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2   **forests**

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Henri E. Cuny[1,2,3*,] Cyrille B. K. Rathgeber[1,2], David Frank[3,4], Patrick Fonti[3], Harri Mäkinen[5],

Peter Prislan[6], Sergio Rossi[7,8], Edurne Martinez del Castillo[9], Filipe Campelo[10], Hanuš

Vavrčík[11], Jesus Julio Camarero[12], Marina V. Bryukhanova[13,14], Tuula Jyske5, Jožica Gričar[6],

Vladimír Gryc[11], Martin De Luis[9], Joana Vieira[10], Katarina Čufar[15], Alexander V.

Kirdyanov[13,14], Walter Oberhuber[16], Vaclav Treml17, Jian-Guo Huang[8], Xiaoxia Li1[8], Irene

Swidrak[16], Annie Deslauriers[7], Eryuan Liang[18], Pekka Nöjd[5], Andreas Gruber[16], Cristina

Nabais[10], Hubert Morin[7], Cornelia Krause[7], Gregory King[19] and Meriem Fournier[1,2]

[1] INRA, UMR 1092 LERFOB, F-54280 Champenoux, France.

[2] AgroParisTech, UMR 1092 LERFOB, F-54000 Nancy, France.

[3] Swiss Federal Research Institute WSL, CH-8903 Birmensdorf, Switzerland.

[4] Oeschger Centre for Climate Change Research, CH-3012 Bern, Switzerland.

[5] Natural Resources Institute Finland, PO Box 18, Vantaa 01301, Finland.

[6] Slovenian Forestry Institute, Vecna pot 2, Ljubljana 1000, Slovenia.

[7] Université du Québec à Chicoutimi, Chicoutimi, QC G7H 2B1, Canada.

[8] Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems, Provincial Key Laboratory of Applied Botany South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China.

[9] Department of Geography and Regional Planning, Environmental Science Institute (IUCA), University of Zaragoza, C/Pedro Cerbuna 12, Zaragoza 50009, Spain.

[10] Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, Coimbra 3000–456, Portugal.

[11] Faculty of Forestry and Wood Technology, Department of Wood Science, Mendel University in Brno, Zemědělská 3, Brno 613 00, Czech Republic.

28   [12] Instituto Pirenaico de Ecología (IPE-CSIC), Avda. Montañana 1005, Zaragoza 50192,

29   Spain.

30   [13] V.N. Sukachev Institute of Forest, SB RAS, Krasnoyarsk 660036, Russia.

31   [14] Siberian Federal University, 660041 Krasnoyarsk, Russia.

32   [15] Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, Ljubljana 1000, Slovenia.

33   [16] University of Innsbruck, Institute of Botany, Sternwartestrasse 15, Innsbruck 6020, Austria.

34   [17] Faculty of Science, Charles University in Prague, Prague CZ-12843, Czech Republic.

35   [18] Key Laboratory of Tibetan Environment Changes and Land Surface Processes and Key

36   Laboratory of Alpine Ecology and Biodiversity, Institute of Tibetan Plateau Research, Chinese

37   Academy of Sciences, Beijing 100101, China.

38   [19] Department of Geography, Queen's University, Kingston, Canada.

39   *e-mail: henri.cuny@wsl.ch

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Plant tissues annually sequester nearly half of the 120 Pg of carbon assimilated by photosynthesis[1], and thus contribute to uptake 15% of anthropogenic CO2 emissions[2]. Most of the carbon is sequestered in the wood, that acts as a long-term storage pool of carbon and represents about 80% of the terrestrial living biomass[3]. Biomass accumulation in northern hemisphere woody plants sensitively follows the seasonal cycles, with modifications that can strongly impact the global carbon cycle[1,4]. However, the seasonal dynamics of woody biomass accumulation remains poorly quantified, limiting our understanding of the terrestrial carbon cycle and its sensitivity to on-going climate change. Here we show a one month delay between radial size increase and carbon sequestration in trees during the season, based upon three years of weekly observations of stem size and carbon storage in a mature temperate forest. We expect the predicted changes in the annual cycle of temperature[5] to shift the phasing of stem size increase and carbon sequestration, as stem size increase closely matches photoperiod cycle, whereas woody carbon sequestration follows the seasonal course of temperature. Comparison of these detailed quantifications with a global dataset of wood formation phenology reveals that such a lag is a common feature in temperate, boreal, alpine, and Mediterranean forest biomes of the northern hemisphere. These results question widely applied definitions of growth and demonstrate that the seasonal dynamics of woody biomass accumulation cannot be inferred from stem size measurements[6-8]. Our study also provides new insights into the processes driving the annual cycle of CO2[9] and long-term terrestrial carbon storage[3], with important implications on future climate-carbon interactions.

< The importance of wood formation in shaping the seasonal carbon cycle >

The atmospheric CO2 concentration undergoes a seasonal cycle in the northern hemisphere with a 6 to 19 ppm amplitude[9], thereby representing intra-annual net fluxes

5

75 greater than the total annual anthropogenic emissions. This seasonal cycle in CO2

76 concentration is predominantly driven by the annual rhythm of terrestrial plant activity,

77 including the delicate balance between carbon uptake by photosynthesis, release by

78 respiration, and sequestration through plant tissue formation. Wood formation in particular is

79 the primary biological process through which carbon is sequestered on multi-annual to even

80 millennial time-scales in plants. In total, the sequestration in forest ecosystems is nowadays a

81 bit higher than the emissions, which contributes to a carbon sink that mitigates climate

82 change[2,10]. However, large uncertainties remain in the response of the global carbon cycle to

83 on-going climate change[11]. For example, enhanced seasonal amplitudes in the CO_2

84 concentration of the northern hemisphere observed over the last decades are suggesting large

85 ecological changes in northern forests and a major shift in the global carbon cycle[9]. In-situ

86 observations are thus required to identify and attribute the individual biological processes

87 controlling carbon cycling in terrestrial ecosystems and consequently the global carbon

88 budget

89 <How carbon fluxes in forests are measured>

90 Since the late 1990s, the "eddy covariance" technique has emerged as the primary

91 method to evaluate net carbon fluxes between forest ecosystems and the atmosphere[12]. This

92 method produces a direct measure of the CO2 exchanges across the interface between the

93 atmosphere and plant canopy from sub-hourly to yearly time scales. Furthermore, carbon

94 allocation among the different ecosystem components can be estimated from repeated stand

95 inventories[6], measurements of tree size changes[7] or litter fall[13], phenocams[14] or remote

96 sensing[15]. Yet, discrepancies between the seasonality of carbon fluxes and tree size

97 measurements[16] are indicative of the large uncertainties of terrestrial carbon dynamics[11]. This

98 is not surprising, as neither the eddy covariance technique nor the indirect methods allow

99 quantification of when carbon is locked in the wood or a mechanistic understanding of the

physiological processes responsible for the terrestrial carbon sink and long-term storage in woody tissues. A more detailed, process-oriented knowledge of woody carbon sequestration is therefore a timely and necessary endeavour to improve our understanding of terrestrial carbon cycle and its interaction with the climate.

<Define the aim of the study >

In this study, we unravel the seasonal dynamics and climatic sensitivity of stem size increase and woody carbon sequestration in trees. We achieve this by performing observations of wood formation dynamics and stem size changes at weekly resolution in three main European conifer species (silver fir, Norway spruce and Scots pine). Our detailed measurements of the forming and mature wood in concert with robust computation of cellular developmental kinetics allow us, for the first time, to quantify the magnitude and dynamics of seasonal carbon sequestration into stem wood with high temporal precision (Extended Data Fig. 1).

<Description of seasonal dynamics of processes>

Wood formation (comprehensive of the processes of cambial activity, xylem size increase and woody carbon sequestration) was active during almost 7 months, from mid-April to the beginning of November (Fig. 1). We found that cambial activity and xylem size increase had maximum activity at the end of May, with a secondary summer maximum for xylem cell production in the cambium (Fig. 1b). The dynamics of xylem size increase well-matched seasonal stem radius changes (Fig. 1a,c; Extended Data Fig. 2). This pattern was mainly shaped by the enlargement of the newly produced cells (80% of the total signal) and only marginally affected by the cell production activity of cambial tissue (Fig. 1c). Consequently, the rate of xylem size increase culminated in spring, at the time of maximal enlarging cells number (Fig. 1c) itself associated with the development of the large earlywood cells. In contrast, the seasonal rate of carbon sequestration in stem wood followed a

symmetric bell-shaped curve culminating in the first half of July (Fig. 1d). We estimated the formation of the primary walls (for cambial cell division and cell enlargement) accounts for only 20% of the total woody carbon sequestration, while the remaining 80% is driven by secondary wall formation and lignification (Fig. 1d). Surprisingly, the rate of woody carbon sequestration was maximal before the formation of the characteristic small and thick-walled latewood cells had even begun. This shows that, contrary to a long-lasting common wisdom[17,18], latewood formation does not coincide with maximum rates of carbon sequestration in wood.

<Exploration of the lag between xylem radial growth and woody carbon sequestration>

The quantification of the seasonal dynamics of xylem size increase and carbon sequestration was used to further explore the temporal divergence between these two processes. We observed a shift of 1.5 months between their respective maximal rates (Fig. 1c,d) and, at mid-September, when the xylem size increase had stopped, the carbon sequestration was still at almost 50% of its maximal rate. The time-lag was also striking when expressing both processes in terms of cumulated budget (Fig. 2a). To reach the same relative advancement, the carbon sequestration needed on average 26 days more than the xylem size increase. In that sense, 90% of the annual xylem size increase was completed by early August, but only 70% of the total carbon was permanently fixed into wood structures.

This temporal divergence is the result of xylogenesis, which chronologically separates size increase and carbon sequestration at the cellular level: a newly produced cell first enlarges and requires little carbon to extend its thin primary wall, and only when it reaches its final size, most of the carbon is fixed into the building and stabilisation of its thick secondary wall. Moreover, the kinetics of cell enlargement and cell-wall thickening follow divergent seasonal patterns, making cell size and cell-wall biomass content evolve differently[19].

149   The detailed quantification of carbon sequestration dynamics exposed in this paper

150   was achieved by microscopic observations of xylem cell development and computation of

151   associated kinetics (Extended Data Fig. 1). We see similar patterns in three common

152   coniferous species from nearby sites in temperate Europe, raising the question if our findings

153   universally apply to extra-tropical forests, including broadleaved species. Unfortunately, it is

154   impossible to directly answer this question owing to an absence of equivalent data from other

155   sites, but a preliminary larger scale assessment can be made from a global analysis of

156   phenological data. Indeed, a rough estimation of the mean lag can be obtained from the

157   differences observed between the beginning and the cessation of the periods of cellular

158   enlargement and wall thickening during the year (Figure 2b,c). From a global dataset of wood

159   formation phenology (53 sites covering the northern hemisphere; Extended data table 1), we

160   thus demonstrated that the lag between radial size increase and woody carbon accumulation is

161   a common feature of northern hemisphere forest biomes: we estimated a lag slightly inferior

162   to a month for alpine, boreal and temperate forests, and a lag about two times longer for

163   Mediterranean forests (Figure 3).

164   Our results question widespread conception and use of the term "growth" in biomass

165   studies. Classically, the term "growth" is used to define indifferently an irreversible increase

166   in organism size or mass due to metabolic processes. However, as plants cannot be weighed

167   easily, their mass gain is usually assessed by size measurement, according to the tight

168   relationships between body size and mass that exist for a wide range of organisms. Actually in

169   trees, allometric equations are thus widely applied to compute biomass or carbon content from

170   size measurements[20,21], and size increase and biomass gain are well related in space[22] or at

171   inter-annual time-scales[6,22]. This conception has further fuelled the assumption that tree size

172   increase and biomass gain (which is also carbon sequestration) are synchronised during the

173   season[6-8]. Importantly, our demonstration of a temporal dissociation between stem volume

174 increase and carbon sequestration invalidates this assumption. This means that at intra-

175 seasonal scale the carbon fixation to stem wood cannot be inferred from stem radial size

176 measurements. And above all, this provides rationale to clarify the misunderstood

177 discrepancies observed between Eddy-covariance and tree size data, like the poor

178 relationships between net ecosystem productivity and tree size changes at short time scales[23]

179 or the positive forest carbon uptake when radial sire increase is over in autumn[16].

180   Our work helps to better understanding the seasonal terrestrial carbon balance,

181 providing clues and methods to link forest atmosphere exchanges to sequestration in woody

182 organs on a seasonal time-step. Modelling the timing of processes that drives carbon fluxes in

183 the different ecosystem components is a key issue, especially for assessing how climate

184 change impacts these processes[24]. Therefore, our detailed mechanistic representation of when

185 and how carbon is seasonally locked in the wood provide crucial information on a major flux

186 component, which is important for further developing the process-oriented biosphere models

187 needed to constrain modern carbon budgets and predict future carbon–climate interactions[24].

188 <Relationships with environmental factors>

189   To explore for a possible different climatic sensitivity between xylem size increase and

190 carbon sequestration processes, we related their seasonal dynamics to seasonal environmental

191 conditions. We observed that the seasonal course of xylem size increase was best

192 synchronised with the light conditions, in particular with the photoperiod and to a slightly

193 lesser extent with the cumulative daily radiation (Fig. 4a). By contrast, the woody carbon

194 sequestration was highly synchronised with the seasonal course of temperature (Fig. 4b).

195 When looking more in details to the shape of the relationships during the season, it appears

196 that the rate of xylem size increase evolved quite linearly with photoperiod (Fig. 4c), while

197 carbon sequestration changed linearly with temperature (Fig. 4d).

198   These results provide new insights into the evolutionary adaptation of plants to their

199   environment. On one hand, the coordination between tree-ring size increase and photoperiod

200   can be interpreted as a way to safely respect the timing of the growing season. Indeed,

201   photoperiod attests a stable indicator of the time of the year that controls many developmental

202   responses in plants[25], including xylem production[26]. On the other hand, the synchronisation of

203   carbon sequestration with temperature could be rather linked to the strong metabolic

204   constraints that limit this process, which is particularly sensitive to temperature[27]. Finally, as

205   climatic changes involve shift in the phase of the annual cycle of temperature (in addition to

206   the global increase in mean annual temperature)[5], the phasing of stem volume increase and

207   carbon sequestration might change, with possible consequences on tree-ring structure, carbon

208   fluxes and carbon-climate interactions in woody ecosystems.

209

210   **Methods Summary**

211   To assess the seasonal dynamics of wood formation, small wood samples (microcores) were

212   collected weekly during 3 years (2007–2009) on the stem of 45 mature trees belonging to

213   three conifer species (silver fir, Norway spruce and Scots pine) and grown in three sites in

214   northeast France. Microcores were prepared in the laboratory, after what anatomical sections

215   were cut, stained, and analysed using an optical microscope to track wood formation process.

216   On the anatomical sections, we counted the cells in the different zones of differentiation along

217   the forming tree ring: cambial cells, enlarging cells, wall thickening and lignifying cells, and

218   mature cells. Generalised additive models were then applied on the cell count data to

219   characterise wood formation dynamics and to calculate the rates of cambial activity, xylem

220   size increase and wall material deposition in the tree ring. This latter was up-scaled at the tree

221   level from tree dimensions, and then was converted into a rate of carbon sequestration based

222   on an apparent density of the wall of 1.100 g cm^{-3}, and on a wood carbon content of 50% of

223 dry weight. The dynamics of stem radial variations was assessed from external measurement

224 of stem size variations using band dendrometers. We used a global xylogenesis phenology

225 dataset to estimate the timings of xylem size increase and woody carbon sequestration in other

226 forest biomes of northern hemisphere. To characterise the environmental conditions of the

227 studied area, daily meteorological data (temperature, precipitation, cumulative global

228 radiation, wind speed, and air relative humidity) of the period 2007–2009 were gathered from

229 three stations, and the soil water balance was modelled. To assess the synchronisation

230 between xylem size increase, carbon sequestration and environmental factors during the

231 season, cross-correlations were performed between the different time series. Relationships

232 between series were further assessed by linear regression.

233

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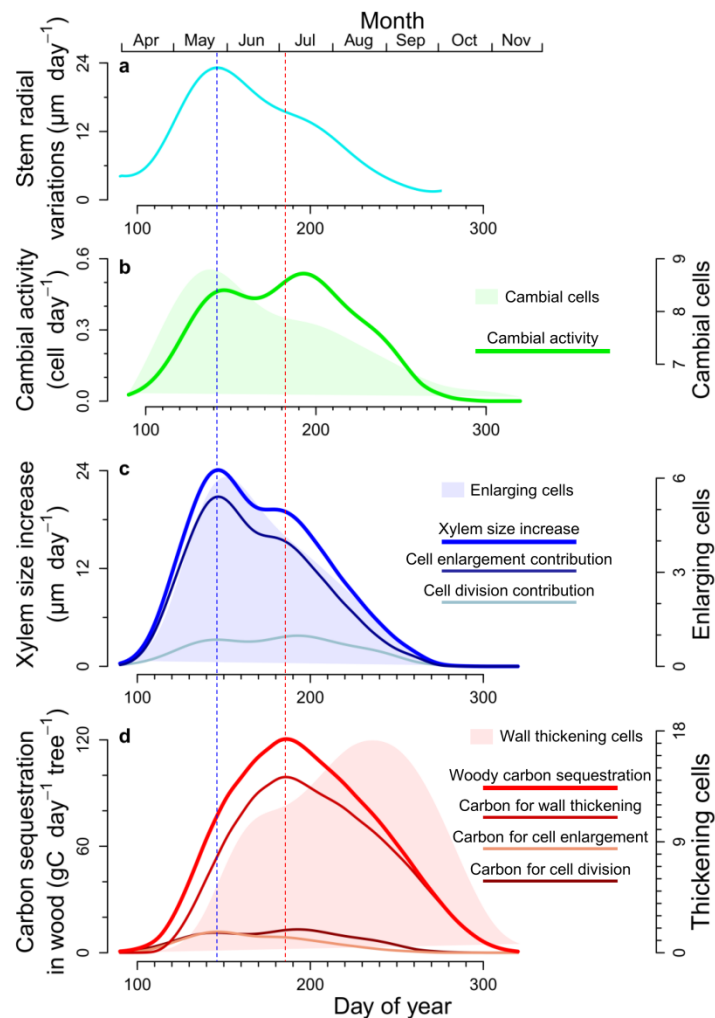
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Author Contributions C.B.K.R. conceived the study with feedbacks from M.F. H.E.C. did the anatomical observations and performed data analysis with the help of C.B.K.R. H.E.C. wrote the manuscript, with the assistance of C.B.K.R., D.F. and P.F. All authors discussed and commented on the manuscript.

319

321 **Figures**

323 **Figure 1: Seasonal dynamics of tree radial size increase and woody carbon sequestration**

324 **processes. a**, Stem radial variations. **b**, Rate of cambial activity. Superimposed (right axis) is

325 the number of cambial cells that produce new xylem cells. **c,** Rate of xylem size increase, with

326 isolated contributions of cambial cell division and cell enlargement. Superimposed (right axis)

327 is the number of enlarging cells.. **d**, Rate of carbon sequestration in the stem wood, which

328 adds the carbon sequestered by wall thickening plus the carbon sequestered by cell

329 enlargement and cell division. Superimposed (right axis) is the number of wall thickening

330 cells. Verical blue and red dashed lines materialise occurrence of maximal rates of radial size

331 increase and carbon sequestration, respectively. Graphics represent the means for 45 trees of

332 three species monitored over three years in three sites.
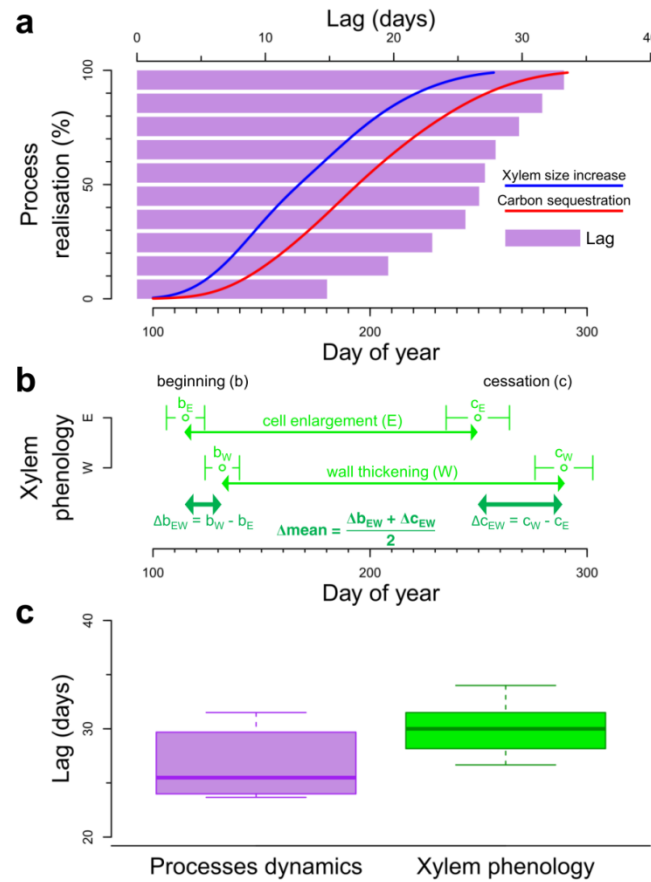
Figure 2: Asynchrony of tree radial size increase and woody carbon sequestration, and xylem phenology. a, Time-lag between the seasonal dynamics of xylem size increase and woody carbon sequestration when expressing both processes in terms of cumulated budget. **b**, Estimate of the mean time-lag from xylem phenology. **c**, Comparison of the mean time-lag calculated from the quantifications of processes dynamics with this estimated from xylem phenology. In **a** and **b**, graphics represent the means (with associated standard deviations in **b**) for 45 trees of three species monitored over three years in three sites. In **c**, boxplots represent 9 values of mean time-lag (1 value per site and year).
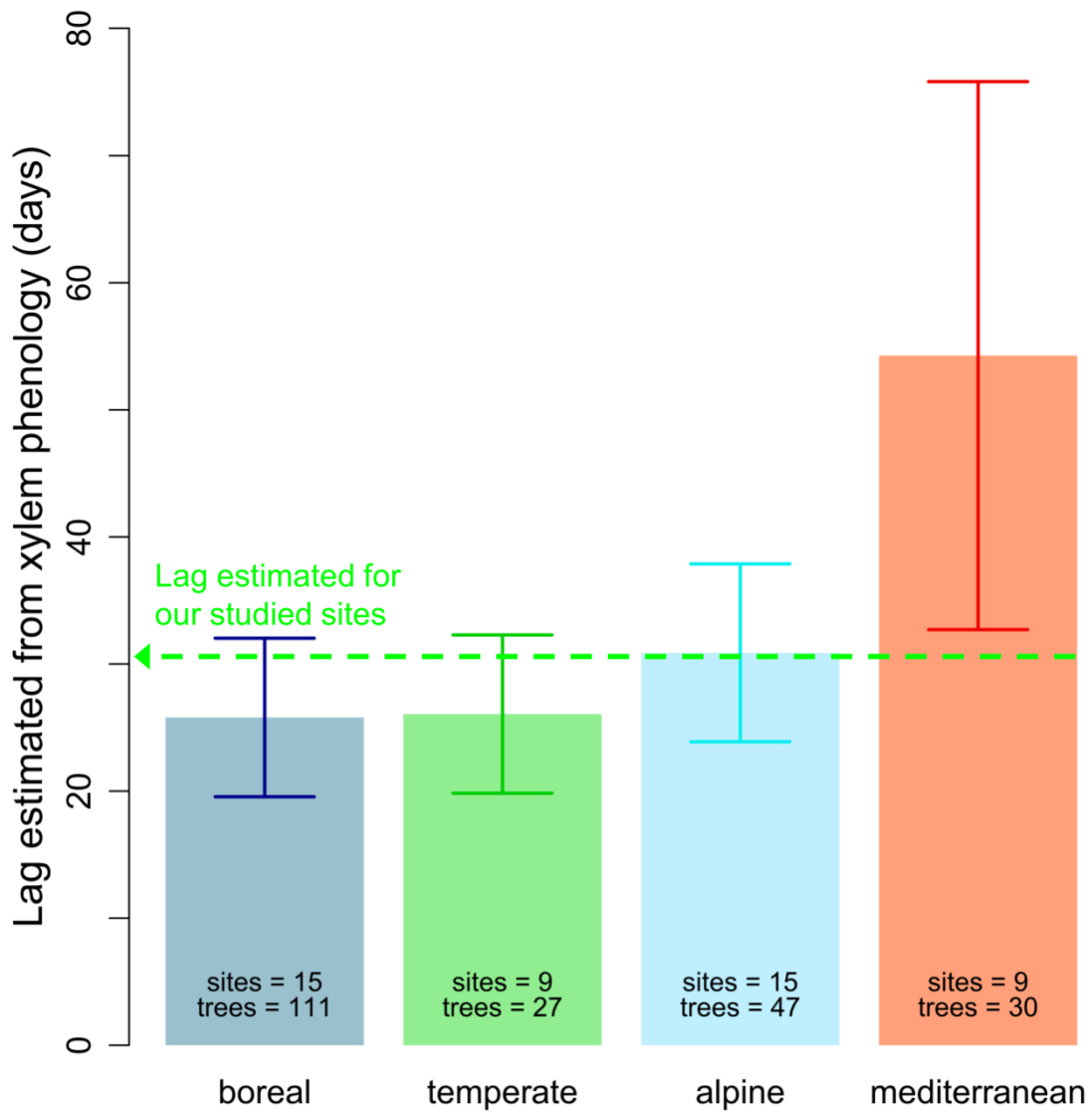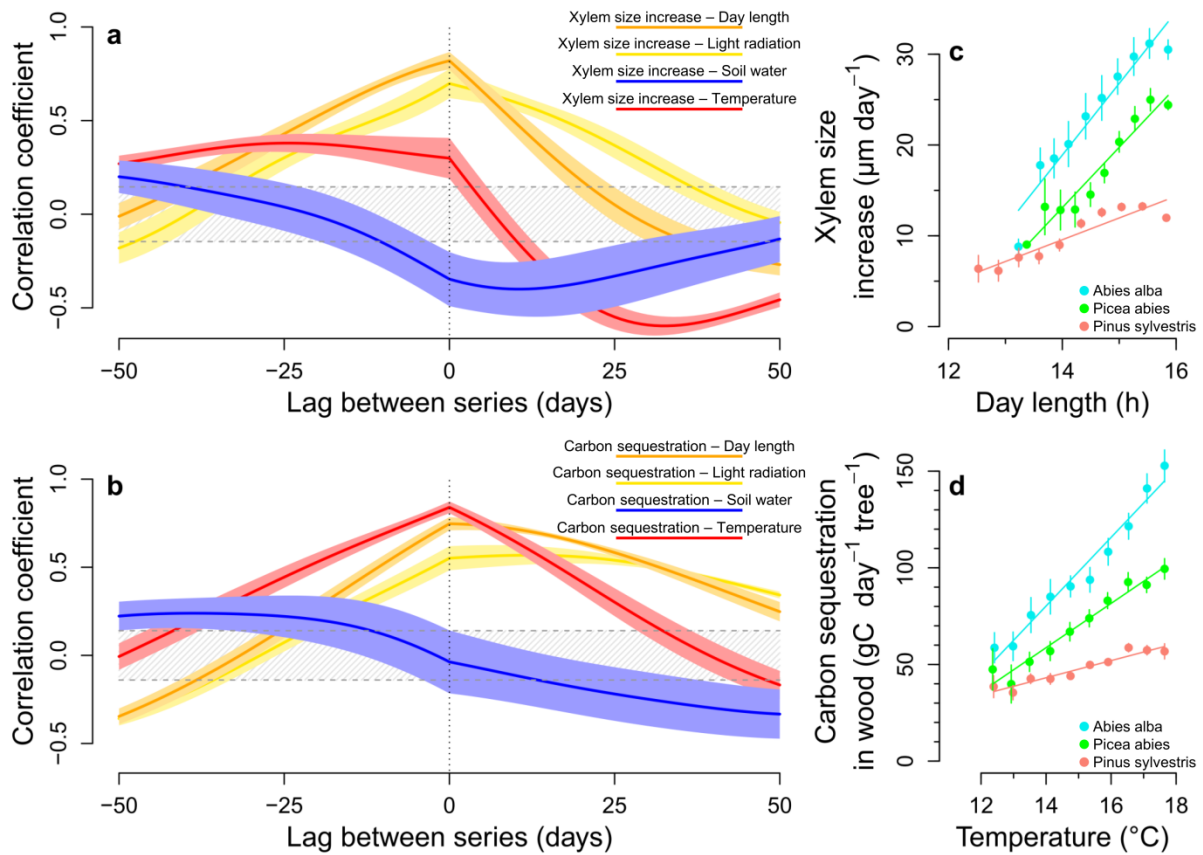
Figure 3: Time-lag between tree radial size increase and woody carbon sequestration estimated from observations of xylem phenology in different forest biomes of the northern hemisphere.

Figure 4: Relationships between the seasonal dynamics of xylem size increase, woody carbon sequestration and environmental factors. **a**, **b**, Cross-correlations between the seasonal course of environmental factors and: xylem size increase (**a**), woody carbon sequestration (**b**). **c, d**, Relationships between the seasonal dynamics of: xylem size increase and day length (**c**), carbon sequestration and temperature (**d**). For **a** and **b**, the different curves represent the mean correlation coefficients for the three species, the three years, and the three sites. Shaded area around lines represents the 95% confident interval. For **c** and **d**, each points represents the mean value of the process rate within a class of the environmental factor, and vertical bars represent the 95% confident interval (Methods).