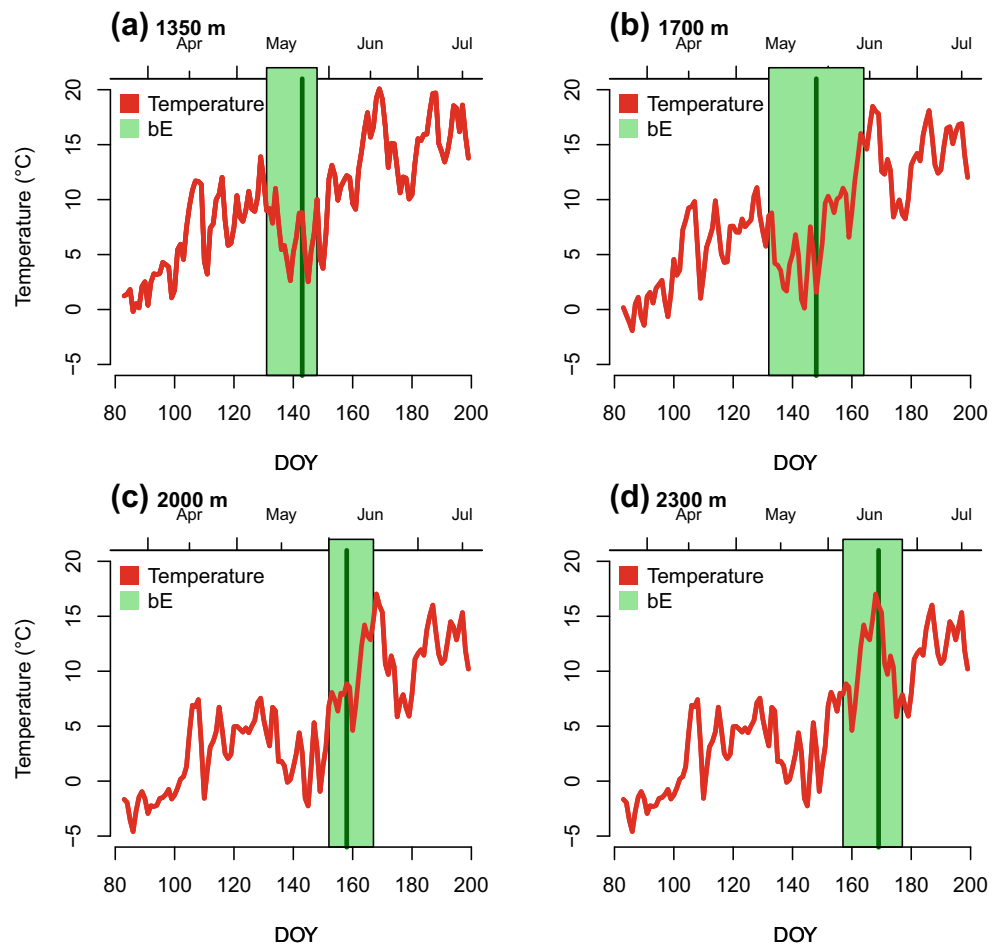
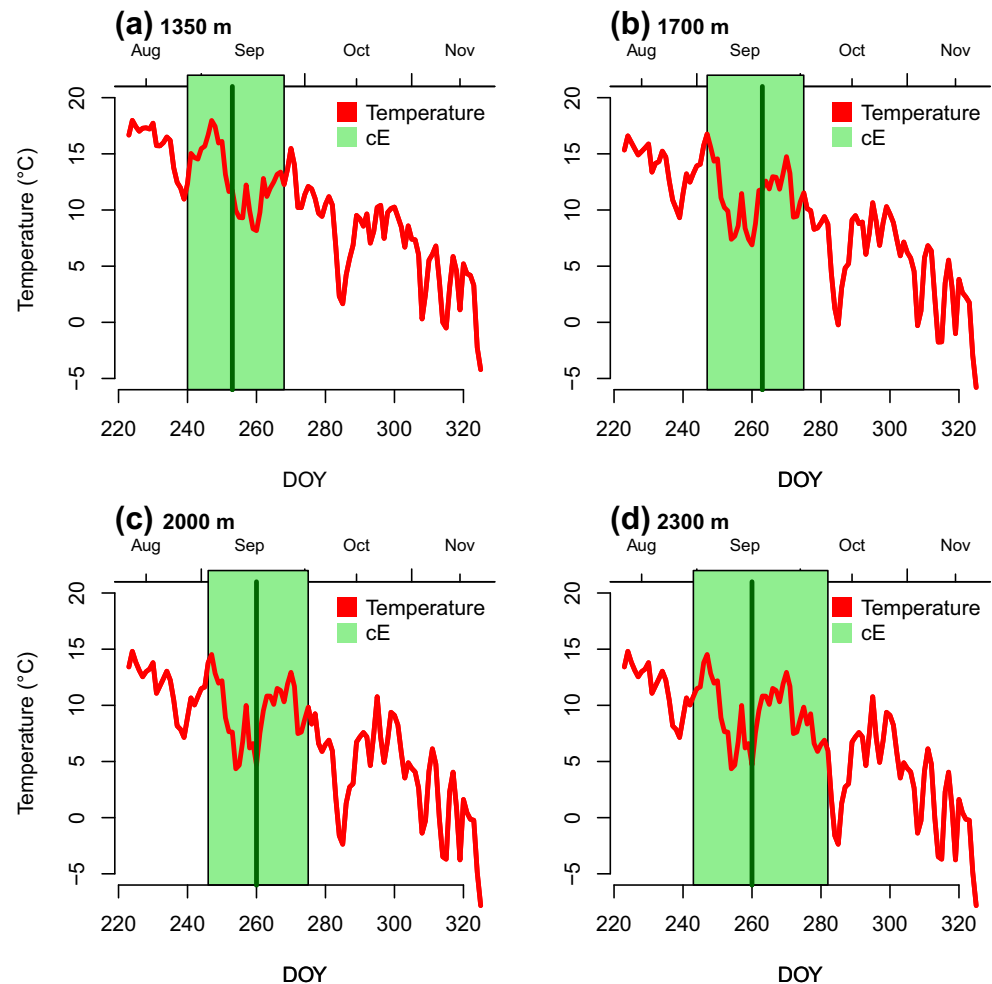


Fig. 6 Course of the mean daily temperatures from August to November at the four studied stands. The dark green lines show the average date for the cessation of the enlargement phenophase (cE) for each stand, while the light green areas show cE full range of variation for all the trees in a stand



The average temperatures of the week before bE exhibited a parabolic trend (Fig. 7a), with large variations in values comprised between 5 and 8 °C (7.07 ± 0.3 °C at 1350 m, 7.3 ± 0.6 °C at 1700 m, 5.3 ± 0.8 °C at 2000 m, and 8.6 ± 0.6 °C at 2300 m). The bootstrap test analysis revealed significant differences between the sites ($P < 0.01$) except for the two lowest elevations.

The average temperatures of the week before the cessation of the enlargement period showed a significant decreasing trend toward the higher elevation (Fig. 7b), with the values of 12.5 ± 0.3 °C at 1350 m, 12 ± 0.3 °C at 1700 m, 9.7 ± 0.14 °C at 2000 m, and 7.2 ± 0.4 °C at 2300 m. cE occurred in September during a period when temperatures were generally decreasing in all the studied stands. However, looking more in details, at 1700, 2000, and 2300 m, cE occurred during a short spell of temperature increase inside this general period of temperature decrease (Fig. 6). The temperatures occurring when enlargement phenophase stopped were much higher than the temperatures observed when it started, except for the highest plot.

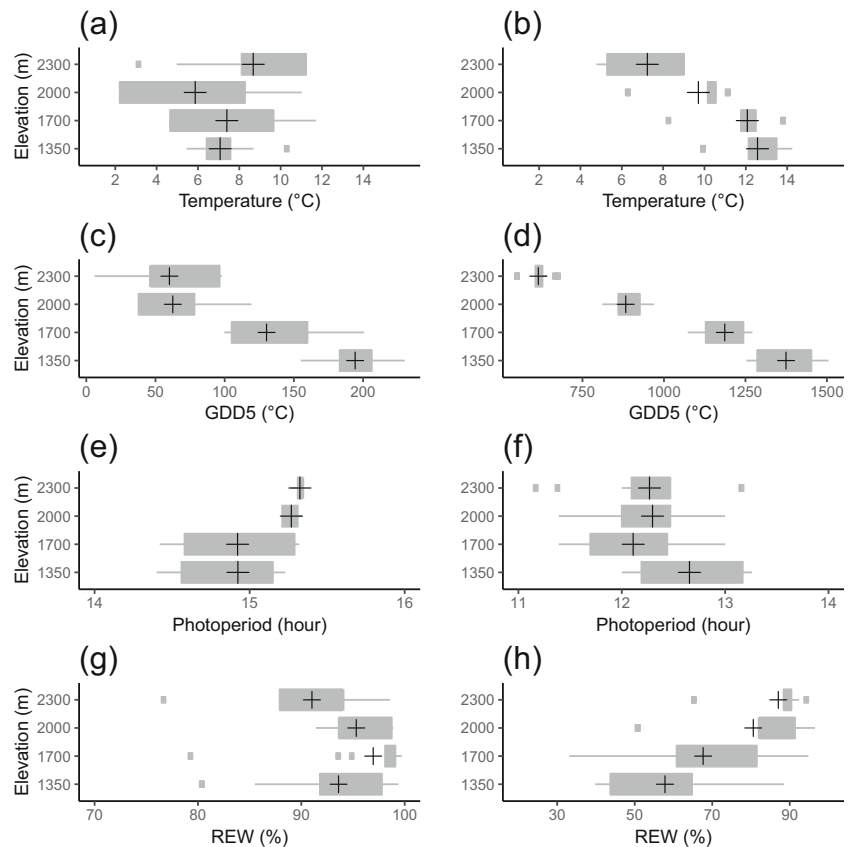
The comparison of the GDD5 requirements for the onset of the enlargement period showed a strong decrease in the

amount of GDD5 with increasing elevation along the gradient (Fig. 7c), with a GDD5 of 194 ± 4 °C at 1350 m, 130 ± 8 °C at 1700 m, 62.5 ± 2 °C at 2000 m, and 60 ± 8 °C at 2300 m. According to the bootstrap tests, the different sites exhibit different GDD5 values for bE. The comparison of the GDD5 requirements for the cessation of the enlargement period also showed a decrease with increasing elevation (Fig. 7d), with GDD5 of 1374 ± 25 °C at 1350 m, 1186 ± 19 °C at 1700 m, 882 ± 12 °C at 2000 m, and 614 ± 8 °C at 2300 m. According to the bootstrap tests, the different plots exhibited different requirements of GDD5 for cE.

3.3.2 Comparison of the photoperiod at the onset and cessation of the enlargement along the elevation gradient

The onset of the enlargement period occurred at a mean day length of about 15 h (Fig. 7e), a bit less for the lowest stands (14.76 ± 0.04 h at 1350 m and 14.64 ± 0.07 h at 1700 m) and a bit more for the highest ones (15.46 ± 0.02 h at 2000 m and 15.56 ± 0.07 h at 2300 m). The bootstrap test analysis confirmed this visual impression ($P < 0.01$). The mean day length

Fig. 7 Change in the studied environmental factors along the elevation gradient for the onset and cessation of the enlargement phenophase. **a** Average temperature of the week before the onset of enlargement (bE). **b** Average temperature of the week before the cessation of enlargement (cE). **c** Growing degree days (GDD5) at bE. **d** Growing degree days at cE. **e** Day length at the bE. **f** Day length at the cE. **g** Relative extractable water (REW) at the bE. **h** REW at the cE



was much lower when the cessation of the enlargement occurred: 12.7 ± 0.14 h at 1350 m, 12.20 ± 0.14 h at 1700 m, 12.38 ± 0.12 h at 2000 m, and 12.40 ± 0.14 h at 2300 m (Fig. 7f). According to the bootstrap tests, the different sites exhibited similar day lengths ($P < 0.01$) except the plot at 1350 m.

3.3.3 Comparison of the water availability at the onset and cessation of the enlargement along the elevation gradient

The relative extractable water (REW) at the onset of the enlargement was very high (Fig. 7g), with values of 93 ± 1 (%) at 1350 m, 96 ± 1 (%) at 1700 m, 95 ± 0.7 (%) at 2000 m, and 91 ± 1 (%) at 2300 m. Comparison of the REW at the cessation of enlargement period showed an increasing trend from the bottom to the top site (Fig. 7h), with values of 57 ± 4 (%) at 1350 m, 67 ± 5 (%) at 1700 m, $80 \pm$ (%) at 2000 m, 87 ± 2 (%) at 2300 m. The results of bootstrap test showed significant differences between the median of all the different sites ($P < 0.05$).

3.3.4 Removing the potential effect of age and diameter on the phenology of wood formation

In order to check that the effects of age and size were secondary compared with environmental factors, we sub-sampled

five trees per elevation that had similar age and DBH (Fig. 1), and we processed the selected data a second time. This verification confirmed all the results we exposed previously, only slightly changing P values, correlation coefficients, and equation parameters.

4 Discussion

4.1 “Natural experiment” of wood formation monitoring along a 6 K temperature gradient

In this study, we took advantage of a 1000-m elevation gradient located in the French Southern Alps to investigate the phenology of wood formation of 60 larch trees growing in four stands along the gradient (at 1350, 1700, 2000, and 2300 m a.s.l.) and experiencing very contrasted climatic conditions. The top and bottom plots, for example, exhibit a difference of 6 °C in mean annual temperature.

Elevation gradients are powerful natural experimental design in ecological studies. They provide unique basis to look at the response of organisms to geophysical factors (such as temperature) in their natural habitat (Korner 2007). One of the difficulties while working with “natural experiments” is to sample comparable individuals (e.g. same age, size, and genotype) all along the gradient, which is hardly possible. In our

case, the age and size of the selected trees were not perfectly balanced along the gradient with younger and thinner trees (about 120 years old and 41 cm in DBH) located at the top site and older ones (about 190 years old and 48 cm in DBH) at intermediate elevation (2000 m). Previous studies have shown that age and size matter in phenology of wood formation. Indeed, Rossi et al. (2008a) have shown that old larch trees (200–350 years old) exhibited 3-week shorter growing seasons than adult (50–80 years old) ones at the tree line in the Italian Dolomites. Additionally, Rathgeber et al. (2011b) have shown that large dominant silver firs exhibited nearly 2-month longer growing seasons than small suppressed ones of the same age in a plantation grown under temperate climate. However, in our monitoring design, all the selected trees were dominant and the differences in age and in diameter, even if significant, are smaller than the one reported in the two previous publications. Anyway, we repeated all the analyses on a sub-sample of 5 trees per elevation, which were comparable in age and in diameter (Fig. 1), and we did not find any noticeable differences with the results obtained on the full dataset. We are then confident that the results we reported concerning the changes in wood formation phenology along our 1000-m elevation gradient are due to the influences of environmental factors and not to tree or stand characteristics.

Several papers showed the absence of clear genetic structure in forest stands along elevation gradients in the Alps (King et al. 2013; Nardin et al. 2015). The low genetic differentiation found among tree populations along the gradients indicates high gene flow and suggests that migration rate is high enough to counteract the selective pressure of local environmental variations. This means that the phenological differences observed between the stands are not due to the genetic structure of the population but can be interpreted directly as the phenotypic response of the trees to the influence of their environment. These papers conclude that elevation gradients are extremely valuable design for understanding climatic-driven changes over time and space.

4.2 Temperature increases in spring trigger the onset of xylem formation of larch trees

The delay in the onset of cambial activity and wood formation phenophases with increasing elevation can be associated with the adiabatic temperature decrease and thus translated into a lengthening of the growing season of about 5 days per °C. Moser et al. (2010) working also with larch trees on a comparable gradient of about 800 m in the Northern Swiss Alps have found a slightly higher delay of 7 days/°C. Wang et al. (2014) have found exactly the same rate of change (7 days/°C) for Qilian juniper (*Juniperus przewalskii*) along a gradient of about 700 m in northwestern China. Zhang et al. (2018), however, have reported a nearly double rate of change of

14.1 days/°C also for the Qilian juniper along a 400-m elevation gradient on the Tibetan Plateau despite a similar lapse rate of -0.58 °C per 100 m. Vitasse et al. (2018) also found the elevation-induced phenological shift (EPS) from 34 days/1000 m in 1960 to 22 days/1000 m in 2016. This EPS of 22 days per 1000-m elevation is very close to our observation of 27 days/1000 m for the onset of cambial activity along our studied gradient.

All these evidences point out to temperature as the main factor controlling the onset of wood formation in trees. Temperature allows the metabolic process of growth to be completed and can influence biomass accumulation in cell walls (Antonova and Stasova 1993; Begum et al. 2007; Rossi et al. 2008b). Applying heat to tree stems induced reactivation of cambium and cell divisions and led to xylem and phloem formation (Oribe and Kubo 1997; Oribe et al. 2001; Gricar et al. 2006). Rossi et al. (2008b) found common critical temperature thresholds for xylogenesis in conifers at different latitudes and altitudes in Europe and Canada, with average values of 8–9 °C for daily mean temperature.

However, looking into details how temperatures influence wood formation did not reveal any clear functional mechanism. Indeed, our results did not add more credit to the threshold value approach (Rossi et al. 2008b) since we showed that the average temperature during the 7 days preceding the onset or cessation of xylem enlargement varied inconsistently between them and along the gradient. Moreover, our results showed that smaller amounts of growing degree days above 5 °C (GDD5) are needed for the onset of cambial activity with increasing elevation. Gricar et al. (2014) also found the same negative relationship between GDD and elevation in Norway spruce. In contrast, Schmitt et al. (2004) found similar GDD values for the onset of cambial activity in Scots pines growing along a latitudinal gradient in the boreal forests in Finland. These contrasted results suggest that growing degree days do not capture correctly the influence of the forcing temperatures on wood formation along elevation gradients. Thus, we confirmed in this study that temperature has a major influence on wood formation phenology, but the mechanisms through which it controls cambium activity remain hidden.

More complete approaches could include the influence of the chilling on the timing of forcing temperature accumulation (Jackson 2009). Indeed, the resting period of the cambium is nowadays seen as composed of three stages of dormancy: paradormancy, endodormancy, and ecodormancy (Begum et al. 2013; Delpierre et al. 2016; Rathgeber et al. 2016). The resumption of cambial activity can be triggered during late winter by an artificial heating of tree stems (Oribe et al. 2003; Begum et al. 2007, 2010). Interestingly, this artificial resumption of cambial activity can only be triggered during

the ecodormancy phase (referred to as the “quiescent phase” in Oribe and Kubo 1997 and Begum et al. 2013), after the chilling requirement has been fulfilled during endodormancy (referred to as the “rest phase” in Little and Bonga 1974 and Begum et al. 2013). This body of results clearly demonstrates that cambium division does not simply respond to the occurrence of certain temperature thresholds but that other environmental cues are involved in the timing of cambium activity. Our results also point in the direction of the influence of chilling requirement for larch. Indeed, a lower requirement of forcing temperatures with increasing elevation for larch may reveal an influence of greater accumulations of chilling temperatures. This approach was developed with success in Delpierre et al. (2019), where it has been shown, using data from our gradient as well as data from three other networks, that the chilling-influenced heat sum model gives the best results for predicting the onset of cambial activity in larch trees. Additionally, the role of other factors such as soil temperature could be explored since some authors found that an increase in the soil temperature is needed for the resumption of cambial activity (Lupi et al. 2012; Jyske et al. 2012; Tremblé et al. 2015).

Interestingly, we saw in our study that the onset of all phenophases of xylem formation (enlarging, wall thickening, and mature) followed the same trend along the elevation gradient, empirically showing a strong relationship between them. This suggests that either they are under the control of the same environmental factors (temperatures) or more probably they follow each other in a predetermined cascade of events (Lupi et al. 2010; Rossi et al. 2012). Temperature may control the onset of cambial activity, which triggers then the onset of cell enlargement and so on. Testing this conceptual model remains however tricky since the observations of the onset of cambial activity are less reliable than observations for the other phenophases (Rathgeber et al. 2016).

4.3 Photoperiod and water stress control the cessation of xylem formation of larch trees

We observed in this study that the cessation of wood formation phenophases occurred in a parabolic pattern along the elevation gradient with trees from the lowest stand finishing first, followed by those from the highest stand, while those from the intermediate elevations finished the last. These results indicate that the cessation of wood formation is probably under the control of several independent environmental factors following different patterns along the gradient. Previous studies on wood formation along elevation gradients reported also that the cessation of cambial activity and wood formation was less related to elevation (Cuny et al. 2012, 2018; Moser et al. 2010; Wang et al. 2014). Our results suggest that for larch trees in the south of the Alps, the cessation of cambial activity occurs before the day length drops to about 12 h. Moser et al. (2010) found parallel cessations of wood

formation phenophase in larch trees across different elevations in Swiss Alps that also support the photoperiod interpretation. The main argument in favor of a control of the cessation of wood formation by photoperiod is provided by the fact that trees at higher latitudes finish their growth earlier in autumn compared with trees at lower latitudes (Jackson 2009; Jyske et al. 2014; Rossi et al. 2014).

Concerning that the photoperiod length is the same along our studied gradient, the much different dates for the onset of cambial activity and quite the same dates for the end suggest that photoperiod has a minor role at the beginning of the growing season and a more prominent role at the end. However, in our site, photoperiod alone could not explain cessation of cambial activity since the bottom stand stops growing more than 10 days before the other stands. Compared with the other stands of our gradient, the bottom stand experienced the longest period of water deficit, the highest drought intensity, and the greater amount of vapor pressure deficit. It is documented now that low soil humidity can trigger an earlier conclusion of wood formation (Ren et al. 2015; Ziaco et al. 2016). Thus, our results suggest that the cessation of cambial activity and wood formation is mainly under the control of photoperiod but with water shortage being able to hasten it for dry sites or years.

4.4 Impact of the climatic changes on the duration of the growing season

The gradual delay in the onsets of cambial activity and wood formation and the parabolic trend in the cessations resulted in decreasing growing season durations with increasing elevations, with the shortest duration occurring at the 2300-m site (about 4 months) and the longest ones at the 1700-m site (about 5 months), are consistent with the results from previous studies. A reduction in the length of the wood formation period from 220 to 50 days was observed in nine conifer species from Europe and Canada that was consistent with the decrease of the site temperature (Rossi et al. 2013). Moser et al. (2010) also observed a decrease from 137 to 101 days in the length of the growing season toward the higher altitude in larch trees growing along a gradient of about 800 m in the Swiss Alps (Moser et al. 2010). Another example is the length decrease of the growing season from 5 to 2 months in Norway spruces from lower elevation to the Alpine tree line (Rossi et al. 2008b). These results agree with our observations of a shortened growing season from the middle to the top elevation, with a smaller order of magnitude of 150 to 126 days.

Trees growing at higher altitudes and latitudes experience less favorable climatic condition during their growth season and need to finish their wood formation in a limited time before the occurrence of very cold temperature. Adjusting

the length of the growing season is an optimization between exposition to frost damage and maximization of annual carbon assimilation (Chuine 2010). Regulating the phenological phases of xylem formation should guarantee enough time for latewood tracheids to finish their wall-thickening period (Lupi et al. 2010; Rossi et al. 2012). When the temperature is under 0 °C, the metabolism of trees decreases in order to maintain the minimum physiological activity and restrict the water uptake and root functioning (Korner 2003). Cuny et al. (2018) studied the cell wood formation in Norway spruce and larch trees growing along an 8 °C thermal gradient. They found longer duration of cell wall formation at colder elevations as a strategy for trees subjected to lower temperatures to maintain similar tree-ring structure.

The observed delay of 5 days/°C and the projected 2.5 to 3.5 °C temperature increase over France (Gosling et al. 2011) allow us to predict an earlier start of cambial activity of about 12–18 days in 2100 compared with 1960. Predicted higher temperature and corresponding earlier resumption of cambial activity will tend to lengthen the growing season. This increase in the length of the growing season should result in an increase in xylem cell production and tree-ring width (Rossi et al. 2014; Prislán et al. 2019). However, the length of the growing season is not enough to predict tree growth and forest production, and other factors such as growth rate need to be considered (Rathgeber et al. 2011b). Moreover, our results suggested that the cessation of cambial activity is more related to the photoperiod but that water stress can hasten the termination of the growing season at lower elevations. The more intense evaporation associated with the ongoing climatic warming will limit even more water availability and increase water demand. According to Pachauri et al. (2014), increased VPD due to higher temperature is the most critical parameter affecting the different ecosystems facing climatic change. Soil water content will decrease along the growing season while water consumption will be maximum at the middle of the growing season (Way and Sage 2008). In conclusion, the predicted climatic changes should affect the larch trees growing in the Southern Alps by lengthening their growing season (earlier onsets at all elevations but also earlier cessations at low elevations) but potentially decreasing their growth rate due to increased evaporative demand. Our results suggest that the optimal elevation for larch growth in the Southern Alps is expected to move upwards in the coming years.

5 Conclusions

Monitoring wood formation in larch trees along a 1000-m elevation gradient in the Southern French Alps, we observed that cambial activity started earlier at the bottom stand before progressing toward higher elevations following a linear trend. The onset of the other phenophases of xylem formation

followed the same trend but with a variable time lag of a few days (for cell enlargement phenophase) up to 1 or 2 months (for cell wall-thickening phenophase). On the other hand, the cessation of wood formation phenophases followed a parabolic trend with the trees from the lowest stand finishing first, followed by those from the highest one, while those from the intermediate elevations finished the last. Wood formation duration also exhibited a parabolic trend, with the shortest growing seasons occurring at 2300 m (about 4 months) and the longest one at 1700 m (about 5 months) which is then currently the optimal elevation for larch growth in the Southern Alps. The observed delay in the cambial activity onset with increasing elevation can be associated with the adiabatic temperature decrease and thus translated into a lengthening of the growing season of about 5 days per °C. On the other hand, the interplay between photoperiod and water stress more probably triggers cambial activity cessation. Indeed, water stress seems to be the main factor at the lowest elevation, while the influence of photoperiod progressively increases from the bottom to the top, where it could play the major role, in association with a minor role of temperature. This leads us to predict that future climatic changes will increase growing season length (but not necessarily tree growth) and shift upwards the optimal elevation for larch growth in the Southern Alps.

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Data availability The datasets generated and/or analyzed during the current study are available in the GloboXylo Initiative data base (<https://www6.nancy.inra.fr/foret-bois-lerfob/Projets/Projets-en-cours/GLOBOXYLO>) which is open from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Appendix

Table 6 Characteristics of the selected trees at each study site

Site (m)	Tree	DBH (cm)	Age (years)	Height (cm)	
1350	4382	45.44	131	2580	
	4383	36.94	154	NA	
	4384	42.19	149	2470	
	4385	41.56	NA	2180	
	4386	40.50	173	NA	
	4387	37.99	139	2230	
	4388	42.99	NA	2400	
	4389	33.02	136	NA	
	4390	39.04	NA	2155	
	4391	38.59	192	2310	
	4392	39.64	157	2175	
	4393	41.40	NA	1730	
	4394	36.30	NA	1960	
	4395	42.86	173	2355	
	4396	32.16	147	NA	
	Mean ± SE	39.38 ± 1	155 ± 6	2231 ± 72	
	1700	4397	55.41	107	2860
		4398	67.99	169	3160
		4399	46.49	150	2985
		4400	52.70	189	2800
4401		61.78	172	2795	
4402		44.90	163	2955	
4403		42.03	170	3020	
4404		39.17	127	2450	
4405		35.19	120	NA	
4406		46.97	137	2720	
4407		41.24	NA	2685	
4408		36.78	202	NA	
4409		40.60	104	2190	
4410		52.86	132	2340	
4411		49.04	181	2560	
Mean ± SE	47.54 ± 2	151 ± 8	2732 ± 78		
2000	4412	43.31	NA	2495	
	4413	43.31	189	3000	
	4414	48.08	192	2990	
	4415	51.75	162	NA	
	4416	34.87	173	1970	
	4417	49.36	190	2755	
	4418	54.93	168	2870	
	4419	43.31	175	2740	
	4420	49.84	208	2870	
	4421	56.05	233	2660	
	4422	56.21	210	2900	
	4423	57.48	217	2655	
	4424	38.69	NA	2410	
	4425	45.22	177	2700	
	4426	49.20	234	2645	
Mean ± SE	48.45 ± 2	194 ± 6	2690 ± 71		
2300	4427	42.67	154	1806	
	4428	39.96	NA	1725	
	4429	41.082	120	1775	
	4430	49.36	90	1930	
	4431	32.80	94	1710	
	4432	36.62	74	1540	
	4433	39.49	132	1335	
	4434	40.12	147	1505	
	4435	54.14	184	1710	
	4436	39.49	122	1660	
	4437	38.53	150	1690	
	4438	38.53	97	1780	
	4439	49.68	177	1950	
	4440	35.82	54	1725	
	4441	45.22	NA	1970	
Mean ± SE	41.57 ± 1	122 ± 10	1720 ± 43		

Table 7 Characteristic of the selected trees with similar age and diameter

Site (m)	DBH (cm)	Age (years)
1350	43 ± 0.6	168.2 ± 7
1700	44 ± 1	168.4 ± 5
2000	44.3 ± 2	171 ± 2
2300	43 ± 2	162.4 ± 7

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