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ECOPHYSIOLOGY OF TREE-RING FORMATION: CONCEPTS, METHODS AND APPLICATIONS

Summary

Wood, the most abundant biological compound on earth, is of crucial importance for Humankind and Biosphere. Although tree rings and the process of xylogenesis that creates them have long been under the microscope of biologists, it is only recently that the invention of new techniques has made it possible to monitor the intra-annual dynamics of xylem formation by the cambium, along with the environmental conditions that influence it.

In most regions of the world cambial activity follows a seasonal cycle. At the beginning of the growing season, when temperatures increase, the cambium resumes activity. During the growing season, new xylem cells produced by the cambium are undergoing profound transformations, passing through successive differentiation stages, which will enable them to perform their future functions in trees. Finally, at the end of the growing season, when climatic conditions deteriorate, cambial activity stops, soon followed by the cessation of all the differentiation processes.

In this manuscript, after reviewing the biological basis of wood formation and describing the methods that allow its monitoring, we investigate and model the relationships between cambium phenology, tree-ring formation dynamics, tracheid differentiation kinetics, and the climatic factors. The results obtained are used to describe the coordination between stem growth in size and in biomass, as well as the relationships between the source (the canopy) and the main sink (the cambium) of carbon in trees.

Finally, three promising research avenues are presented, exploring the relationships between: (1) the intra-annual dynamics of woody biomass production and gross primary productivity; (2) the intra-annual dynamics of water conduction in xylem and stem sap flow and stand evapotranspiration; (3) the kinetics of tracheid differentiation and the creation of the isotopic signals in tree-ring archives.

In the context of accelerating global changes, it is crucial to investigate what is ruling tree-ring formation and wood production, in order to better evaluate how the ongoing modifications of the environmental factors are impacting trees, biogeochemical cycles, human societies, and ultimately the climate itself.

Key words:

Xylogenesis, Wood anatomy, Tree radial growth, Cambium phenology, Forest productivity, Dendroecology, Climate change, Carbon and water cycles

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CHAPTER 1 | GENERAL INTRODUCTION

1.1 | The importance of forest biomes for the Earth

Forests are the most widely distributed biomes on earth. They cover one third of the emerged lands, host more than 50 % of the world biodiversity, and contain more than 60 % of the terrestrial carbon pool {Groombridge and Jenkins 2002}.
The exchanges of carbon dioxide between the forest ecosystems and the atmosphere are crucial processes that influence the balance and the dynamics of the global carbon cycle {Beer et al. 2010}.
Terrestrial plant photosynthesis (i.e. atmospheric CO2 uptake by the leaves) captures about 120 petagrams of carbon per year {Lal 2008}. Plant growth fixes half of this carbon (i.e. 60 petagrams) in form of biomass {Zhao and Running 2010}, while the other half is released back in the atmosphere by autotrophic respiration {Lal 2008}.
Concomitantly, plant transpiration releases 40 000 petagrams of water into the atmosphere {Holbrook and Zwieniecki 2003}, influencing global precipitation and heat flux {Seneviratne et al. 2006}.
In average, the forest ecosystem photosynthesis draws more carbon from the atmosphere than auto- and hetero-trophic respiration pump into it. So, at the global scale, world forests constitute a large and persistent net sink of carbon {Pan et al. 2011}.
Moreover, the sequestration of carbon into forest woody biomass uptakes 15 % of anthropogenic CO2 emissions, slowing down climate warming {Pan et al. 2011}. Indeed year, about 50 gigatonnes of atmospheric carbon are accumulated by plants in the newly formed wood {Zhao and Running, 2010}. Since the amount of carbon released into the atmosphere through forest ecosystem respiration is significantly lower, carbon sequestration in wood contributes to a carbon sink that offsets atmospheric carbon emissions from human activities, thereby mitigating climate change {Canadell and Raupach, 2008}.

1.2 | The importance of wood for trees

It has been estimated that woody plant material (arising from cambial cells) accounts for more than half of Earth's biomass {Bar-On et al. 2018}.
A large part of the terrestrial carbon pool is stored in wood, which is the most abundant biological compound on earth, representing about 80 % of the terrestrial biomass {Lal 2008}.
Wood performs four essential functions in trees: (1) it supports and spatially distributes the photosynthetic tissues above ground; (2) it conducts the raw sap (i.e. water and nutrients) from the roots up to the leaves; (3) it stores carbohydrates, water, and other compounds; and finally (4) it protects the tree from pathogens, by storing and distributing defensive compounds {Kozlowski and Pallardy 1997}.
The ability of trees to face environmental stress strongly depend on wood capacity to provide these four vital functions. The anatomy, structure and function of the wood formed under normal circumstances usually fit the environmental conditions

trees are living in. However, under a strongly and rapidly changing climate these characteristics need adjustments that might lie outside the current natural phenotypic plasticity and/or evolutionary adaptation of trees.

1.3 | Wood is an essential resource for humankind

For humans, dependence on wood is so strong that, throughout history, the availability of wood has been closely linked to the success or decline of civilizations {Perlin, 1989}.
Today, wood still plays a crucial role in our societies by being a preferred material in many fields such as building construction, furniture manufacturing or energy.
With the increase in the world population, there is a growing demand for wood products and consumption has never been so high, making it one of the major products on the world market {FAO, 2007}.
This market will continue to grow in the future as wood, a renewable natural material, is expected to become an alternative to fossil fuels and petrochemical compounds of high environmental cost.
To promote a sound future management of forest resources it is fundamental that tree breeders and forest managers select and promote individuals, provenances and species that best fit uncertain future conditions. It is therefore important and urgent that plant scientists provide tools and knowledge helping them to identify and select the most relevant adaptive traits.

1.4 | The importance of tree rings in global change sciences

We have now entered the Anthropocene, the new geological era when human activities have irremediably altered the Earth's environment {Steffen et al., 2015}. Nowadays, all organisms and ecosystems are confronted with unprecedented conditions that are challenging their functioning and performance at the most basic adaptive and evolutionary levels {Stocker, 2014}. In this context of rapid transition there are great uncertainties in how forest ecosystems will respond to, and feedback upon, the climate system {Reichstein et al., 2013; Frank et al., 2015}.
Woody plants, via the formation of annual rings of wood, not only provide an abundant natural material that fixes atmospheric carbon contributing to mitigate climate warming, but also records and archives in their perennial structure, at annual resolution, their responses to changing developmental stages and environmental conditions.
Tree-rings are one of the most important and prominent high-resolution natural archives at the disposal of ecologists and climatologists to investigate long-term plant-environment interactions {Fritts, 1976; George, 2014}. On the one hand, tree-ring data (including wood density and isotope ratios) are key for reconstructing climate variability over centuries to millennia {e.g., Esper et al., 2012; Ahmed et al., 2013; Neukom et al., 2014}. On the other hand, tree-ring data are used in new ways to assess the responses of forest ecosystems to environmental change {e.g., Anderegg et al., 2013; Rigling et al., 2013; Babst et al., 2014; Granda et al., 2014; Tognetti et al., 2014} and to quantify their interactions with the climate system {e.g., Frank et al., 2015}. Indeed, tree-rings provide indications on how growing

season conditions influence wood structures and composition, and in turn, how these changes, by modifying the xylem functions (e.g., hydraulic, mechanical, and storage capacities), impact the ability of a tree to perform and survive {Levanic et al., 2011; Lachenbruch & McCulloh, 2014}.
Advances in monitoring wood formation {Gricar et al., 2011} are offering new opportunities to link causes and consequences in the complex world of climate-growth relationships, and to build a novel mechanistic framework of radial growth {Rathgeber, 2017}.
Results from these efforts have demonstrated that climate not only affects the period when wood formation occurs {Moser et al., 2010}, but also impacts the kinetics (timings and rates) of wood cell differentiation {Cuny et al., 2013, 2016}, and thus directly influences tree-ring structure {Cuny et al., 2014, 2018}, as well as carbon sequestration into wood {Cuny et al. 2015}. The above studies demonstrate the possibility to directly link environmental factors to the differentiation and resulting morphology of every single tracheid produced during the growing season. This can yield an improved mechanistic understanding of the interactions between climate and tree growth (along with resulting wood structure, composition, and function), forest ecosystem productivity, and associated impacts on biogeochemical cycles.

1.5 | The importance of wood formation monitoring in tree-ring science

So, in the context of global changes, we believe, it is crucial to investigate what is ruling tree-ring formation and wood production, in order to better evaluate how the ongoing modifications of the environmental factors are impacting trees, forests, biogeochemical cycles, and ultimately the climate itself.
Cambium activity and xylem formation has been studied for more than a century {Hartig, 1892}. Early studies mainly focused on description of the process of wood formation {Priestley 1930; Wilson 1964; Wilson 1970}, while later studies explored how cambial activity shaped wood anatomy {Skene 1969} and, more recently, how wood formation responds to the environment {Wodzicki 1971; Denne 1976; Ford et al. 1978; Denne and Dodd 1981}.
These questions received a renewed attention during the last decade because of the pervasive problem of global changes, leading to a rapid increase in the number of scientific studies involving trees growing in natural or experimental conditions {Gricar et al. 2011, Rathgeber et al. 2019}.
Direct observations of tree-ring formation are impossible since cambial activity takes place under the bark of a tree. However, several approaches have been developed to assess wood production dynamics. Techniques such as stem-dimension monitoring (using dendrometers), and repeated marking (generally called pinning) and sampling (generally called microcoring) of the stem at regular intervals during the growing season have been used to study wood formation. All these approaches have their strengths and weaknesses and the potential for further improving them is high. In this manuscript, however, we will focus on wood-formation-monitoring based on microcoring, which is the best approach to link wood formation dynamics and tree-ring structure and properties.

In the context of global changes, a more detailed mechanistic understanding of the processes leading to tree-ring formation, including features that record the environmental signals, is therefore crucial to better exploit this wonderful natural archive, as well as to forecast tree adaptation to such a fast-changing climate. Questions such as: "which environmental factor produced this feature?"; "when during the growing season does it occurred?"; "what processes are involved in the creation of such a feature"; are fundamental questions to link effects to causes and structures to functions.

1.6 | The importance of the cambium in the carbon cycle: the source-sink controversy

Up to now, scientists have considered photosynthesis as the main driver of plant growth and have put much effort in better understanding this process.
However, another view, which emerged recently {Fatichi et al. 2014}, is claiming that, under normal conditions, it is not the source (i.e. photosynthesis) that limit plant growth, but the sinks (i.e. the ability of meristems to convert carbon into biomass). In other words, carbon can only be sequestered into wood to the extent cambial activity and environmental conditions permit it {Körner 2015}.
However, if after decades of researches on photosynthesis, source activity is very well known and quantified nowadays, while cambium functioning is still poorly understood {Fatichi et al. 2018}.

1.7 | Presentation of the outline of the manuscript

Following this general introduction (chapter 1), the chapter 2: 'Biological Basis of Wood Formation' reviews the cellular processes involved in xylogenesis, as well as their control by physiological, developmental and environmental factors.
The chapter 3 of this manuscript: 'Methods for Monitoring Wood Formation' describes the technical work involved in wood-formation-monitoring studies: from the selection of the trees, to the preparation of the samples, the observation of the anatomical slides, and the collection and verification of the raw data.
The chapter 4: 'Phenology of Cambial Activity and Tree-Ring Formation' presents the numerical methods we developed for quantifying wood formation phenology, as well as the significant results we obtained in this domain. These results mainly concern the influence of tree characteristics and environmental factors on wood formation phenology. A first process-based modeling approach, which could be generalized in the future, is developed for the onset of xylem formation.
The chapter 5: 'Seasonal Dynamics of Tree-Ring Formation in Conifers' presents the numerical methods we developed for quantifying the intra-annual dynamics of wood formation, as well as the significant results we obtained in this domain. These results mainly concern the influence of site conditions and environmental factors on xylem development. Sensitivity analyses allowed us to disentangle the contribution of duration vs. rate of tracheid production and better understand wood production machinery.
The chapter 6: 'Kinetics of Tracheid Differentiation in Conifers' presents the numerical methods we developed for quantifying cell differentiation kinetics, as well as the significant results we obtained in this domain. These results mainly

concern the influence of developmental and environmental factors on tracheid differentiation kinetics. An innovative mechanistic modeling framework is presented to challenge the classical hypotheses on the role of the auxin morphogenetic gradient in structuring the differentiation zones of the developing xylem.
The chapter 7: 'Intra-Annual Dynamics of Woody Carbon Sequestration' presents the numerical methods we developed for quantifying the intra-annual dynamics of woody carbon sequestration, as well as the significant results we obtained in this domain. These results exploring the coordination between stem growth in size and in biomass, as well as the influence of the environmental factors, eventually leads to a better understanding of the relationships between the carbon source in trees (the canopy) and its main sink (the cambium).
The chapter 8: 'Frontiers in Tree-Ring Formation Dynamics' present three promising research avenues we are currently developing. The first avenue prolongs chapter 7 approach, exploring the coordination between intra-annual dynamics of woody biomass production and gross primary productivity. The second avenue explores the intra-annual dynamics of water conduction in wood in relation with stem sap flow and stand evapotranspiration. The third avenue uses all the knowledge previously acquired concerning the kinetics of tracheid differentiation to better understand the physiological and environmental meanings of the isotopic signals in tree-ring archives. This chapter also present our thoughts for a broad, integrated approach to tree-ring sciences and wood quality.
Finally, the chapter 9 briefly presents our 'General Conclusions and Perspectives', including some thoughts concerning potential future projects.

1.8 | References

This reference list is incomplete and not properly formatted!

Barceló AR Lignification in plant cell walls - International review of cytology, 1997 - Elsevier

Begum, S., Nakaba, S., Yamagishi, Y., Oribe, Y., and Funada, R. (2012). Regulation of cambial activity in relation to environmental conditions: understanding the role of temperature in wood formation of trees. Physiologia Plantarum 147, 46–54. doi: 10.1111/j.1399-3054.2012.01663.x.

Bonan, G. B. (2008). Forests and climate change: forcings, feedbacks, and the climate benefits of forests. Science 320, 1444–1449. doi: 10.1126/science.1155121.

Boyce CK, Zwieniecki MA, Cody GD, Jacobsen C, Wirick S, Knoll AH, Holbrook NM. 2004. Evolution of xylem lignification and hydrogel transport regulation. Proceedings of the National Academy of Sciences, USA 101: 17 555–17 558.

Breitsprecher, A., and Bethel, J. S. (1990). Stem-Growth Periodicity of Trees in a Tropical Wet Forest of Costa Rica. Ecology 71, 1156–1164.
Bollhöner, B., Prestele, J., and Tuominen, H. (2012). Xylem cell death: emerging understanding of regulation and function. Journal of Experimental Botany 63, 1081–1094. doi:10.1093/jxb/err438.

Cosgrove, D. J. (2005). Growth of the plant cell wall. Nature Reviews. Molecular Cell Biology 6, 850–861. doi: 10.1038/nrm1746.

Cuny, H. E, Rathgeber, C. B. K., Frank, D. C., Fonti, P., Mäkinen, H., Prislan, P., et al. (2015). Woody biomass production lags stem-girth increase by over one month in coniferous forests. Nature Plants 1, 15160. doi: 10.1038/nplants.2015.160.

Cuny, H. E., and Rathgeber, C. B. K. (2016). Xylogenesis: coniferous trees of temperate forests are listening to the climate tale during the growing season but only remember the last words! Plant Physiology 171, 306–317. doi: 10.1104/pp.16.00037.

Cuny, H. E., Rathgeber, C. B. K., Frank, D. C., Fonti, P., and Fournier, M. (2014). Kinetics of tracheid development explain conifer tree-ring structure. New Phytologist 203, 1231–1241. doi: 10.1111/nph.12871.

Delpierre, N., Vitasse, Y., Chuine, I., Guillemot, J., Bazot, S., Rutishauser, T., and Rathgeber, C. B. K. (2015). Temperate and boreal forest tree phenology: from organ-scale processes to terrestrial ecosystem models. Annals of Forest Science 73(1), 5–25. doi: 10.1007/s13595-015-0477-6.

Denne, M. P., and Dodd, R. S. (1981). The environmental control of xylem differentiation. Xylem cell development. Castle House Publications: Tunbridge Wells, 236–255.

Donaldson, L. A. (2001). Lignification and lignin topochemistry — an ultrastructural view. Phytochemistry 57, 859–873. doi: 10.1016/s0031-9422(01)00049-8.

Evert, R. F. (2006). Esau's Plant Anatomy (3rd ed.). John Wiley & Sons. 624p.

Expansion of the plant cell wall – D.J. Cosgrove, Pennsylvania State University, USA..Annual Plant Reviews, Volume 8, The Plant Cell Wall Jocelyn K. C. Rose (Editor)ISBN: 978-1-405-14773-6 February 2009 Wiley-Blackwell 400 Pages

Griçar, J., Rathgeber, C. B. K., and Fonti, P. (2011). Monitoring seasonal dynamics of wood formation. Dendrochronologia 29, 123–125. doi: 10.1016/j.dendro.2011.01.007.

Groombridge, B., and Jenkins, M. D. (2002). World Atlas of Biodiversity. Prepared by the UNEP World Conservation Monitoring Centre. University of California Press. 360p.

Groover, A., and Jones, A. (1999). Tracheary element differentiation uses a novel mechanism coordinating programmed cell death and secondary cell wall synthesis. Plant Physiology 119, 375–384.

Körner, C. (2015). Paradigm shift in plant growth control. Current Opinion in Plant Biology 25, 107–114. doi: 10.1016/j.pbi.2015.05.003.

Kozlowski, T. T., and Pallardy. S. G. (1997). Physiology of Woody Plants. Academic Press. 411p.

Lachaud, S., Catesson, A.-M., and Bonnemain, J.-L. (1999). Structure and functions of the vascular cambium. Comptes Rendus de l'Académie des Sciences - Series III - Sciences de la Vie 322, 633–650. doi: 10.1016/S0764-4469(99)80103-6.

Lal, R. (2008). Sequestration of atmospheric CO2 in global carbon pools. Energy & Environmental Science 1, 86–100.

Larson, P. R. (1994). The Vascular Cambium: Development and Structure. Springer-Verlag. 725p.

Pan, Y., Birdsey, R. A., Fang, J., Houghton, R., Kauppi, P. E., Kurz, W. A., et al. (2011). A Large and persistent carbon sink in the world's forests. Science, 333, 988–993. doi: 10.1126/science.1201609.

Perrot-Rechenmann, C. (2010). Cellular Responses to Auxin: Division versus Expansion. Cold Spring Harbor Perspectives in Biology 2, a001446. doi: 10.1101/cshperspect.a001446.

Plomion, C., Leprovost, G., and Stokes, A. (2001). Wood formation in trees. Plant Physiology 127, 1513–1523. doi: 10.1104/pp.010816.

Prislan, P., Čufar, K., Koch, G., Schmitt, U., and Griçar, J. (2013). Review of cellular and subcellular changes in the cambium. IAWA Journal 34, 391–407. doi: 10.1163/22941932-00000032.

Rowe, N., and Speck, T. (2005). Plant growth forms: an ecological and evolutionary perspective. New Phytologist 166, 61-72.

Seneviratne, S.I., Lüthi, D., Litschi, M., and Schär, C. 2006. Land-atmosphere coupling and climate change in Europe. Nature 443, 205-209.

Sperry, J. S., Hacke, U. G., and Pittermann, J. (2006). Size and function in conifer tracheids and angiosperm vessels. American Journal of Botany 93, 1490–1500.

Stals, H., and Inze, D. (2001). When plant cells decide to divide. Trends in Plant Science 6, 359–364.

The composition and structure of plant primary cell walls – M. A. O'Neill and W.S. York, Complex Carbohydrates Research Center, Athens, Georgia, USA.. Annual Plant Reviews, Volume 8, The Plant Cell Wall Jocelyn K. C. Rose (Editor)ISBN: 978-1-405-14773-6 February 2009 Wiley-Blackwell 400 Pages

Ursache, R., Nieminen, K., and Helariutta, Y. (2013). Genetic and hormonal regulation of cambial development. Physiologia Plantarum 147, 36–45. doi: 10.1111/j.1399-3054.2012.01627.x.

Vaganov, E. A., Hughes, M. K., and Shashkin, A. V. (2006). Growth Dynamics of Conifer Tree Rings. Springer. 354 p.

Wang H, Zhao Q, Chen F, Wang M, Dixon RA. 2011. NAC domain function and transcriptional control of a secondary cell wall master switch. Plant Journal 68: 1104–1114.

Wang HZ, Dixon RA. 2012. On-off switches for secondary cell wall biosynthesis. Molecular Plant 5: 297–303.

Wang J, Kucukoglu M, Zhang L, Chen P, Decker D, Nilsson O, Jones B, Sandberg G, Zheng B. 2013. The Arabidopsis LRR-RLK, PXC1, is a regulator of secondary wall formation correlated with the TDIF-PXY/TDR-WOX4 signaling pathway. BMC Plant Biology 13: 94.

Wilson, J. W. (1970). The Growing Tree. The University of Massachusetts Press. 152 p.

Zhong, R., and Ye, Z.-H. (2009). Secondary cell walls. eLS. doi: 10.1002/9780470015902.a0021256/full.

Zimmermann M. H. (1983). Xylem Structure and the Ascent of Sap. Springer-Verlag. 143 p.

CHAPTER 2 | BIOLOGICAL BASIS OF WOOD FORMATION

2.0 | Synthesis

2.0.1 | Related papers

Cuny, H., Rathgeber, C.B.K., 2014. Une synthèse sur le fonctionnement et la régulation des processus cellulaires de la formation du bois. *Revue Forestière Française* LXVI, 761–777.

Rathgeber CBK, Cuny H, Fonti P, 2016. Biological basis of tree-ring formation: a crash course. *Frontiers in Plant Science* 7: 734: 1–7.

Deslauriers A, Fonti P, Rossi S, Rathgeber CBK, Griçar J, 2017. Ecophysiology and plasticity of wood and phloem formation. In: Amoroso MM, Daniels LD, Baker, PJ, Camarero JJ (Eds.), *Dendroecology*. Springer International Publishing, pp. 13–33.

De Micco V, Carrer M, Rathgeber CBK, Camarero JJ, Voltas J, Cherubini P, Battipaglia G, 2019. From xylogenesis to tree rings: wood traits to investigate tree response to environmental changes. *IAWA Journal* 40 (2): 155-182.

Rathgeber CBK, Fonti P, Shishov VV, Rozenberg P, 2019. Wood formation and tree adaptation to climate. *Annals of Forest Science* (in preparation a)

Rathgeber CBK, Pérez-de-Lis G, Fernández-de-Uña L, Ponton S, Deslauriers A, Fonti M, Rossi S, Gessler H, Treydte K, Fonti P. Anatomical, developmental and physiological basis of tree-ring formation in relation with environmental factors. In Siegwolf R, Brooks JR, Roden J, Saurer M (Eds), *Stable Isotopes in Tree Rings: Inferring Physiological, Climatic and Environmental Responses*, Tree Physiology Series, Springer (in preparation b).

2.0.2 | Summary

Wood is of crucial importance for man and biosphere. Moreover, wood performs four essential functions in trees: it supports and spatially distributes the photosynthetic tissues above ground; it conducts the raw sap (i.e. water and nutrients) from the roots up to the leaves; it stores carbohydrates, water, and other compounds; and finally, it protects the tree from pathogens, by storing and distributing defensive compounds.

In this chapter, we present the fundamental processes involved in intra-annual dynamics of wood formation, along with their relationship with the developmental, physiological and environmental factors.

During wood formation, new xylem cells produced by the cambium are undergoing profound transformations, passing through successive differentiation stages, which will enable them to perform their future functions in trees.

Xylem cell formation can be divided in five major phases: (1) the division of a cambial mother cell that creates a new cell; (2) the enlargement of this newly

formed cell; (3) the deposition of its secondary wall; (4) the lignification of its cell wall; and finally, (5) its programmed cell death.
Later on, heartwood formation completes the process providing natural durability and ornamental values to wood products as well as antimicrobial properties to protect living stems from decay.
Without a clear understanding of the xylem formation process, it is not possible to comprehend how annual growth rings and typical wood structures are formed, recording normal seasonal variations of the environment as well as extreme climatic events.

2.0.3 | Keywords

Cambial activity, Tree growth, Tree-ring structure, Quantitative wood anatomy, Climatic factors, Xylem, Lignin, Cellulose

2.1 | Introduction

2.1.1 | Wood formation monitoring: a fast-growing discipline

As we have seen, wood is of crucial importance to the functioning of forest ecosystems, the carbon cycle and our human societies. This is why Man has long sought to know the process of its formation in trees. The introduction of the term "cambium" in a botanical sense dates back to the 17th century and is attributed to Nehemiah Grew in 'The Anatomy of Plants' {1682} {for a more detailed history of cambium study, see for example Larson, 1994}. However, the study of cambium activity and xylogenesis has progressed at a very slow pace for a long time due to the difficulty of studying these microscopic and fragile tissue hidden under tree bark.
Since the beginning of the 21st century, however, research on wood formation has accelerated {Savidge et al., 2000}. This intensification has been made possible by technological advances, which allow to sample and preserve the integrity of the cambium and the forming xylem, and then to study the processes of xylogenesis on a very fine scale.
The monitoring of the seasonal dynamics of xylogenesis started, more than 50 years ago, with few pioneering works, which aimed to better understand the influence of climate on tree growth, cambium phenology, and wood formation dynamics {Wilson, 1970; Denne and Dodd, 1981}. These questions received a renewed attention during the last decade because of the pervasive problem of global changes, leading to a rapid increase in the number of scientific studies involving trees growing in natural or experimental conditions {De Micco et al. 2019; Griçar et al., 2011; Rathgeber et al. in prep. a}.
Three major challenges motivated the acceleration of work on xylogenesis. The first challenge consists to better understand the process of xylogenesis along with its relationships with developmental and environmental factors, in order to better reconstruct past climates (more reliable, accurate and detailed reconstructions). The second challenge consist to better assess the potential impact of climate change on wood formation in trees. The third challenge consist to successfully meet the growing demand for wood while preserving forests and their resources. The degree of understanding of the xylogenesis process will indeed influence our ability

to address these three challenges. For example, a thorough knowledge of xylogenesis would allow trees to be grown in highly controlled environments typical of conventional agriculture, in the same way as corn or wheat, in order to obtain the desired wood quality according to future use and thus optimize the wood supply {Plomion et al., 2001}.

2.1.2 | Aims and outline of the chapter

The objective of this chapter is to review fundamental knowledge on the xylogenesis process in order to help ecologists to better interpret results from wood formation monitoring studies.
After reminding the structure and function of the cambium, we describe the mechanisms and intrinsic pathways (inside the tree) of regulation of the five main stages of xylem cell differentiation.
Moreover, we review the current knowledge about the influence of the environmental factors on the cellular processes, the intra-annual dynamics, and the phenology of wood formation.
However, aspects related to genetics or genomics will not be addressed in this chapter, as they are not the authors' speciality, and have already been the subject of several syntheses {see for example, Plomion et al., 2001; Zhang et al., 2014}.

2.2 | Wood: structures and functions

2.2.1 | Wood cells provide vital functions for trees

Wood performs four essential functions for the tree, it provides: (1) mechanical support {Fournier et al., 2006}; (2) conduction of raw sap (i.e. soil water and the diluted mineral elements it contains) from roots to leaves {Sperry et al., 2008}; (3) transport and storage of reserve compounds {Kozlowski and Pallardy, 1997}; and (4) transport and storage of defense compounds (ibidem).
In conifers, only one type of cell: the tracheids, fulfills both functions of water conduction and mechanical support.
In hardwood trees, two different types of cells share these two different functions. The vessel elements, whose stacking forms long vertical tubes in the trunk (the vessels), are dedicated to the conduction of raw sap; while the fibres mainly provide the mechanical support.
In both soft- and hard-wood trees, parenchyma cells provide the radial transport of reserve and defense compounds, as well as their storage.
Tracheids, fibres and vessel elements are dead elongated cells, surrounded by a thick, rigid and impermeable wall, which delimits an empty central space called "lumen".
On the other hand, parenchyma cells are alive, exhibiting an active metabolism in their dense cytoplasm, which is surrounded by a thin wall.

2.2.2 | Xylogenesis: the complex process that produces wood

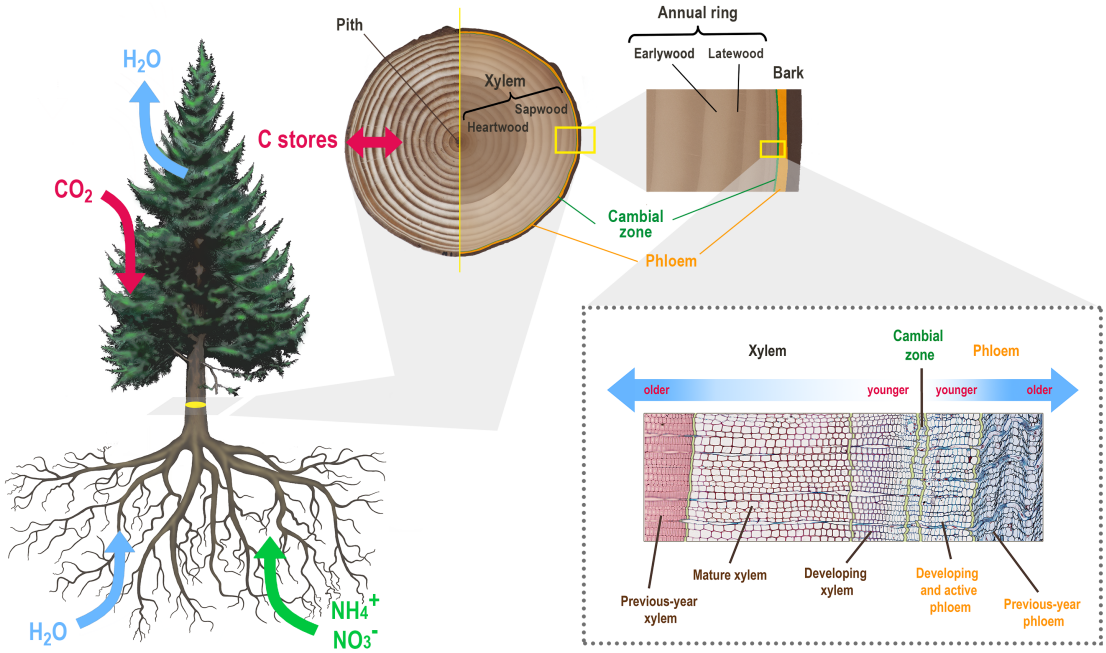
Xylogenesis is the term used to designate the process of wood (i.e. xylem) formation in woody plants.

Xylogenesis appeared about 350 to 400 million years ago as the result of the development of the vascular cambium and the former appearance of lignin {Rowe and Speck, 2005; Weng and Chapple, 2010}.
The vascular cambium is composed of a thin layer of meristematic cells located between the secondary xylem (i.e. the wood) and the secondary phloem (i.e. the living bark), forming a continuous envelop all around the stems, branches, and roots of woody plants {Evert 2006}. The cambium gives rise to the xylem inwards (i.e. towards the pith), and to the phloem outwards (i.e. towards the bark).
The ability to form wood has been an essential evolution for the adaptation of plants to the aerial environment and has led to a major transformation of the plant world {Rowe and Speck, 2005}. Indeed, the formation of a tissu that can provide both the mechanical support necessary for stability in the low load-bearing air environment, and the ability to transport water upwards and over long distances, have contributed to the emergence of the 'tree' life form. Trees colonized then earth emerged lands, developing sometimes gigantic architectures.
Xylogenesis involves the production of wood cells by the cambium, as well as the differentiation of these cells. Differentiation itself involves several morphological and physiological transformations that aim to give xylem cells a structure adapted to the realization of their functions.
During xylogenesis xylem cells undergo five successive stages of differentiation: (1) birth of the new xylem cell; (2) enlargement of the new xylem cell; (3) secondary cell-wall formation; (4) primary and secondary cell-wall lignification; (5) programmed cell death {Wilson, 1984}.
The parenchyma cells, which remain alive at maturity, also follow this program, but 'escape' the final stage of programmed cell death. However, after several years of functioning, parenchyma cells also die, while in the same area tracheids and vessels lose their ability to conduct raw sap. This process is called duraminisation and marks the transition between hardwood (dead, non-functional wood at the core of the tree), and sapwood (alive, functional wood at the periphery of the tree) {Hillis, 1987; Taylor et al., 2002}.

2.2.3 | The vascular cambium: structure and function

The term 'cambium' was coined by Nehemaia Grew in the early 1600s, because this Latin name means a place of trade. Indeed, Grew believed that cambium was to be a kind of sap (rather than living cells) capable of secreting materials both inside and outside, to form wood and bark, respectively.
The vascular cambium is a bifacial lateral meristem that produces wood in the inside and bast (living bark) towards the outside {Larson, 1994}. It is composed by a thin layer of a few living cells (called "initials") located between the wood and the bark in the stem of woody plants [Fig. 2.1] {Lachaud et al., 1999}.
The cambial initials are able to divide to form the secondary xylem towards the pit and the secondary phloem towards the bark.
Two types of cambial initials can be found in the cambium [Fig. 2.1]: (1) the radial initials, which are short and isodiametric cells (about 40 µm in length); and (2) the fusiform initials, which are long, spindle-shaped cells (about 0.4–4.0 mm in length, 30 µm in radial diameter, and 5 µm in tangential diameter).
Radial initials are the least numerous (10-40 %) and produce the radial elements of xylem and phloem (i.e. the elements that are arranged perpendicular to the stem

in the direction of their length, such as the cells of the ray parenchyma). The fusiform initials are the most numerous (60-90%) and produce the longitudinal elements of the xylem and phloem (i.e. the elements that are arranged parallel to the stem of the tree in the direction of their length).
Cambial initials can divide in several directions responding to different needs. Anticlinal divisions (divisions occurring along the tangential direction) allow the cambium to increase in circumference as the stem grows and maintain the balance between the two types of initials. Periclinal divisions (divisions occurring along the radial direction), which represent 90% of the cambial divisions {Lachaud et al., 1999}, give rise to xylem and phloem cells. A cambial initial that divides in periclinal orientation first produces a mother cell of xylem or phloem. This mother cell is able to divide itself to produce daughter cells that will either retain the characteristics and function of the mother cell and thus continue to divide, or differentiate into xylem or phloem cells. In general, xylem mother cells divide more often than phloem mother cells, resulting in a much higher wood production than bark {Evert, 2006; Gricar et al., 2009}. Additionally, fusiform cells can also follow transverse divisions to give rise to two radial initials.



1 — Figure 2.1 | Basic organization of the tree stem and main fluxes of elements.
Water absorption by roots and leaf water transpiration (blue arrows) are related to H and O isotope signals; atmospheric C uptake by leaves and reserve storage-mobilization (red arrows) are related to C and O isotope signals; while nitrate and ammonium absorption by roots (green arrow) is related to N isotope signal. The transverse micro-section (bottom right) shows the relative position and age (double blue arrow) of developing and mature zones of xylem (left, inwards) and phloem (right, outwards) that are originated from periclinal divisions in the cambial zone. Figure from Rathgeber et al. {in preparation b}, prepared by G. Perez-de-Lis.

2.2.4 | The different types of xylem cells

In gymnosperms (i.e. conifers, or softwoods), xylem is made of a simple and homogeneous tissue, mainly composed of two types of cells: (1) tracheids, which represent more than 90 % of the total number of cells, and perform both the mechanical support and the water conduction; and (2) parenchyma cells, which are in charge of the storage and radial transport of various compounds.
Tracheids are elongated, spindle-shaped cells of 3 to 6 mm in length and 6 to 60 µm in diameter {Sperry et al., 2006}.
In angiosperms (i.e. hardwoods), xylem is made of a more complex and heterogeneous tissue, composed of several types of cells.
Vessels take care of the water conduction, fibers of the mechanical support, and parenchyma cells of the storage.
In diffuse-porous trees, vessel dimensions range from 1 to 30 cm in length, and from 15 to 150 µm in diameter; in ring-porous trees, vessel dimensions range from 1 cm to 10 m in length, and from 15 to 300 µm in diameter {Zimmermann, 1983}.
In both angiosperms and gymnosperms, almost all xylem cells die off at the end of their development to fulfill their functions, only parenchyma cells stay alive for a couple of years.

2.2.5 | The different types of xylem cell walls

Cell wall composition

The wall of wood cells consists in three main components: cellulose, hemicelluloses and lignin, which represent more than 90% of wood dry matter {Pettersen, 1984}.
Cellulose is the world's most abundant biopolymer and a key structural component of the plant cell wall {Mutwil et al., 2008}.
The cellulose is a long molecule (like a rope), consisting of a linear glucose chain, which presents high tensile strength {Brett, 2000}.
In the wall, cellulose molecules are assembled to form macromolecules: the 'cellulose microfibrils', which are even longer and stronger than individual cellulose molecules {Somerville, 2006}.
Lignin is a molecule resulting from a complex and heterogeneous assemblage of units called "monolignols" {Freudenberg, 1959}. Lignin permeates the walls, acting as a cement. The matrix it forms, reinforces, stiffens and waterproofs the cellulosic network of the secondary wall {Sarkanen and Ludwig, 1971}.
Hemicelluloses are smaller and more diverse branched molecules {Scheller and Ulvskov, 2010}, which closely connect the wall components into a coherent whole {Keegstra, 2010}.
A wide variety of proteins can also be found in the walls of wood cells {Keegstra, 2010}.

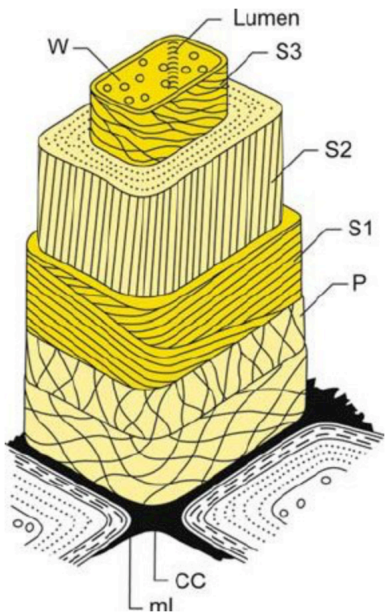
The primary cell-wall

Unlike animal cells, plant cells are surrounded by a primary wall, which is flexible but resistant, regulates cell volume and content, defines and maintains the shape of the cell, stores nutrients and provides a protective barrier {Rose, 2009}.
Primary cell walls of land plants are thin (approx. 1 µm), well hydrated, flexible and extensible. They are principally composed of cellulose microfibrils (15–40 % of

the dry mass), linked via hemicellulosic tethers (20–30 % of the dry mass) to form cellulose-hemicellulose networks, which are embedded in the pectin matrix (30–50 % of the dry mass), and associated with structural proteins (1–10 % of the dry mass) {O'Neill and York, 2009}.

The secondary cell-wall

Unlike the primary wall, the secondary wall only surrounds some particular plant cells {Evert, 2006}. Indeed, they form remarkable structures in many specialized plant cells, and they are particularly important for woody plants, to which they provide mechanical support, water transport, and biological resistance.
Moreover, secondary cell walls represent the major constituent of wood, which is the most abundant component of terrestrial biomass.
Secondary walls are thick (2-10 µm), poorly hydrated (approx. 30 %), rigid, and multi-layered structures. Their principal components are celluloses (40-60 % of dry mass), hemicelluloses (10-40 %), and lignins (15-35 %) {Cosgrove and Jarvis, 2012}.
Cellulose microfibrils together with hemicellulose form the main load-bearing network, in which lignin is impregnated to form another cross-linked matrix ensuring hydrophobicity, rigidity, and durability {Zhong and Ye, 2009}.
Secondary cell walls are commonly composed of three layers: S1 (external), S2 (medium), and S3 (internal), which present almost similar composition, but differ in the thickness and orientation of their cellulose microfibrils [Fig. 2.2] {Evert, 2006; Beck, 2010}.
While the microfibrils are oriented transversally in the S1 layer (from 60° to 80° with regard to the cell axis), they change to a longitudinal orientation in the S2 (from 5° to 30° with regard to the cell axis), before coming back to a transversal orientation in the S3 {Chaffey et al. 2001; Plomion et al., 2001}.
It is mainly the secondary wall, due to its much greater thickness, that provides trees sufficient mechanical support to grow vertically above the ground {Speck and Burgert, 2011}.
The rigidity of the secondary cell wall allows vessels to withstand the intrinsic negative pressure generated by transpiration. Tensile stiffness is provided by oriented cellulose microfibrils, and compression resistance by the lignified matrix {Wang & Dixon, 2012}. In addition, the hydrophobicity of lignin may influence the hydraulic properties of the xylem vessels {Boyce et al., 2004}.
The secondary wall is also the largest biomass stock in terrestrial plants {Zhong and Zheng-Hua, 2009}.



2 — Figure 2.2 | Schematic depiction of tracheid cell wall structure in black spruce wood.
W thin warty layer in cell lumen; S3, S2, S1 secondary wall layers; P, ml, CC primary wal, middle lamella, and cell corner middle lamella. Layer thickness is: S3, ~ 0.1 µm; S2, 1–5 µm; S1, 0.2–0.3 µm; P, 0.1–0.2 µm; mL, 0.2–1.0 µm. Parallel and wavy lines indicate different organization of cellulose microfibrils. From Argawal {2006}.

2.2.6 | Concluding remarks

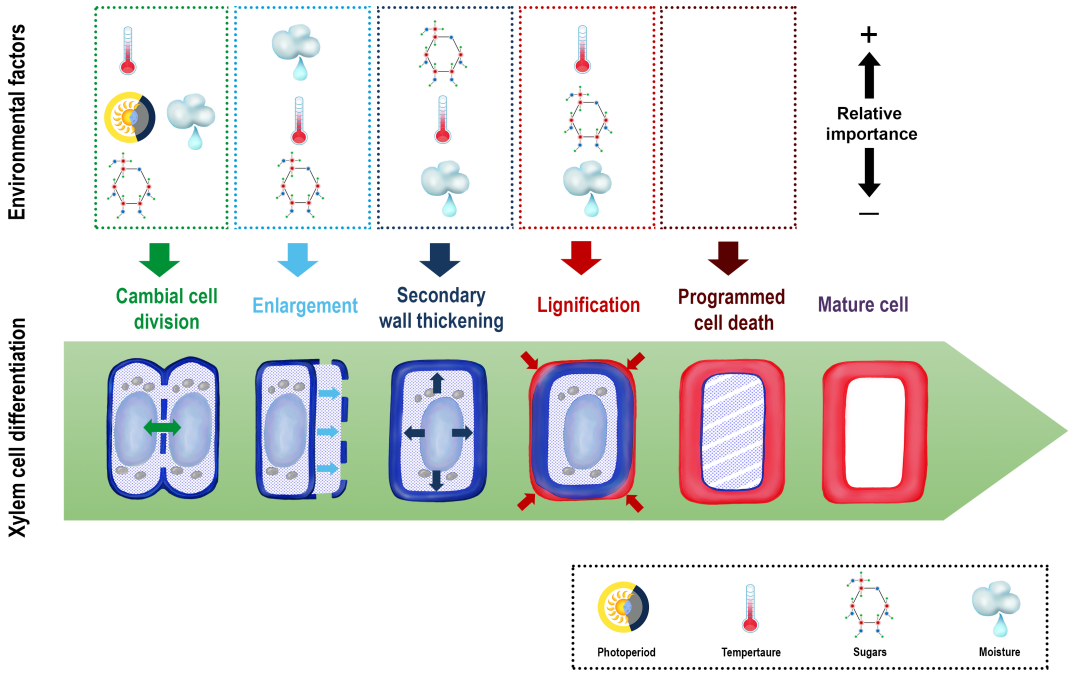
Although their main functions differ, xylem and phloem are closely associated both spatially and functionally {Evert 2006; Steppe et al. 2015}. Due to their tight coupling, any changes in water–carbon relationships have impact on xylem and phloem formation processes {Gricar et al. 2015b; Jyske and Hölttä 2015; Hinckley and Lassoie 1981}. Therefore, carbon gain and whole-tree survival depend on the functioning and interplay between these two vascular tissues {Sevanto et al. 2014}. Wood and phloem-anatomical features thus offer a direct link for understanding physiological processes in a tree {Savage et al. 2016}.

2.3 | Cellular processes involved in wood formation

2.3.1 | Introduction and overview

Xylogenesis consists in the production and differentiation of new xylem cells into mature and functional wood cells.
During their differentiation, xylem cells undergo profound morphological and physiological transformations, which will craft them according to their future functions in trees.
The formation of a xylem tracheary element can be divided in five major stages: (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 deposition of cellulose and hemi-cellulose to build the secondary cell-wall; (4) the impregnation of the primary and secondary cell-walls with lignin; and finally, (5) the programmed cell death (also called apoptosis) {Wilson, 1970} [Fig 2.3].

This sequence is common to both angiosperms and gymnosperms but variations in duration and intensity of the differentiation phases, as well as in molecular components involved, finally result in different cell types and tree-ring structures.



3 — Figure 2.3 | Differentiation stages of tracheids and related environmental and endogenous cues.
Tracheids in each developmental stage are shown in their transverse section, indicating cambial division, enlargement, wall thickening, lignification, and programmed cell death prior to full maturation. Key environmental (photoperiod, temperature, water content) and endogenous (sugar availability) factors are placed according to their relative influence on each process. Figure from Rathgeber et al. {in preparation b}, prepared by G. Perez-de-Lis.

2.3.2 | Cambial cells proliferation: the division process

Introduction

Cell division is the elementary process through which the cell number is increased into a forming tissue. In all organisms whose cells contain a nucleus (i.e. the eukaryotes) dividing cells follow a highly ordered sequence of successive events called the cell cycle {Taiz and Zeiger, 1998; Evert, 2006}.
During this cycle, the meristematic mother cell undergoes several stages of development encompassing cellular growth and DNA synthesis, division of the nucleus, and separation of the cytoplasm, in order to give birth to two daughter cells {Lachaud et al., 1999}.

Mechanisms of cell division

During the cell cycle, the cell expands and the genetic material contained in the nucleus is duplicated and then divided into two identical batches. The two batches are then individually partitioned, resulting in the formation of two daughter cells. The division of the nucleus is called 'mitosis' or 'karyokinesis', while the separation of the cell into two daughter cells is called 'cytokinesis'.

Note that the enlargement of cambial cells is similar to the enlargement followed by differentiating cells and described in the next section. The only difference is that the enlargement of a cambial cell triggers cell division when the cell reaches a certain dimension, while the enlargement of a differentiating cell leads to an irreversible increase in its diameter without triggering division.
The formation of a new primary wall is necessary to separate the two daughter cells.
At the end of mitosis, a complex structure (combination of microtubes, actin microfilaments and endoplasmic reticulum), called phragmoplast, forms at the equatorial level of the cell, between the two separate sets of chromosomes. The phragmoplast is used as a framework for the formation of the new wall. Vesicles containing precursors of the wall components are combined to form the cellular plate, a primitive partition that leads to the definitive separation of the two daughter cells. The vesicles come from the Golgi apparatus, a cellular organelle that serves as a transit point and reservoir for proteins and lipids. Subsequently, the vesicles continue to flow: the membranes of the vesicles fuse together and with the plasma membrane of the dividing cell to form the new plasma membranes of the two daughter cells, while the contents of the vesicles provide the materials from which the common lamella (primary membrane that plays a role in the adhesion between the cells and on which the primary wall will settle) and the primary wall are assembled.
Cambial cell cytokinesis is very particular. Indeed, a cell generally divides in the direction of its smallest dimension. However, during periclinal division, a spindle-shaped cambial cell, which can be several hundred times longer than wide, is divided along its length. It must then form an abnormally high amount of new primary wall, several hundred times greater than that deposited for cell division in primary meristems, for example {Lachaud et al., 1999}.
This could explain why the process of cell division is slow in the cambium {Mellerowicz and Sundberg, 2008}, with cell cycle duration ranging between 10 and 50 days, depending on tree species, developmental stages, and environmental conditions {Skene, 1969; Cuny et al., 2012; Larson, 1994}.
To achieve high cell production rates, the tree must therefore increase the size of the cambial cell population {Saderi et al. in preparation}, hence the very close relationships obtained between the number of cambial cells and the cell production rate {Cuny et al., 2012}. As a result, the number of cells per developing radial file can only increase by about 1 cell per day for the most productive trees under the most favorable conditions.
Another particularity related to the large size of the fusiform cambial initials is that they contain a large central vacuole filled with water and solutes. According to Lachaud et al. {1999} and Cosgrove {2000a}, such a vacuole provide a cost-effective way to divide, as it reduces the amount of material and energy required for cytoplasm biosynthesis for each mitosis.

Regulation of cell division by internal factors

The division process is controlled by various plant hormones such as auxins, cytokinins and gibberellins {Sorce et al., 2013; Ursache et al., 2013}.
Auxins are the most well-known plant hormones and have been involved in regulating many aspects of plant development {Taiz and Zeiger, 1998}. In

particular, auxins play a fundamental role in the induction and progression of the cell cycle {Perrot-Rechenmann, 2010}.
At the cambium level, it has been shown that auxins initiate and maintain cell division activity {Little and Wareing, 1981}, influence the cambial division rate {Uggla et al., 1998}, as well as the relative proportions of xylem and phloem {Aloni, 1987}.
However, the presence of cytokinins is also essential to stimulate cell division {Matsumoto-Kitano et al., 2008}.
Finally, gibberellins are also known to promote cellular division {Aloni, 2001}.
Auxins, cytokinins and gibberellins act in stimulating the synthesis of key proteins: the cyclin-dependent kinases (CDKs), whose enzymatic activity is essential to trigger the start of the cell cycle, and to guarantee its smooth running {Stals and Inze, 2001}.
Differentiation, cell-cycle control, and cell size regulation form an intricate network of dependencies and feedback loops, which regulatory cascades are beginning to emerge {Harashima & Schnittger, 2010}.

Influence of environmental factors on cell division

Temperature exerts a direct control on cambial cell division, most probably via the polymerization-depolymerisation of the microtubules, which constitute a major component of the cytoskeleton, and play important roles in the division and differentiation of cells. Microtubules are sensitive to temperature: while favorable temperatures allow their polymerization, chilling temperature tends to depolymerize or disassemble them in plant cells {Begum et al., 2012; 2013}.
The increase of spring temperatures also promotes the enzymatic conversion of starch to sugar, which can then supply the energy for the biosynthesis of the new cell walls {Begum et al., 2010; 2013}.
Temperature also influences the division process via hormonal regulation operated by various phyto-hormones such as auxins, cytokinins, and gibberellins {Ursache et al., 2013}.

2.3.3 | Xylem cell radial growth: the enlargement process

Introduction

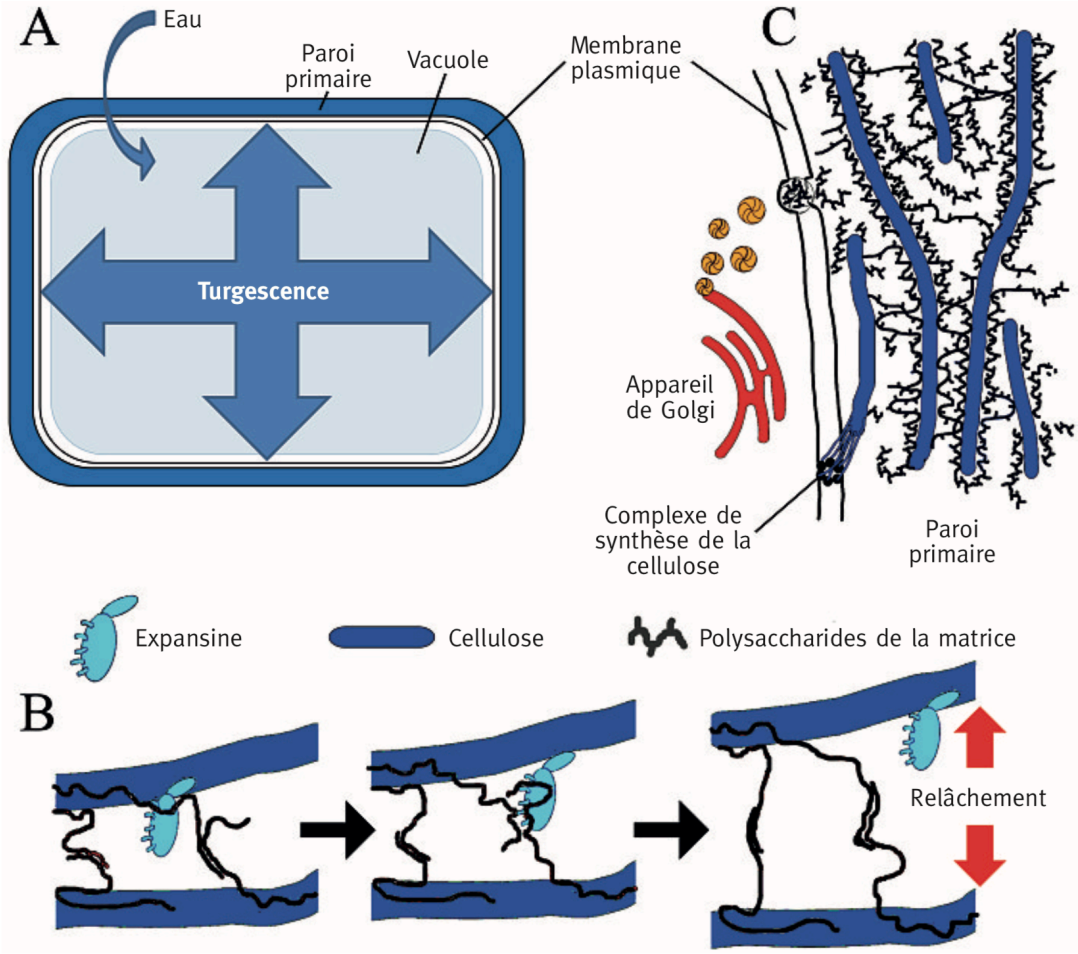
Cell enlargement, a process common to all types of plant cells, is the first stage of wood cell differentiation. It consists of an irreversible increase in cell volume (i.e. cell growth) not followed by any cell division.
The primary wall and the cell membrane are the theatre where the enlargement process takes place, the turgor pressure is the engine of this process, while auxins and other phytohormones are the drivers.
The orientation of cell growth is determined by the orientation of cellulose microfibrils, which is determine by the orientation of microtubules in the cytoplasm {Taiz & Zeiger, 2010}.

Mechanisms of cell enlargement

In nature, water tends to move from the least concentrated solution to the most concentrated one, this phenomenon is called osmosis.

In plants (because of the combined effects of active transport, which import solutes, and cell membrane semi-permeability, which keeps them from getting out) the solution inside the cell is more concentrated than the one outside. Water is therefore attracted into the cell and exerts a pressure (the so-called osmotic pressure) on the inside of the cell membrane and primary wall, which constitutes a thin but elastic barrier around the cell.
However, In the state of equilibrium, the primary cell-wall withstand the osmotic pressure, exerting a pressure in the opposite direction (the turgor pressure, also called turgidity) {Schopfer, 2006}.
The enlargement of the cell results from: (1) the relaxation of the primary cell wall; which (2) creates a passive inlet of water, which (3) is counter-balanced by an active influx of solutes in order to maintain a high turgor pressure {Cosgrove, 2005}. The first three steps of this mechanical-hydraulic process lead to an irreversible enlargement of the cell, but its completion also requires (4) the biosynthesis and deposition of building material to restore the integrity of the stretched primary cell walls [Fig. 2.4].
The relaxation of the primary cell wall is carried out under the coordinated action of several enzymes that break the bonds between the compounds of the wall {Rose and Bennett, 1999; Cosgrove, 2000b}.
The release of the wall causes the turgor pressure to drop, allowing water to enter the cell by osmosis, restoring the initial equilibrium between osmotic pressure and turgidity.
However, water absorption results in dilution of solutes and a reduction in the osmotic potential of the cell, which reduces its water absorption capacity and should lead to a rapid cessation of its enlargement.
To overcome this problem, the enlarging cells maintain a constant osmotic pressure by actively absorbing solutes in parallel with the water withdrawal. Regular cell enlargement can thus be maintained for several hours or days {Schopfer, 2006}.
Cell enlargement is made possible by the remarkable combination of elasticity and rigidity of the primary wall, which allows it to withstand distension while resisting the high mechanical forces imposed by turgor pressure {Cosgrove, 2000b}.
The thinning of the primary cell wall during its stretching is prevented by the concomitant deposition of newly synthesized wall material {Cosgrove, 2000a}.
Cellulose is comprised of hydrogen-bonded beta-1,4-linked glucan chains that are synthesized at the plasma membrane by large cellulose synthase (CESA) complexes. These complexes are composed of 6 subunits, each containing many units of cellulose-synthase, the enzymes that assemble the linear glucose chains that make up microfibrils {Mutwil et al., 2008}.
Hemicelluloses and pectins are synthesized by enzymes in the Golgi apparatus and then brought to the wall in tiny vesicles {Scheller and Ulvskov, 2010}.
The cessation of wall expansion that occurs during cell maturation is generally irreversible and is typically accompanied by a reduction in wall extensibility {Taiz & Zeiger, 2010}. Several modifications of the maturing wall may contribute to this rigidification: (1) a reduction in wall loosening processes; (2) an increase in wall cross-linking polysaccharides that may form tighter complexes; or (3) a modification of the composition of the wall, making for a more rigid structure or one less susceptible to wall loosening {Cosgrove 1997}.

It is important to underline that, for xylem (as for phloem) cells, the enlargement process only consist in an increase in the radial diameter of the cell. Nevertheless, this differentiation phase is particularly important for xylem tracheary elements, since their radial diameter can be multiplied by approx. 10 (gymnosperms) to 100 (angiosperms) during it.
As with the division, the enlargement of xylem cells would be carried out in a cost-effective way by filling the cell with a large central vacuole containing only water and solutes {Cosgrove, 2000a}.



4 — Figure 2.4 | Schematic description of the mechanism of cell enlargement. In A, the cell is in a state of full turgidity, with a large central vacuole filled with water that exerts pressure on the wall that is under tension. In B, the wall is relaxed by weakening the bonds between the polymers under the action of enzymes (expansins), which allows its extension and water entry. In C, the new wall material is deposited to prevent rupture during prolonged expansion. Modified from Cosgrove {2000b}. Figure from Cuny et al. {2014}.

Regulation of cell enlargement by internal factors

Like division, cell enlargement is regulated by phytohormones, primarily auxins but also cytokinins and gibberellins {Taiz and Zeiger, 1998}. These hormones would all act by increasing the extensibility of the primary cell wall, but using many different control pathways.

The action of auxins is closely related to key cell-wall loosening proteins called 'expansins' {Cosgrove, 2000b}. Auxin stimulates proton pumps in the plasma membrane, which activity lower the pH in the cell wall {Perrot-Rechenmann, 2010}. This increase of acidity stimulates expansins' enzymatic activity, which enables the relaxation of the wall by releasing the bonds between cross-linking polysaccharides (cellulose and hemicellulose in particular). With the loosening of the cellulose-hemicellulose network, the cell wall rigidity drops and it can expand under turgor pressure.
On the contrary, the mode of action of gibberellins and cytokinins has not been associated with acidification {Taiz and Zeiger, 1998}. The molecular mechanism of their action is still poorly understood but may be related to the activity of the xyloglucan-endotransglycosylase enzyme {Rose and Bennett, 1999; Cosgrove, 2000a}.

Influence of environmental factors on cell enlargement

Under normal conditions, the action of hormones (suc as auxin) and environmental conditions (light and water availability) modulate cell expansion by altering wall extensibility and/or yield threshold, controlling the final radial diameter of xylem cells.
However, since osmotic pressure is the 'engine' of cell wall expansion, water shortage must undoubtedly influence cell growth.
Considering the mechanisms behind cell enlargement, we expect that the effects of water stress are manifested as rapid decrease in enlargement rate leading to abrupt change in cell diameter (resulting in IADF for example), rather than progressive seasonal trends (as visible in EW-LW transition).

2.3.4 | Xylem cell secondary wall deposition: the thickening process

Introduction

The deposition of the secondary cell wall constitutes the second stage of xylem cell differentiation, its start also marks the end of cell enlargement.

Mechanisms of secondary wall deposition

The formation of the secondary wall begins with the deposition against the primary wall of a dense matrix of cellulose microfibrils associated with hemicelluloses and forming the S1 layer.
As with the formation of the primary wall, cellulose is synthesized at the level of the plasma membrane rosettes, while hemicelluloses are synthesized in the Golgi apparatus and then brought to the wall.
While the angle of the microfibrils with the cell axis is almost transverse in the S1 layer, the microfibrils are then deposited according to the longitudinal arrangement that characterizes the S2 layer [Fig. 2.2] {Beck, 2010}.
The S3 layer is finally formed against the S2 layer with a sudden reorientation of the microfibrils in the transverse helix.
Changes in the orientation of microfibrils during the formation of successive layers of the cell wall go hand in hand with the reorientation of fibres constituting the

cytoskeleton (filamentous network inside the cell) called "microtubules". This supports the hypothesis that microtubules control the orientation of cellulose microfibrils in the wall {Abe et al., 1995; Chaffey et al., 1999; Oda and Fukuda, 2012}.
The secondary wall is not covering the whole cell surface, but is absent around the pits. Pits thus form microscopic openings in the secondary wall, where the modified primary wall (the pit membrane) allows the passage of water and solutes from one cell to the next —making possible the upward sap flow from the root tips to the leaves.

Regulation of secondary cell wall deposition by internal factors

The formation of the secondary cell wall is a complex developmental process supported by the expression a significant number of genes activating the biosynthesis, transport, deposition, and assembly of the wall constituents {Mellerowicz et al., 2001; Reiter, 2002; Brown et al., 2005; Mellerowicz and Sundberg, 2008; Zhong and Ye, 2009; Zhong et al., 2011}.
The construction of the secondary wall must be a highly regulated process because cells need a coordinated expression of all these genes. Therefore, transcription factors —proteins that bind to specific DNA sequences and thus regulate gene expression— play a crucial role in regulating secondary wall formation {Mellerowicz and Sundberg, 2008; Zhong et al., 2011}.
However, there is much less knowledge about hormonal regulation of secondary wall formation compare to cell division or expansion {Mellerowicz et al., 2001}.
Auxin would play an indirect, inhibiting role. Indeed, this hormone is distributed according to a gradient between the different xylem zones: the concentration of auxins is highest in the cambial zone, decreases in the enlarged zone and reaches a very low or even zero level in the formation zone of the secondary wall {Uggla et al., 1996; Tuominen et al., 1997}. This gradient was interpreted as a positioning signal that would delimit the different differentiation zones. Auxin would act as a morphogen, i.e. a signal molecule that induces specific cellular responses according to its concentration. A low or zero concentration would favor the beginning of the formation of the secondary wall.
Finally, it has been shown for xylem cells in culture that brassinosteroids, a class of phytohormones, are necessary for the induction of secondary wall development {Yamamoto et al., 2001}.

Influence of environmental factors on secondary cell wall deposition

The influence of the environmental factors on the deposition of the secondary cell wall remains unexplored. However, we can be sure that this complex metabolic process needs favourable physiological conditions (e.g., temperature, water availability, pH), a good source of energy (ATP), and abundant building material (sugars) for its intense enzymatic activity of biosynthesis.

2.3.5 | Xylem cell wall lignification: the lignification process

Introduction

Cell wall lignification is a complex process occurring exclusively in higher plants to strengthen the plant vascular body.

The lignification of the common lamella, the primary wall, and the secondary wall corresponds to the deposition of phenol polymers, the so-called lignins, in the empty spaces of the extracellular poly-saccharidic network {Barcelo 1997}. Indeed, lignin permeates the network formed by the association between cellulose microfibrils and hemicelluloses, making the wall more rigid and resistant {Sarkanen and Ludwig, 1971}. As lignin is a hydrophobic molecule (which repels water), it also makes the wall waterproof {Zhong and Zheng-Hua, 2009}.

Mechanisms of cell wall lignification

Cell wall lignification starts at the cell corners, in the middle lamely and the primary wall, at about the same time as the deposition of the S1 layer. Then it extends along the middle lamella and the primary wall, before progressing inwards into the secondary wall following its deposition {Donaldson, 2001}.

After being synthesized in the cytosol (liquid fraction of the cytoplasm) from phenylalanine, the basic components of lignin (monolignols) are transported to the wall by a mechanism still yet unknown {Boerjan et al., 2003; Vanholme et al., 2010}. They are oxidized and polymerized to form complex cross-linked phenol polymers of lignin (a linear chain 13 to 20 monolignol units) that are incorporated into the wall (*ibidem*). Lignin is then deposited in the intercellular layers, in the spaces between cellulose microfibrils, where it forms chemical bonds with hemicelluloses, acting as a cement that strengthens and waterproofs the cell walls {Sarkanen and Ludwig, 1971}.

Recently, some authors suggest that a significant part of the lignification would occur postmortem {Pesquet et al., 2013; Smith et al., 2013}, which could be explained by two mechanisms: (1) a significant amount of monolignols is stored in the vacuole and released upon death {Pesquet et al., 2010}; (2) living xylem cells such as parenchyma can synthesize monolignols and export them to the lignified walls of neighbouring dead cells —this mechanism referred to as non-autonomous lignification— {Pesquet et al., 2013; Smith et al., 2013}.

As a consequence of the timing of the lignification process and the structure of the walls, the proportion of lignin decreases from the most external layers of the cell walls (i.e. the middle lamella and the primary wall) to the most internal ones (i.e. the secondary wall) {Donaldson et al. 2018}.

This well-designed structure perfectly fits the physiological functions of the xylem tracheary elements. The heavy impregnation of the middle lamella and primary wall by the rigid and hydrophobic lignin molecules, allows xylem tracheary elements to form strong, rigid and self-supporting networks of waterproof « pipes », while the lighter impregnation of the secondary wall allows tracheary element lumens to keep essential capillarity properties that can support sap ascent.

Regulation of xylem cell lignification by internal factors

As for the other components of the cell walls, transcription factors play an essential role in the biosynthesis, transport, and deposition of lignin {Schuetz et al., 2013; Vanholme et al., 2010; Zhong and Ye, 2009; Zhong et al., 2011}.

A direct hormonal effect may also exist on lignification because auxins have been shown to increase the lignification of secondary xylem in tobacco plants (*Nicotiana tabacum* (L.)) {Sitbon et al., 1999}. However, this stimulation of auxins was

associated with an increase in ethylene production which is known to induce several enzymes involved in lignin biosynthesis.

Influence of environmental factors on xylem cell lignification

The influence of the environmental factors on the deposition of the secondary cell wall remains almost unexplored. The lignification process is generally described as sensitive to temperature {Donaldson, 2001; Piermattei et al. 2015}; but, we can be sure that this complex metabolic process needs also favourable physiological conditions (e.g., water availability, pH), a good source of energy (ATP), and abundant building material (sugars) for its intense enzymatic activity of biosynthesis.

2.3.6 | Xylem cell apoptosis: the programmed cell death process

Introduction

Programmed cell death (also called apoptosis) marks the end of xylem cell differentiation and the advent of mature, fully functional, xylem elements (e.g., tracheids for gymnosperms, vessels and fibbers for angiosperms). In xylem, only parenchyma cells escape programmed cell death and remain alive for several years in the sapwood.

This process involves a highly controlled sequence (hence the term "programmed") of events that leads a cell to kill itself. This "suicide" process is followed by many cells in multicellular organisms (e.g., plants, fungi, or animals), because it is essential to life {Ellis et al., 1991}. For example, in plants, the process plays a central role in the development and defence against pathogens {Greenberg, 1996; Jones, 2001}. However, the case of xylem elements is a remarkable example of programmed cell death. Indeed, while most differentiated cells perform specialized functions until death, xylem-conducting cells die before they perform their function. This is therefore paradoxical: the death of wood cells is vital, because it is it that, by emptying the cell lumen, makes them suitable for sap conduction.

Mechanism of xylem cell apoptosis

Although they have similarities, the molecular mechanisms of programmed cell death differ according to the organisms and cell types considered. Programmed cell death of xylem elements is a specific mechanism in which the vacuole plays a central role {Fukuda, 2000}.

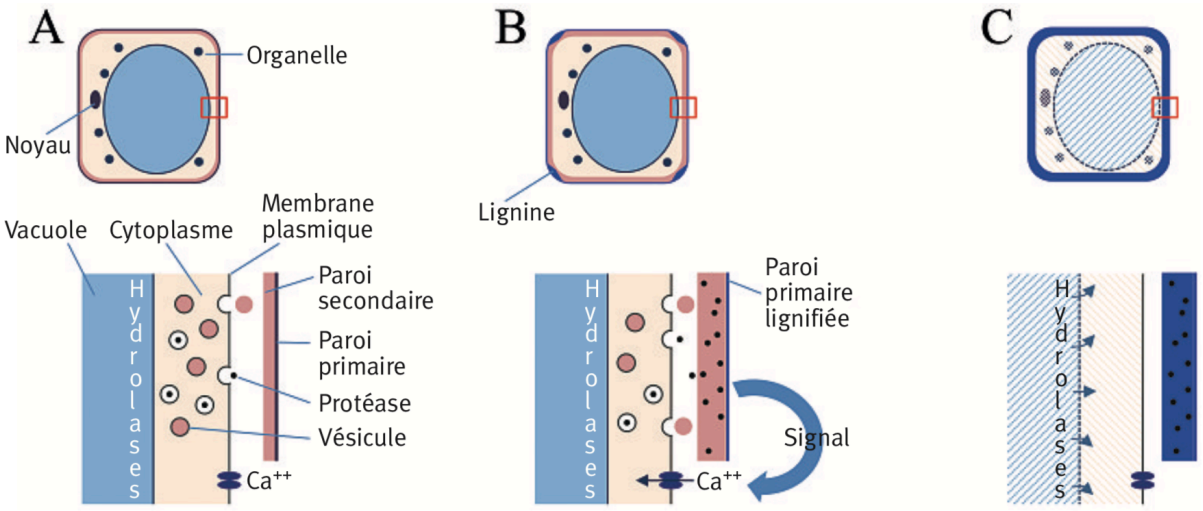
The main trigger for programmed cell death is a regulated entry of calcium ions (Ca2+) into the cell, probably through the channels of the plasma membrane {Groover and Jones, 1999; Jones, 2001}. Death then manifests rapidly (in few minutes or so) as a sudden break-up of the vacuole and the cessation of the cytoplasmic streaming {Groover and Jones, 1999; Fukuda, 2000}.

The break-up of the vacuole releases hydrolases, which attack and degrade the cell organelles and clean up the cell content (autolysis) {Bollhöner et al., 2012}.

After a couple of days, the cell is finally left as an empty space (the lumen) surrounded by a thick wall pierced with punctuations.

Regulation of xylem cell programmed cell death by internal and external factors

Once again, transcription factors would play a crucial role in regulating programmed cell death {Escamez and Tuominen, 2014}. However, the mechanisms regulating apoptosis and secondary wall formation appear to be inseparable, as most of the agents that regulate the first process also regulate the second {Bollhöner et al., 2012; Schuetz et al., 2013; Escamez and Tuominen, 2014}.
For example, identical transcriptional factors are involved in the regulation of both the secondary wall and programmed cell death {Ohashi-Ito et al., 2010}. Similarly, brassinosteroids, which initiate the formation of the secondary wall, also initiate apoptosis {Fukuda, 2000; Kuriyama and Fukuda, 2001; Yamamoto et al., 2001}.
In their seminal work, Groover & Jones {1999} proposed a biological mechanism linking together xylem cell apoptosis and secondary wall formation [Fig. 2.5]. In this mechanism, the deposition of parietal material is accompanied by the secretion of an enzyme (protease) that accumulates in the extracellular matrix. When the activity of the protease reaches a critical threshold, it triggers the influx of calcium ions, which, in turn, triggers the process of programmed cell death.
The influence of the environmental factors on programmed cell death remains completely unexplored.



5 — Figure 2.5 | Schematic description of the mechanism of programmed cell death.
In **A**, parietal material and a protease are brought by vesicles to the secondary wall formation site. In **B**, the protease concentration in the extracellular matrix exceeds a threshold, which triggers an influx of calcium ions (Ca++) through the plasma membrane channels. In **C**, the entry of CA++ caused the break-up of the vacuole and the release of hydrolases, which degraded the cell content. This process eventually forms a dead and empty cell capable of transporting water and providing mechanical support. Figure from Cuny et al. {2014}.

Conclusion

Apoptosis is an essential step of xylem cell differentiation, allowing mature xylem cells to perform their specific functions in trees. The cell walls (in particular in tracheids and fibers) endow the function of mechanical support to the wood, while

the empty cell lumens and the pits (in particular in tracheids and vessels) offer the necessary pathway for water transport into the plant.

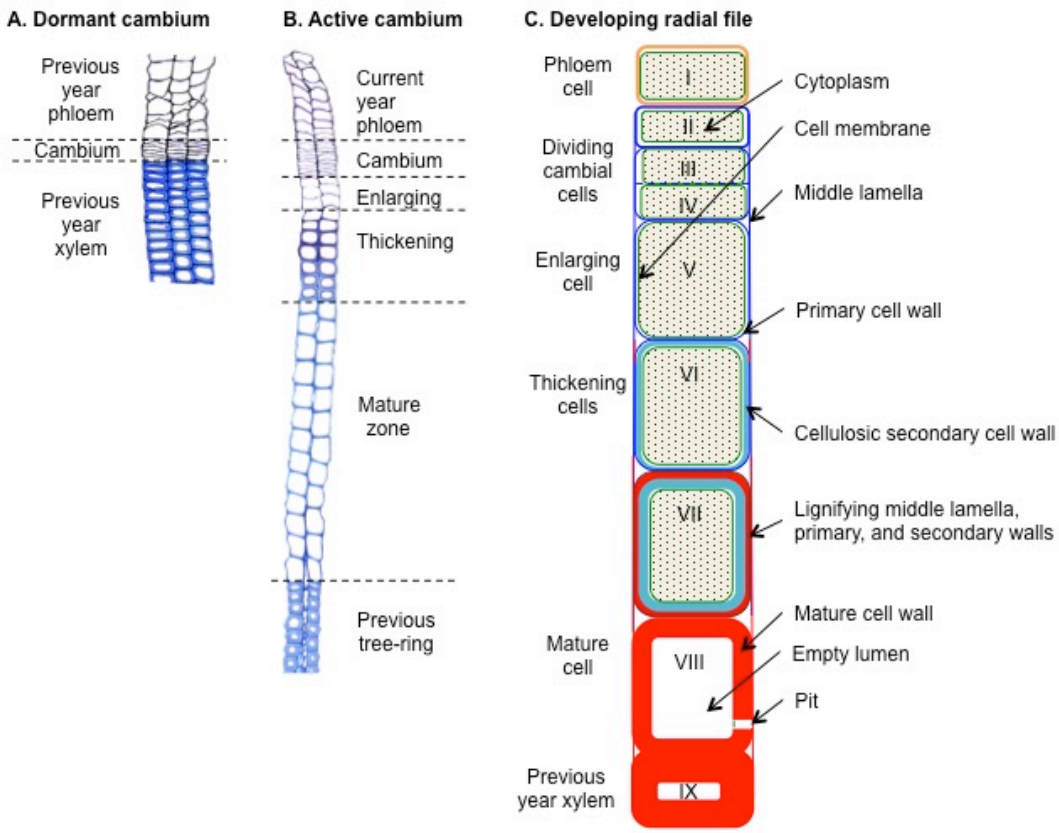
2.4 | Seasonal changes of cambium and developing xylem and phloem

2.4.1 | Introduction

Cambial activity follows the cycle of the seasons {Denne and Dodd, 1981}. In extra-tropical regions, the cambium is dormant during winter and active during summer {Delpierre et al., 2015}, while in tropical regions it may rest during the dry season and be active during the wet season {Breitsprecher and Bethel, 1990}. Annual growth rings and typical tree-ring structures, both result from these periodical changes in cambial activity {Evert, 2006}.
The cambial initials contain many small vacuoles (during the winter rest period) or a large central vacuole (during the growth period) {Prislan et al., 2011}. They are surrounded by a thin primary wall (about 0.1–1.0 µm thick) and have a narrow diameter.

2.4.2 | The resting cambium

During winter, the dormant cambium is composed of a few layers of resting cells (3-6), presenting thickened primary cell walls [Fig. 2.6A]. Each spring, when day length increases and temperature rises, cambium resumes activity preparing the first divisions of mother cells {Prislan et al., 2013}.
Cambial initials are small cells that contain very many small vacuoles (during the winter rest period) or a large central vacuole (during the growing season); they are surrounded by a thin primary wall (about 0.1-1 µm thick).



6 — Figure 2.6 | Schematic cross-sections through resting and developing radial xylem cell files in Scots pine (*Pinus sylvestris* L.).
A. Dormant cambium during winter, composed of a thin strip of 4–6 reinforced cambial cells layers, looking like a "pile of plates", and embedded between the xylem and the phloem formed during the previous growing season. B. Active cambium and associated developing radial cell files at the beginning of summer. The active cambium, composed of a wide strip of 11–13 cambial cells layers, exhibiting wavy radial cell walls, is embedded between the newly formed phloem and xylem. The developing xylem, which is composed of an enlarging zone (3-4 cell layers), a thickening zone (7 cell layers), and a mature zone (12-13 cell layers), is embedded between the cambium and the previous tree ring. C. Schematic view of a developing xylem radial file. Cell I represents a freshly formed phloem cell of the current year. Cells II to IV represent cambial cells, of which cells III and IV are dividing. Cell V represents an enlarging cell. Cells VI to VII represent thickening cells (note the beginning of the lignification at the corners of cell VI). Finally, cell VIII represents a mature, dead, and fully functional xylem cell (note the disappearance of its cytoplasm). Cell IX represents a latewood cell belonging to the previous tree ring (i.e. formed during the previous growing season). Green backgrounds represent cytoplasm, green lines represent cell membranes, blue lines represent cellulosic cell walls (dark blue for primary walls, light blue for secondary walls), and red areas represent lignified cell walls.

2.4.3 | The beginning of the growing season

During the growing season, the active cambium is composed of numerous dividing cells (6-18), presenting thin tangential cell walls [Fig. 2.6B].

A couple of days or weeks after the start of cambial cell divisions, newly created xylem cells appeared in the developing xylem. The enlarging cells still only consist of primary cell walls, but present much wider radial diameters than dividing cells. The appearance of these first enlarging cells marks the onset of xylem radial growth and wood formation. A couple of weeks after their birth, these first cells start to thicken, building their secondary walls.
Because secondary walls hold most of the biomass, the appearance of the first thickening cells can be seen as the effective beginning of carbon sequestration into wood {Rathgeber et al. 2016}.
Finally, one or two months after their birth, differentiating xylem cells reached their final mature state. Mature and fully functional xylem treachery elements are composed of thickened secondary cell walls surrounding empty lumens.

2.4.4 | The structure of the developing xylem during the main part of the growing season

During the growing season, new xylem cells, resulting from cambial cell division, are disposed along radial files, and successively undergo their differentiation program according to their identity and their place in the "queue" (i.e. the radial file).
At the tissue level, the succession in time of cells belonging to the different stages of differentiation is well coordinated between all the radial files, creating a characteristic spatial pattern composed of strip-like developmental zones [Fig. 2.6C]. Once established, this organization remains rather stable throughout the growing season {Vaganov et al., 2006}.
Provided that no environmental stress comes into play, cambial activity and xylem radial growth rate generally peak around the summer solstice, when the photoperiod is maximal {Rossi et al. 2006}. This period also generally marks the transition between early- and late-wood {Cuny et al., 2014}.

2.4.5 | The end of the growing season

At the end of the growing season, in autumn —or even earlier if water or temperature conditions are not favorable anymore— cambial activity stops, soon followed by cell enlargement (flagging the end of stem radial growth).
However, the completion of wood formation (marking the end of carbon sequestration) only occurs a couple of months later {Cuny et al., 2015}. Indeed, lignification is a slow process, so the last xylem cells need up to two months for completing cell wall maturation and reaching full maturity {Cuny & Rathgeber, 2016; Cuny et al, 2018}.

2.4.6 | Conclusion

The cambium and the developing xylem and phloem form a complex dynamic system that periodically produces living bark and wood according to the cycle of the seasons.
Without a profound knowledge of the biological processes at play in each component of this system, it is not possible to understand how xylogenesis

responds to environmental conditions, and how it creates typical tree-ring structures endowing specific functions to the wood.
Furthermore, considering the interactions between the environmental drivers, the physiological state of the trees, and the developmental stage of the forming xylem, is required to comprehend the creation of the typical tree-ring structures during normal seasonal cycles, as well as special anatomical wood features formed under exceptional conditions (e.g., extreme climatic events).

2.5 | Heartwood formation

2.5.1 | Introduction

Heartwood in tree stems provides natural durability and ornamental values to wood products as well as antimicrobial properties to protect living stems from decay. Control of the amount and quality of heartwood is required because of its importance in wood utilization. However, despite its importance, the mechanism of heartwood formation has been poorly understood.
A number of years after being formed, the parenchymal cells also die while the conductive elements of the wood become clogged. This marks the transition from a conductive wood: the sapwood, which contains living cells and energy reserve materials; to an inert wood: the heartwood (also called heartwood because it is located more in the centre of the stem), which no longer contains living cells or reserve materials (which were disposed or recycled), and which no longer conducts raw sap {Wilson, 1970}.

2.5.2 | Mechanism of heartwood formation

Non-structural biochemical compounds, called extractives, are responsible for the formation of heartwood. Extractibles can be of various kinds (e. g., polyphenols, essential oils, resins) and most are easily eliminated (hence their name) with neutral solvents {Pereira et al., 2003 — Wood Quality and its Biological Basis}.
The presence of extractives and the absence of reserve compounds increase the durability of heartwood by making it less attractive to decomposing organisms. Heartwood formation would also reduce tree's maintenance costs by optimizing the volume of sapwood, whose living parenchyma cells involve significant maintenance costs {Taylor et al., 2002}.
The extractives are synthesized by the living parenchyma cells of the sapwood at the border with the heartwood. They are formed from compounds available locally or brought from the sapwood or phloem, then transferred to the heartwood by passing from cell to cell through the punctuations. The extractives then impregnate the cell walls in a way that resembles lignification, i.e. starting with the middle lamella and then continuing with the primary and secondary walls. They block the pits that connect adjacent cells, which explains the loss of conduction in heartwood {Kuroda et al. 2009}.
Thyloses, bubble-shaped parenchyma projections in the lumen of the conductive elements, can also occur significantly during heartwood formation {De Micco et al. 2016}. Thyloses are formed as a result of enzymatic hydrolysis that breaks the pit membrane between a parenchyma cell and an adjacent conductive element. A part

of the parenchyma cell invades then the light of the conductive element, thus blocking this element.

2.5.3 | Regulation of heartwood formation by internal factors

Heartwood formation appears to be highly genetically controlled. Indeed, heartwood develops at a more or less advanced age depending on the species and is added more or less regularly over the years. As a result, some species have little heartwood in proportion (i.e. heartwood develops in the very old sapwood); while others have many (i.e. heartwood develops in the young sapwood) {Taylor et al., 2002}.

In addition, heartwood formation is linked to the action of many enzymes specific to the sapwood–duramen border. These enzymes are involved in the degradation of reserve compounds (e.g., sugars, starch and lipids) and the synthesis of extractives {Magel, 2000 —Biochemistry and physiology of heartwood formation. In: Cell and molecular biology of wood formation}.

Hormonal control of heartwood formation is also suspected, particularly by ethylene, which is associated with processes such as cell-wall lignification, fruits ripening, or flowers and leaves senescence. Significant ethylene production has been observed at the duramen–aubier border in many species. Ethylene is associated with increases in enzyme activity and the production of phenolic extractives from heartwood {Magel, 2000}.

2.5.4 | Influence of environmental factors on heartwood formation

Dehydration of tracheids at the boundary between sapwood and heartwood wood occurred continuously during calendar year. The death of ray parenchyma cells at the border between sap- and heart-wood occurred from spring to summer. The deposition of heartwood substance took place in autumn to winter. Thus, different components of heartwood formation were active in different seasons {Nakada, 2012}.

2.5.6 | Concluding remark

Since heartwood formation is a system consisting of several components within a series of continuing processes, understanding the seasonal change of heartwood formation will require monitoring these components simultaneously.

2.6 | Synthesis and conclusion

Xylogenesis produces wood cells that are adapted to perform vital functions in woody plants. It involves several processes and represents an extremely complex example of cellular specialization.

Xylogenesis has led to a major transformation of our planet by forming a tissue that provides both the mechanical support necessary for stability in the unbearable air environment, as well as the ability to transport water upwards and over long distances; it has given trees a competitive advantage that has allowed them to colonize the Earth's emerged lands, developing sizes that can be huge.

In this section we provided a synthesis of current knowledge on the functioning and regulation of the five main cellular processes of xylogenesis: cell division, cell enlargement, secondary wall formation, wall lignification and programmed cell death.
This knowledge at the cellular level still needs to be further developed and integrated with recent advances in genetics and genomics, as well as a more global understanding of xylogenesis in relation with tree functioning.
This should ultimately lead to a more complete knowledge of the functioning and regulation of the xylogenesis process, which is particularly important in view of the need to protect forest biodiversity and optimise wood supply in a context of global change.

2.7 | References

This reference list is incomplete and not properly formatted

Barceló AR Lignification in plant cell walls - International review of cytology, 1997 - Elsevier

Begum, S., Nakaba, S., Yamagishi, Y., Oribe, Y., and Funada, R. (2012). Regulation of cambial activity in relation to environmental conditions: understanding the role of temperature in wood formation of trees. Physiologia Plantarum 147, 46–54. doi: 10.1111/j.1399-3054.2012.01663.x.

Bonan, G. B. (2008). Forests and climate change: forcings, feedbacks, and the climate benefits of forests. Science 320, 1444–1449. doi: 10.1126/science.1155121.

Boyce CK, Zwieniecki MA, Cody GD, Jacobsen C, Wirick S, Knoll AH, Holbrook NM. 2004. Evolution of xylem lignification and hydrogel transport regulation. Proceedings of the National Academy of Sciences, USA 101: 17 555–17 558.

Breitsprecher, A., and Bethel, J. S. (1990). Stem-Growth Periodicity of Trees in a Tropical Wet Forest of Costa Rica. Ecology 71, 1156–1164.

Bollhöner, B., Prestele, J., and Tuominen, H. (2012). Xylem cell death: emerging understanding of regulation and function. Journal of Experimental Botany 63, 1081–1094. doi:10.1093/jxb/err438.

Cosgrove, D. J. (2005). Growth of the plant cell wall. Nature Reviews. Molecular Cell Biology 6, 850–861. doi: 10.1038/nrm1746.

Cuny, H. E, Rathgeber, C. B. K., Frank, D. C., Fonti, P., Mäkinen, H., Prislan, P., et al. (2015). Woody biomass production lags stem-girth increase by over one month in coniferous forests. Nature Plants 1, 15160. doi: 10.1038/nplants.2015.160.

Cuny, H. E., and Rathgeber, C. B. K. (2016). Xylogenesis: coniferous trees of temperate forests are listening to the climate tale during the growing season but only remember the last words! Plant Physiology 171, 306–317. doi: 10.1104/pp.16.00037.

Cuny, H. E., Rathgeber, C. B. K., Frank, D. C., Fonti, P., and Fournier, M. (2014). Kinetics of tracheid development explain conifer tree-ring structure. New Phytologist 203, 1231–1241. doi: 10.1111/nph.12871.

Delpierre, N., Vitasse, Y., Chuine, I., Guillemot, J., Bazot, S., Rutishauser, T., and Rathgeber, C. B. K. (2015). Temperate and boreal forest tree phenology: from organ-scale processes to terrestrial ecosystem models. Annals of Forest Science 73(1), 5–25. doi: 10.1007/s13595-015-0477-6.

Denne, M. P., and Dodd, R. S. (1981). The environmental control of xylem differentiation. Xylem cell development. Castle House Publications: Tunbridge Wells, 236–255.

Donaldson, L. A. (2001). Lignification and lignin topochemistry — an ultrastructural view. Phytochemistry 57, 859–873. doi: 10.1016/s0031-9422(01)00049-8.

Evert, R. F. (2006). Esau's Plant Anatomy (3rd ed.). John Wiley & Sons. 624p.

Expansion of the plant cell wall – D.J. Cosgrove, Pennsylvania State University, USA..Annual Plant Reviews, Volume 8, The Plant Cell Wall
Jocelyn K. C. Rose (Editor)ISBN: 978-1-405-14773-6 February 2009 Wiley-Blackwell 400 Pages

Griçar, J., Rathgeber, C. B. K., and Fonti, P. (2011). Monitoring seasonal dynamics of wood formation. Dendrochronologia 29, 123–125. doi: 10.1016/j.dendro.2011.01.007.
Groombridge, B., and Jenkins, M. D. (2002). World Atlas of Biodiversity. Prepared by the UNEP World Conservation Monitoring Centre. University of California Press. 360p.
Groover, A., and Jones, A. (1999). Tracheary element differentiation uses a novel mechanism coordinating programmed cell death and secondary cell wall synthesis. Plant Physiology 119, 375–384.
Körner, C. (2015). Paradigm shift in plant growth control. Current Opinion in Plant Biology 25, 107–114. doi: 10.1016/j.pbi.2015.05.003.
Kozlowski, T. T., and Pallardy. S. G. (1997). Physiology of Woody Plants. Academic Press. 411p.
Lachaud, S., Catesson, A.-M., and Bonnemain, J.-L. (1999). Structure and functions of the vascular cambium. Comptes Rendus de l'Académie des Sciences - Series III - Sciences de la Vie 322, 633–650. doi: 10.1016/S0764-4469(99)80103-6.
Lal, R. (2008). Sequestration of atmospheric CO2 in global carbon pools. Energy & Environmental Science 1, 86–100.
Larson, P. R. (1994). The Vascular Cambium: Development and Structure. Springer-Verlag. 725p.
Pan, Y., Birdsey, R. A., Fang, J., Houghton, R., Kauppi, P. E., Kurz, W. A., et al. (2011). A Large and persistent carbon sink in the world's forests. Science, 333, 988–993. doi: 10.1126/science.1201609.
Perrot-Rechenmann, C. (2010). Cellular Responses to Auxin: Division versus Expansion. Cold Spring Harbor Perspectives in Biology 2, a001446. doi: 10.1101/cshperspect.a001446.
Plomion, C., Leprovost, G., and Stokes, A. (2001). Wood formation in trees. Plant Physiology 127, 1513–1523. doi: 10.1104/pp.010816.
Prislan, P., Čufar, K., Koch, G., Schmitt, U., and Griçar, J. (2013). Review of cellular and subcellular changes in the cambium. IAWA Journal 34, 391–407. doi: 10.1163/22941932-00000032.
Rowe, N., and Speck, T. (2005). Plant growth forms: an ecological and evolutionary perspective. New Phytologist 166, 61-72.
Seneviratne, S.I., Lüthi, D., Litschi, M., and Schär, C. 2006. Land-atmosphere coupling and climate change in Europe. Nature 443, 205-209.
Sperry, J. S., Hacke, U. G., and Pittermann, J. (2006). Size and function in conifer tracheids and angiosperm vessels. American Journal of Botany 93, 1490–1500.
Stals, H., and Inze, D. (2001). When plant cells decide to divide. Trends in Plant Science 6, 359–364.
The composition and structure of plant primary cell walls – M. A. O'Neill and W.S. York, Complex Carbohydrates Research Center, Athens, Georgia, USA.. Annual Plant Reviews, Volume 8, The Plant Cell Wall
Jocelyn K. C. Rose (Editor)ISBN: 978-1-405-14773-6 February 2009 Wiley-Blackwell 400 Pages
Ursache, R., Nieminen, K., and Helariutta, Y. (2013). Genetic and hormonal regulation of cambial development. Physiologia Plantarum 147, 36–45. doi: 10.1111/j.1399-3054.2012.01627.x.
Vaganov, E. A., Hughes, M. K., and Shashkin, A. V. (2006). Growth Dynamics of Conifer Tree Rings. Springer. 354 p.
Wang H, Zhao Q, Chen F, Wang M, Dixon RA. 2011. NAC domain function and transcriptional control of a secondary cell wall master switch. Plant Journal 68: 1104–1114.
Wang HZ, Dixon RA. 2012. On-off switches for secondary cell wall biosynthesis. Molecular Plant 5: 297–303.
Wang J, Kucukoglu M, Zhang L, Chen P, Decker D, Nilsson O, Jones B, Sandberg G, Zheng B. 2013. The Arabidopsis LRR-RLK, PXC1, is a regulator of secondary wall formation correlated with the TDIF-PXY/TDR-WOX4 signaling pathway. BMC Plant Biology 13: 94.
Wilson, J. W. (1970). The Growing Tree. The University of Massachusetts Press. 152 p.
Zhong, R., and Ye, Z.-H. (2009). Secondary cell walls. eLS. doi: 10.1002/9780470015902.a0021256/full.
Zimmermann M. H. (1983). Xylem Structure and the Ascent of Sap. Springer-Verlag. 143 p.

CHAPTER 3 | METHODS FOR MONITORING WOOD FORMATION

3.0 | Synthesis

3.0.1 | Related papers

Harroué M, Cornu E, Rathgeber CBK. 2011. Méthode de prélèvement et de préparation des échantillons pour l'étude de l'activité cambiale et de la formation du bois. *Cahier des Techniques de l'INRA* 73: 15–62.

Rathgeber CBK, Longuetaud F, Mothe F, Cuny H, Le Moguédec G. 2011. Phenology of wood formation: Data processing, analysis and visualisation using R (package CAVIAR). *Dendrochronologia* 29: 139–149.

Rathgeber CBK, Santenoise P, Cuny H. 2018. CAVIAR: an R package for checking, displaying and processing wood-formation-monitoring data. *Tree physiology* 38: 1246–1260.

De Micco V, Carrer M, Rathgeber CBK, Camarero JJ, Voltas J, Cherubini P, Battipaglia G, 2019. From xylogenesis to tree rings: wood traits to investigate tree response to environmental changes. *IAWA Journal* 40 (2): 155-182.

3.0.2 | Summary

In the last decade, the pervasive question of climate change impacts on forests has revived investigations on intra-annual dynamics of wood formation, involving disciplines such as plant ecology, tree physiology, and dendrochronology.
This resulted in the creation of many research groups working on this topic worldwide and a rapid increase in the number of studies and publications.
Wood-formation-monitoring studies are generally based on a common conceptual model describing xylem cell formation as the succession of four differentiation phases (cell division, cell enlargement, cell wall thickening, and mature cells).
They generally use the same sampling techniques, sample preparation methods, and anatomical criteria to separate between differentiation zones and discriminate and count forming xylem cells, resulting in very similar raw data. However, the way these raw data are then processed, producing the elaborated data on which statistical analyses are performed, still remains quite specific to each individual study.
Thereby, despite very similar raw data, wood-formation-monitoring studies yield results, which are still quite difficult to compare.
CAVIAR —an R package specifically dedicated to the verification, visualisation, and manipulation of wood-formation-monitoring data— can help to improve this situation. Initially, CAVIAR was built to provide efficient algorithms to compute critical dates of wood formation phenology for conifers growing in temperate and cold environments.
Recently, we developed it further to check, display, and process wood-formation-monitoring data.

Thanks to new and upgraded functions, raw data can now be consistently verified, standardised, and modelled (using logistic regressions and Gompertz functions), in order to describe wood phenology and intra-annual dynamics of tree-ring formation.
We believe that CAVIAR will help strengthening the science of wood formation dynamics by effectively contributing to the standardisation of its concepts and methods, making thereby possible the comparison between data and results from different studies.

3.0.3 | Key words

Tree-ring formation dynamics, Wood anatomy, Cambium activity, Xylem development, Gompertz function, Tree growth

3.1 | Introduction

3.1.1 | Background and scientific context

In the current context of high uncertainties about the impacts of climate change on forest functioning, tree growth and wood production, investigations concerning intra-annual dynamics of wood formation have received a renewed interest in the last 15 years from disciplines such as plant ecology, tree physiology, and dendrochronology.
Research groups working on tree-ring formation dynamics emerged in many countries all over the world, leading to a rapid increase in the number of studies and publications on the topic {Griçar et al. 2011}.
Over times, microcoring {Rossi et al. 2006a} has been adopted by most of the research groups, establishing it as the main technique for sampling forming wood. Most of the groups also use the same, well-documented, sample treatment techniques to prepare anatomical slides {Rossi et al. 2006b}.
Moreover, most of wood-formation-monitoring studies are based on the same conceptual model of xylem cell differentiation developed by Wilson et al. {1966}.
Finally, most of these groups also use the same anatomical criteria to discriminate between the different zones of the developing xylem and to count the number of forming cells in each stage of differentiation {Rossi et al., 2006b}.
Thanks to this common practical framework, research groups performing wood-formation-monitoring studies produce very similar raw data all over the world.
In that sense, the situation is quite comparable to the one found in dendrochronology. Both disciplines produce homogenous raw data, and process these raw data into elaborated data that will be further analysed.
In the field of wood formation dynamics, typical elaborated data on which analyses are often performed are critical dates of xylem development (e.g., onset of cell enlargement, occurrence of maximum growth rate, cessation of cell wall maturation), and critical rates of xylem formation (e.g., mean or maximum rate of cell production).
However, in contrast to dendrochronology {e.g., Grissino-Mayer, 2001; Bunn, 2008, 2010; Zang and Biondi, 2014}, no method was published until very recently {Rathgeber et al., 2018} to allow checking wood-formation-monitoring raw data, pointing out potential errors, and assessing overall dataset quality.

Moreover, to date, no method has established itself as the standard to compute elaborated data. The consequence is that despite homogeneous raw data, results from wood-formation-monitoring studies are still quite difficult to compare directly, for example, to highlight global ecological trends in a global analysis {but see Rossi et al., 2016}.
We believe that CAVIAR —an R package specifically dedicated to check, visualise, and process wood-formation-monitoring data— can help to improve this situation. Initially, CAVIAR was built to implement objective definitions and provide efficient algorithms to compute and analyse critical dates and durations of wood formation phenology for conifers growing in temperate and cold environments {Rathgeber et al., 2011a}.
However, the most recent version of CAVIAR, has been further developed to tackle the challenges mentioned here above {Rathgeber et al., 2018}.
This new version provides a set of functions designed to import formatted datasets, check raw data, pinpoint potential errors and outliers, and assess overall data quality. It also provides new functions to visualise and model intra-annual dynamics of wood formation. Moreover, former functions allowing estimating, displaying, and analysing wood formation critical dates have been upgraded.

3.1.2 | Aim of the chapter

In this chapter we briefly reviewed the most common techniques and methods used in wood-formation-monitoring studies, from sample collection, preparation and observation, to data checking, visualisation and analyses.

3.2 | Sampling design

3.2.1 | Instrumented sites

Introduction

Wood-formation-monitoring studies can involve single instrumented sites {e.g., Rathgeber et al., 2011b} or more frequently networks of sites, organised in altitudinal {e.g., Cuny et al., 2014; Moser et al., 2010} or latitudinal gradients {e.g., Jyske et al., 2014, Rossi et al., 2014}.

Study sites

Concerning single sites, we hope that our flux tower site in Hesse forest will become a reference {Andrianantenaina, 2019}. In this temperate lowland forest we monitor the wood formation of European beech (and sessile oak) since 2015.
Concerning altitudinal gradients, the most impressive research design is, without any doubt, the network of instrumented sites of the Lötschental valley in Switzerland {Moser et al., 2010}. This network is composed of nine mixed irregular stands, containing each 1 or 2 mountain coniferous species (Larch and Norway spruce), monitored since 2007, and spread along two altitudinal gradients, on north and south facing slopes, from 800 to 2200 m a.s.l.
Concerning latitudinal gradients, the most emblematic research design is, without any doubt, the network of instrumented sites of Québec province in Canada {Rossi et al., 2014}. This network is composed of five pure, mature and even-aged stands,

monitored since 2002 and spread from the 48th to 53rd parallels to cover the whole closed black-spruce distribution in the boreal forest.

Tree selection

Most of the wood-formation-monitoring studies are based on groups of five (3–15) healthy trees grown in the same conditions.
These groups are generally used to investigate the influence of environmental factors on tree-ring formation.
But they are also often used in combination to compare the behaviour of two or three species {e.g., Cuny et al., 2012, Michelot et al., 2012}, age or dimension classes, or social status {e.g., Rathgeber et al. 2011a, Rossi et al. 2008} according to the constitution of the groups.

Tree sampling

Wood samples (i.e. microcores of 2 mm in diameter and 15–20 mm in length) are taken at breast height on the stem of the selected trees using a Trephor® tool {Rossi et al., 2006a} and following an ascending spiral pattern {Deslauriers et al., 2003}.
Microcores are generally collected weekly over one or preferably three (or even more) growing seasons. Generally selected trees are monitored over at least one complete growing season, and changed after a couple of years in order to reduce the impact of sampling-related stimulation and wound reaction.
They are taken at about 2 cm apart to avoid wounding tissues and resin ducts, without increasing too much stem circumferential variability {Wodzicki and Zajaczkowski, 1970}.
To avoid deterioration of living tissues, microcores are generally placed in eppendorfs containing solution of ethanol 50 % and then stored at 5 °C.

3.2.2 | Experimentation

Introduction

Alternatively, to the direct observation of the tree response to the natural changes of their environment, experiments were conducted either in situ or in greenhouses in order to better control environmental parameters and measure tree response more accurately.

Experimentation in natural sites

Experimentation in natural site mainly involve localized heating or cooling {e.g., Gricar et al., 2006; Begum et al., 2016}.
In these experimental studies, a « blancket and a pipe » are wrapped around the stem a tree, while the blancket insulate the portion of stem from the external environment, the pipe heat or cool the stem according to the study protocol.
Small blocks are collected from both treated and non-treated « control » portions of the stems and stored at 5 °C in ethanol 50% before being processed.

Experimentation in green houses

Greenhouse experiments mainly concern conifer saplings submited to water or temperature manipulations.
The most impressive experiment in greenhouse was conducted during the 2011 growing season in Chicoutimi (Canada) on 4-year-old black spruce saplings {Balducci et al., 2013, 2015, 2016}.
In April 2011, more than 1000 saplings of uniform size were selected, fertilized, and transferred into three greenhouses. During the experiment, the saplings were grown in the same type of pots, under natural daylight and local photoperiod. Three different thermal treatments were applied in the three greenhouses: the control plus two warming treatments (warming during the day or during the night). Moreover, two water treatments were applied for each thermal condition: irrigation (soil water content was maintained above 80 % of field capacity), and no irrigation (irrigation was withheld for 25 days in June). So in total there were six combinations of treatments (three thermal conditions × two irrigation regimes). This experiment was conducted during three years adujusting conditions for better results.

Wood sampling

In saplings experiments, few individuals (3-7) selected randomly have to be sacrificed regularly because repeted coring is not possible.
In the Chicoutimi experiment {Balducci et al., 2013, 2015, 2016}, a destructive sampling of six saplings, randomly selected each week from every treatment (i.e. 36 saplings per week), was performed from May to October.
Stem disks were collected 2 cm above the root collar of each sapling.
Blocks containing phloem, cambium and forming xylem were cut and placed in a solution of ethanol 50 % and stored at 5 °C before being processed.

3.3 | Wood sample preparation and anatomical section observation

3.3.1 | Wood sample preparation

To allow microscopic observation of cambial activity and wood formation, cross-microsections are prepared from collected blocks or microcores.
To start, samples are checked under a stereomicroscope at ×10–20 magnifications and the transverse side is marked with a pencil to keep a correct orientation all along the process.
Samples are then successively cleaned, dehydrated and infiltrated by immersion in baths of ethanol, d-limonene and paraffin, using an automatic tissue processor (in our case: STP121, MM France, Francheville, France) according to Rossi et al. {2006a} protocol.
Samples are then embedded in paraffin blocks with an embedding station (in our case: EC 350, MM France), and 10-mm-thick (5–15) transverse sections are cut with a rotary microtome (in our case: Leica RM2255, Leica France).
Sections are then stained with cresyl violet acetate {Rossi et al., 2006a} or with safranin and astra blue (FASGA) {Gricar et al. 2006} and permanently mounted on glass slides by using a balm (e.g., Histolaque LMR, Euparal).

3.3.2 | Anatomical section observations

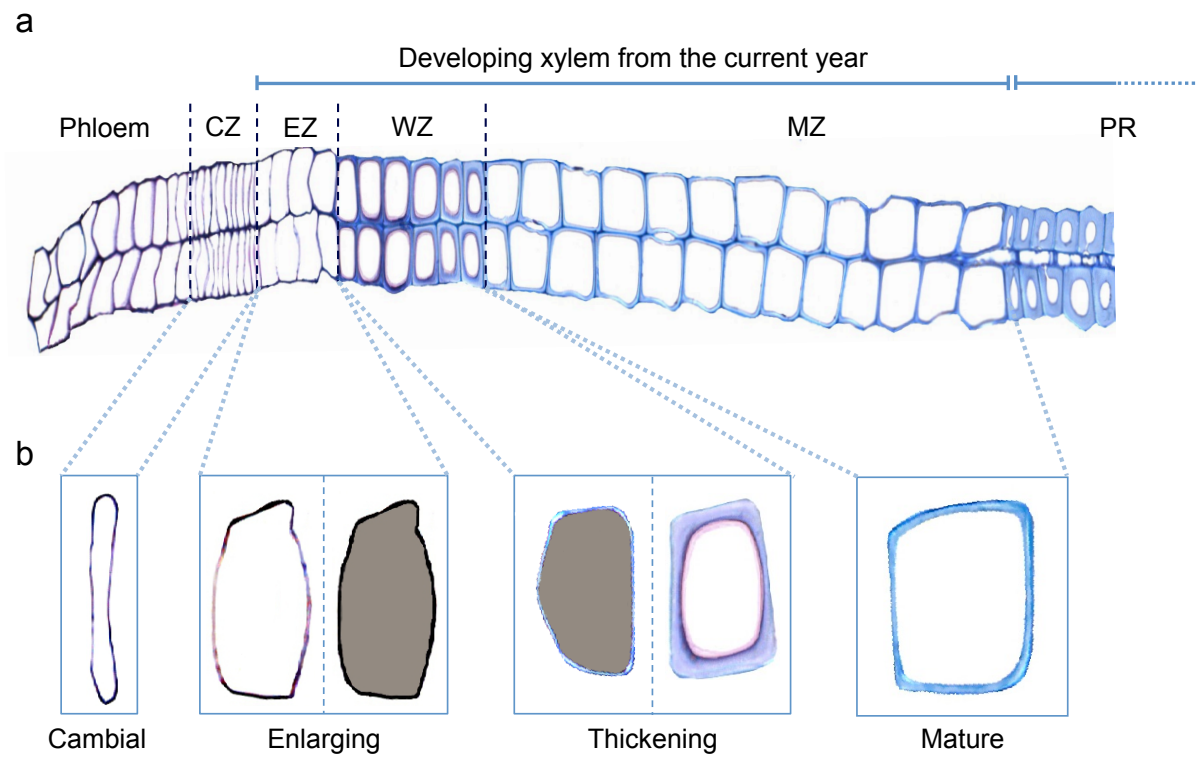
Xylem cell differentiation phases (reminder)

In wood-formation-monitoring studies, the formation of a xylem cell is generally divided in four major differentiation phases: (1) the dividing phase, during which the division of a cambial mother cell creates a new daughter cell; (2) the cell-enlarging phase, during which the newly formed xylem cell expands in the radial direction; (3) the wall-thickening phase, during which secondary walls are built and cell walls are lignified; and finally, (4) the mature stage, in which programmed cell death marks the end of cell differentiation and the advent of mature, fully functional tracheary element {Rathgeber et al., 2016; Wilson, 1970}.

Cellular criteria of discrimination

Anatomical cross-sections are generally observed using an optical microscope (in our case: AxioImager.M2; Carl Zeiss SAS, Marly-le- Roi, Marly-le-Roi, France), under visible and polarized light at ×100–400 magnifications to distinguish cells in different differentiation phases.

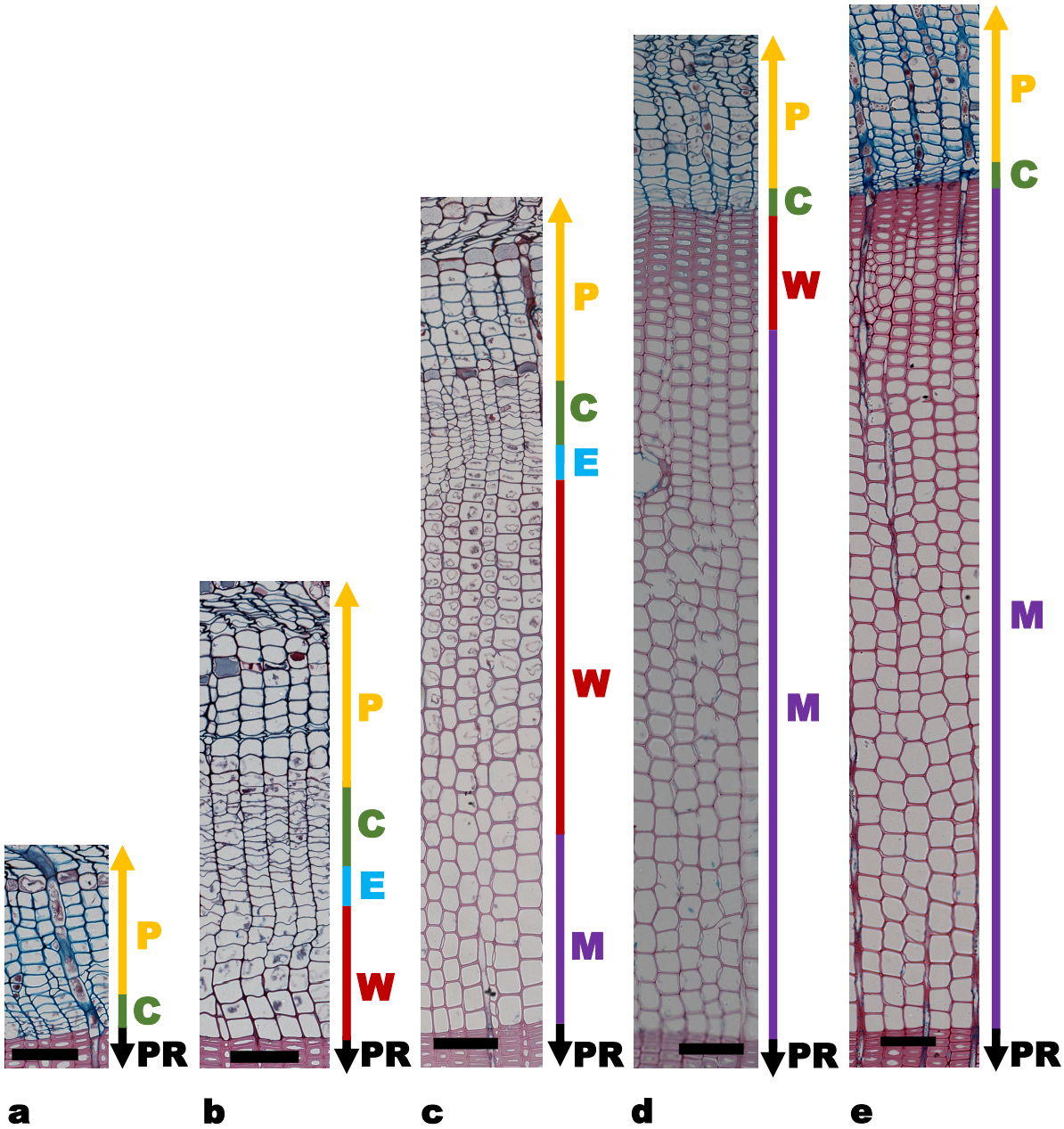
Cells in the cambial, enlarging, thickening, and mature zones are generally sorted out according to criteria reviewed by Rossi et al. {2006b}. In cross section, cambial cells are characterised by thin cell walls and small radial diameters. Cells in the radial enlargement phase are at least twice larger than cambial cells and exhibited thin walls [Fig. 3.1]. Cells in the wall thickening and lignification phase are birefringent under polarised light —because of the orientation of cellulose microfibrils {Abe et al., 1997}, while presenting walls stained in two colours — cresyl violet acetate stains cellulose in violet and lignin in blue, while FASGA stains cellulose in blue and lignin in red. Xylem cells are considered mature when walls are completely stained in one uniform colour (blue with cresyl violet acetate, red with FASGA).



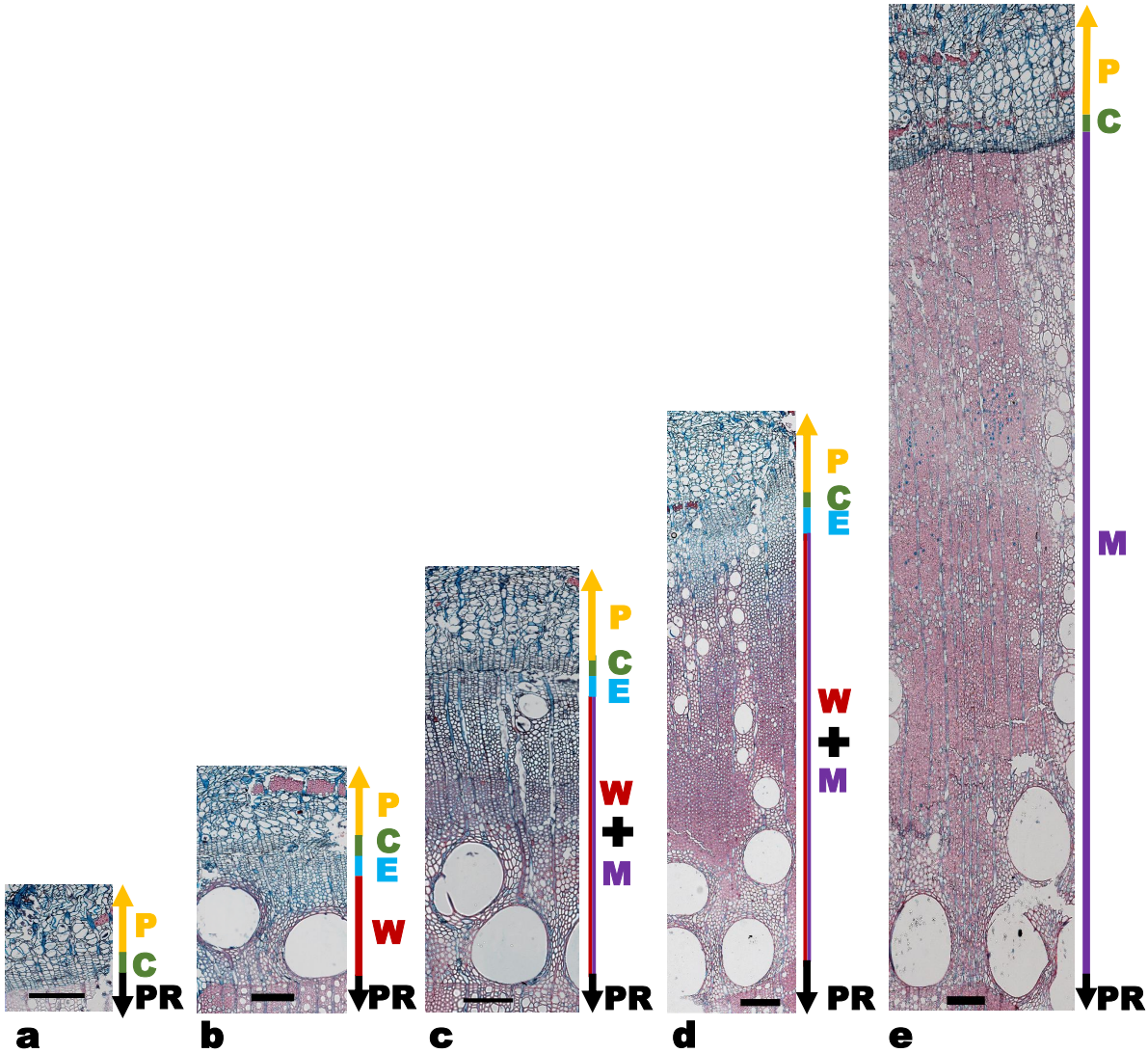
7 — Figure 3.1 | Scots pine developing radial files.
(a) Two developing radial files with marked differentiating zones: cambial zone (CZ), cell-enlarging zone (E), wall-thickening zone (WZ), mature zone (MZ), and previous ring (PR). (b) Isolated cells showing anatomical criteria that allow to discriminate between cells in successive differentiation phases: (1) small thin-walled cambial cells; (2) large thin-walled enlarging cells; (3) thick-walled, birefringent thickening cells; and (4) empty-lumen mature cells. Figure from Cuny et al. {2012}.

Data collection and database construction

The number of cells in the cambial (nC), enlarging (nE), thickening (nW), and mature zone (nM), along with the number of cells of the previous ring (i.e. the tree-ring formed during the previous growing season, nPR), are counted along at least three radial files, according to criteria described here above. Alternatively, for angiosperms, the widths of the four differentiation zones (wC, wE, wW, wM) and of the previous ring (wPR) can be measured [Fig. 3.2]. Finally, these raw data are generally saved in Excel workbooks.

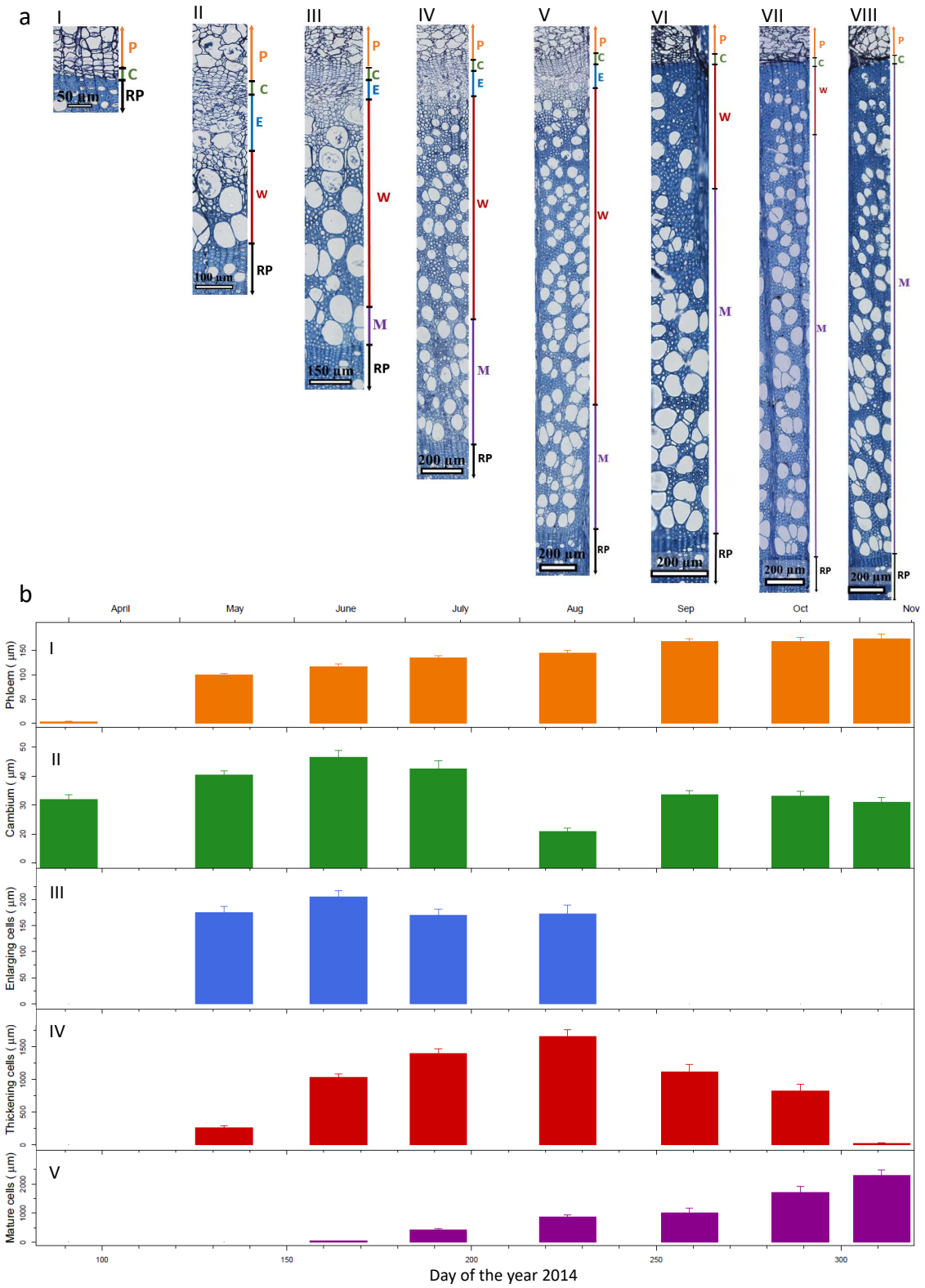


8 — Figure 3.2 | Seasonal dynamics of wood formation in Norway spruce in Hesse forest (Lorraine, France).
These transversal anatomical sections (stained using FASGA) illustrate: (a) the resting cambium (C) sandwiched between the phloem (P) on the outside, and the previous xylem ring on the inside (PR) — sampled on 24 March 2015; (b) the dividing cambium along with enlarging (E) and wall-thickening (W) xylem zones — sampled on 12 May 2015; (c) the dividing cambium along with xylem enlarging, wall-thickening and mature zone — sampled on 9 June 2015; (d) the resting cambium along with xylem cells in wall-thickening and mature zones — sampled on the 1 September 2015; (e) fully mature xylem — sampled on the 4 November 2015. The scale bars (black line) represent 100 µm. Figure prepared by A. Andrianantenaina.



9 — Figure 3.3 | Seasonal dynamics of wood formation in sessile oak in Hesse forest (Lorraine, France).

These transversal anatomical sections (stained using FASGA) illustrate: (a) the resting cambium (C) sandwiched between the phloem (P) on the outside, and the previous xylem ring on the inside (PR) — sampled on 30 March 2016; (b) the dividing cambium along with enlarging (E) and wall-thickening (W) xylem zones — 25 May 2016; (c) the dividing cambium along with xylem enlarging, wall-thickening and mature zone — sampled on 15 June 2016 and 20 July 2016; (d) the resting cambium along with xylem cells in wall-thickening and mature zones — sampled on the 1 September 2015; (e) fully mature xylem — sampled on the 26 October 2016. The scale bars (black line) represent 100 µm. Figure prepared by A. Andrianantenaina.



10 — Figure 3.4 | Seasonal dynamics of wood formation in european beech in Hesse forest (Lorraine, France).

(a) Beech anatomical slides in transversal section, stained with cresyl violet acetate and illustrating: (i) cambium reactivation, sampled on 1 April 2014; (ii) dividing cambium along with enlarging and maturing xylem, sampled on 13 May 2014; (iii-iv-v) dividing cambium, xylem cells in enlarging and wall-thickening phases, and mature xylem cells, respectively sampled on 13 June, 10 July and 14 August 2014; (vi-vii) no cambial division, no xylem cells in enlarging phase, xylem cells in wall-thickening phase and

mature xylem cells respectively sampled on 16 September and 16 October; (viii) Xylem with mature cells sampled on 7 November. With P = phloem, C = cambium, E = enlarging xylem cells, W = wall-thickening xylem cells, M = mature xylem cells, RP = xylem ring of the previous year. (b) Measured monthly xylem and phloem increments with. Figure modified from De Micco et al. {2019}. Figure prepared by A. Andrianantenaina.

3.4 | Meteorological data, site conditions and eco-physiological measurements

3.4.1 | Meteorological data

In order to characterise the annual cycle of climatic factors, daily meteorological data (e.g., temperature, precipitation, cumulative global radiation, wind speed, and relative humidity) are generally gathered from local institutional meteorological stations [Fig. 3.5].

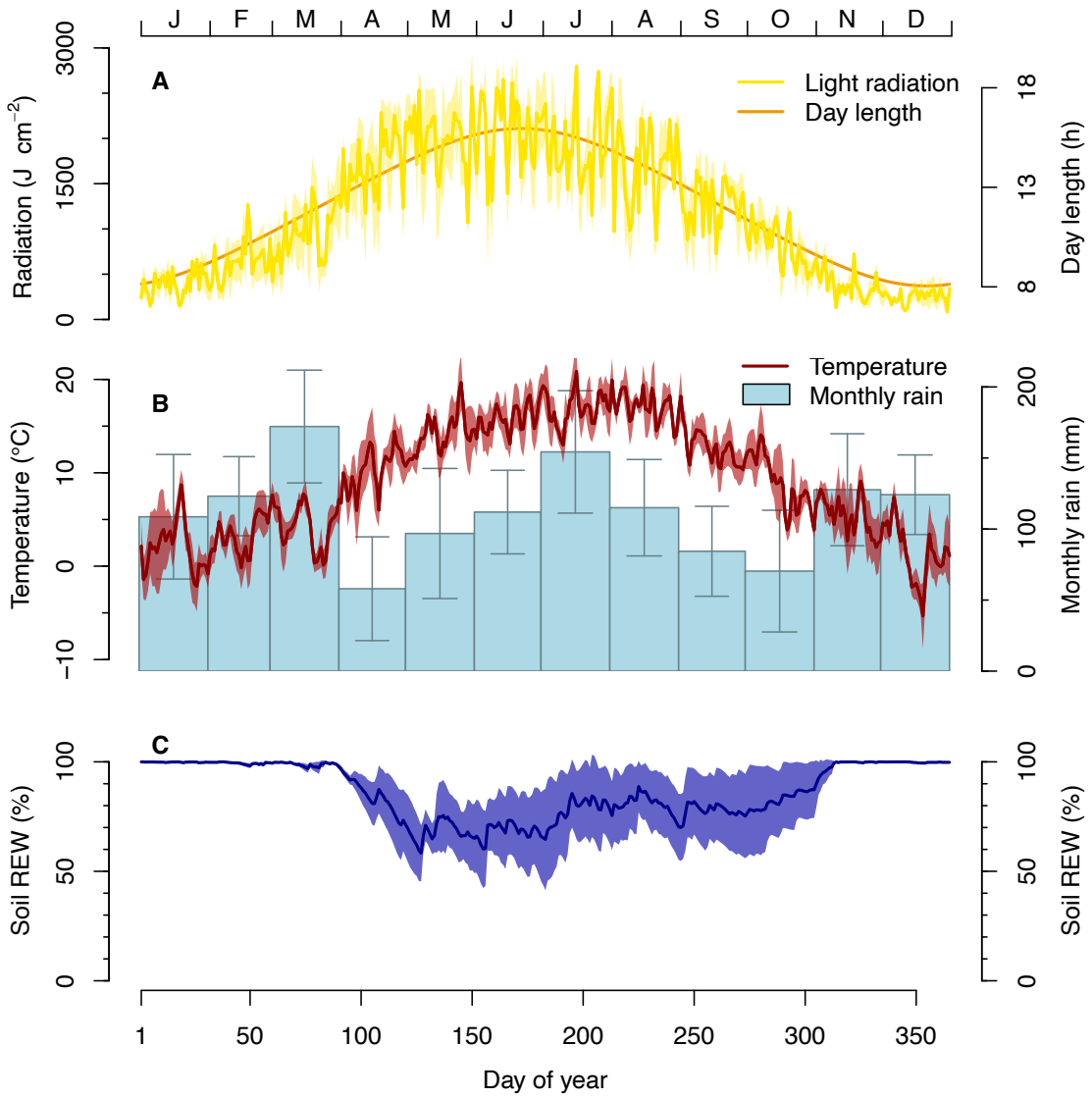
Alternatively, meteorological data can be measured in-situ during the monitoring period directly under the canopy or in small open area close by.

Local meteorological stations can provide long records, which are of interest to characterise both the general climatic conditions of the site and the meteorological conditions that occurred the year before the start of the monitoring; but they are usually not adapted to describe environmental gradients (e.g., elevation gradient) because of poor spatial coverage.

When deployed adequately, in-situ weather stations can provide a good description of the changing conditions over an environmental gradient; but they usually lack of temporal depth, and specially data just preceding the (wood-formation) monitoring period.

Ideally, in-situ and local meteorological data could then be blended to better describe both spatial and temporal climatic variations. Moreover, gridded climate data —e.g., the CRU TS3.10 Dataset {Harris et al., 2014}, WorldClim 2 {Fick et al., 2017} — can be added to further improve the blend.

However, each type of data (tree, stand, local, gridded) has its own specificity that must be considered in order to avoid introducing noise in the final dataset.



11 — Figure 3.5 | Seasonal variations of the monitored environmental factors. (A) Light radiation and daylength. (B) Temperature and monthly sums of rain. (C) Soil relative extractable water (REW). Lines represent the means for the 3 years (2007–2009) and the three sites, and coloured areas around lines represent 95% confident intervals around means. Figure from Cuny et al. {2016}.

3.4.2 | Site conditions and soil water balance modelling

Site conditions

Site conditions (e.g., slope, aspect, topography) are undoubtfully of high importance for cambial activity and wood formation, but they are generally poorly documented in wood-formation-monitoring studies. Indeed, studying the effect of these environmental factors would require a large number of plots organized according to an adequate design, which has been out of reach so far {but see Gricar et al., 2018}.

Soil moisture measurements

However, wood-formation-monitoring studies were interested very early in the effect of soil water balance on xylogenesis {Abe et al. 2003}.

Soil conditions (e.g., soil type, maximal soil water capacity, rooting depth) and variables (e. g., temperature, moisture) are, for some detailed studies, measured directly on the site. This allows to compute the seasonal cycle of relative extractable water, i.e. the actual quantity of water available for tree growth.

Water balance modelling

Alternatively, the forest water balance model Biljou© {Granier et al., 1999} was used to assess the daily water balance of our stands. In addition to daily meteorological data (e.g. temperature, precipitation, global radiation, air humidity), the model takes as input some soil (e.g., number and depth of layers, and proportion of fine roots per layer), and stand (forest type and maximum leaf area index) parameters, and gives as output the daily relative extractable water (REW).

REW is a relative expression of the filling state of the soil: REW is 100 % at field capacity, and 0 % at the permanent wilting point. Water stress is assumed to occur when the relative extractable soil water drops below a threshold of 40 %, under which transpiration is gradually reduces due to stomata closure {Granier et al., 1999}.

3.4.3 | Stand and tree characteristics

Stand characteristics

As for site conditions, stand characteristics (e. g., pure or mixed, even or un-even, managed or un-managed stands, tree density, stem basal area) are of high importance for cambial activity and wood formation, but they are also generally poorly investigated for the very same reason {but see Grotta et al., 2005; Linares et al., 2009}.

Tree characteristics

Tree characteristics (e. g., DBH, height, age) are of primary importance because most of them (as a cause or a result) are directly related to cambial activity and wood formation. For example, it has been shown that, under the same conditions, old trees have a shorter growing season and differentiation period than adult trees {Rossi et al., 2008}. This could explain why old trees are more sensitive to climate variability, and constitute therefore the preferred biological material of dendroclimatologists {Carrer & Urbinati, 2004}.

We have also shown that in an even-age stand, cambial activity started earlier, stopped later and lasted longer in dominant trees compare to intermediate and suppressed trees {Rathgeber et al., 2011}. Moreover cambial activity is more intense in dominant than in intermediate and suppressed trees. Furthermore, we found that growth duration was correlated to tree height, while growth rate was better correlated to crown area. Interestingly, this study {Rathgeber et al., 2011} shows that the variations of the growing season duration between trees of the same age (grown in the same site but belonging to different social status classes), may be higher than the variations observed between dominant trees grown in temperate vs. boreal forests.

Finally, it can be noted that all vigour-related tree characteristics are also related to cambial activity and must be considered in wood-formation-monitoring studies.

3.4.4 | Tree phenology and growth data

Cambium does not work in isolation but in perfect coordination with the other organs of the tree {Wilson, 1970}. Therefore, it is very interesting to associate wood-formation monitoring with observations on the phenology or growth of other parts of the tree. However, only few published wood-formation-monitoring studies present companion data on stem, shoot, or root growth or on leaf phenology {but see Cuny et al. 2012}.

Stem circumference variations — Manual band dendrometers

Manual band dendrometers are convenient and cheap tools, which allow monitoring directly stem circonference variations of the selected trees, preventing mistakes on the field, allowing a better management of the sampling campaigns, and finally providing important companion data.
Manual band dendrometers (in our case: DB-20, EMS Brno, Czech Republic) are generally installed at breast height on the stem of all the selected trees, after the removal of most part of the dead bark. After a period of installation, they can be read weekly (with a precision of 0.1 mm), recording accurately stem circumference variations.

Apical growth

Height growth of mature trees can be measured using a tacheometer (in our case: Leica, TPS400).
Like a theodolite, the tachometer measures horizontal and vertical angles to determine the direction of a target point, moreover it also measure its distance using a laser. These measurements allow to attribute relative coordinates to a target point. From these coordinates it is straightforward to compute the distance between two target points {see Cuny, 2013 for more details}.
In order to measure the apical growth of a tree, we draw a target at the base of its stem, which is used as a reference for the measurements. After recording the coordinates of the reference point, we measure the coordinates of the apex at the top of the tree. The distance separating the apex from the reference point can then be calculated. The increase, week after week, in the distance between the reference point and the tree apex represent the height growth of the tree.

Shoot growth

Using binoculars, the length of new shoots in the upper tier of the crown can be assessed visually by comparison with previous year shoots {Cuny et al 2012}. Repeating this assessment every week allow to monitor shoot growth quite accurately.
To assess shoot elongation dynamics, length of new shoots can be fitted with a logistic curve {Karkach, 2006} using the SSlogis function of the R statistical software {R Development Core Team, 2011}. From this model, the maximal (rx, S) and mean (rm, S) shoot elongation rates can be estimated.
For each tree, onset and cessation of shoot elongation can be defined, respectively, as the date at which 50 % of shoots began to lengthen and 50 % of shoots reached their final length; duration of shoot elongation is computed as the time between onset and cessation.

Fine root growth

Despite the fact that several authors expect a strong relationship between underground activity and stem growth, we are not aware of any scientific paper investigating the relationship between fine root development and wood formation dynamics {Delpierre et al., 2015}.

The hidden fine root compartment can be investigated by endoscopy, a technique that is currently developped by J. Levillain in our team (EcoSilva team, Silva UMR, INRA Grand Est – Nancy). To this end, transparent tubes (minirhizotrons) are installed in the soil near the selected trees, allowing a camera to access the roots of the tree. Sequential images provided by the camera allow to assess root vertical distribution and growth dynamics. At the beginning and at the end of the growing season, soil cores can be taken to measure root biomass distribution and turn-over.

Leaf phenology

Leaf phenology is also classically monitored in the upper tier of the crown using binoculars. In wood-formation monitoring studies leaf phenology is generally monitored at a weekly time, which, without being perfectly adapted, provide good results.

Leaf unfolding can be describe using three successive phenological stages occurring after winter bud dormancy: (i) budburst, (ii) maturing leaves and (iii) mature leaves. Dormant buds are totally enclosed by scales and recognizable by their small size. However, budburst differs between species, we distinguish it according to the criteria of the phenological observations protocol of the French national long-term monitoring network of forest ecosystems {Ulrich and Cecchini, 2009}. Maturation of leaves is indicated by their colour, which progressively changed from light to dark green. Current-year leaves are considered mature when they present the same colour than the old leaves from previous years.

For each tree, onset and cessation of leaves unfolding can be defined, respectively, as the date at which 50 % of buds are broken and 50 % of leaves are mature; duration of leaves unfolding is computed as the time between onset and cessation.

3.4.5 | Eco-physiological measurements

Introduction

Several type of ecophysiological measurements can be made in order to better understand the relationship between wood formation, ecophysiological constraints and environmental factors.

Cambium temperature measurements

Temperature sensors can be inserted under the bark of a tree in order to measure the temperature actually felt by the cambium {Cuny & Rathgeber, 2016}.

Stem radius variation measurements

Automatic point dendrometers are quite frequently installed on wood formation monitoring sites {e.g. Cuny, 2013; Saderi, 2018; Andrianantenaina, 2019; King et al., 2013} — either on the stem of the monitored trees or on comparable trees close

by. Automatic dendrometers records stem radius (for point dendrometer) or circumference (for band dendrometers) variations at an hourly time step.
Automatic dendrometers are a valuable source of tree physiological information, which is still not fully exploited yet {Zweifel et al., 2016}. Time series of stem radius variations offer information about radial stem growth and tree water relations in unmatched quality and resolution (Steppe et al. 2006, Zweifel et al. 2006). However, the task of turning raw stem radius changes into physiologically meaningful measures is more complicated than it appears at first sight.
Thus, there is still a great potential waiting to be discovered in terms of interpreting dendrometer readings. Studies associating both dendometers records and wood formation monitoring may be of great help.

Sap flow measurements

Sap flow measurements are today the most common method to determine transpiration at the tree level {Steppe et al. 2015}.
Sap flow sensors are occasionally installed on wood formation monitoring sites in order to investigate the relationship between tree growth and stem water conduction {Peters et al. in prep, Fernadez-de-Una et al. in prep}.

Non-structural carbohydrate measurements

Non-structural carbohydrates (NSC) are products of photosynthesis, provide substrates for growth and metabolism and can be stored by the plant. Consequently, NSC play a central role in plant response to the environment {Chapin et al., 1990; Kozlowski, 1992}.
Several major questions about the role and regulation of stored carbohydrates in woody plants remain unanswered, such as their role in indicating plant carbon balance, helping plants cope with stress, and whether control of storage and use is active, passive or more complex {Chapin et al., 1990; Sala et al., 2011, 2012; Wiley and Helliker, 2012}.
Few studies associate wood formation monitoring and NSC repeated measurements. However, these studies can help to answer previous questions and understand the relationship between cambial activity and carbon availability.

3.4.6 | The GloboXylo database

The GloboXylo database gathers data from 51 wood-formation-study sites spread at various altitudes and latitudes, on three continents (America, Europe, Asia), in extra tropical parts of the Northern Hemisphere (Boreal, Temperate and Mediterranean bioclimatic zones).
Wood formation was monitored at a weekly time-step using histological sections of forming wood collected from the stems of 15 different conifer species.
Climatic data (temperature, precipitation, radiation) were collected from companion weather stations installed in a forest gap within or close to the study sites. Alternatively, data from local weather stations or gridded dataset were used to complete records or replace missing data.
This database already feed several research studies {e.g., Cuny et al., 2015, Rossi et al., 2016; Delpierre et al., 2018} and more are to come {Rathgeber et al. in prep}.

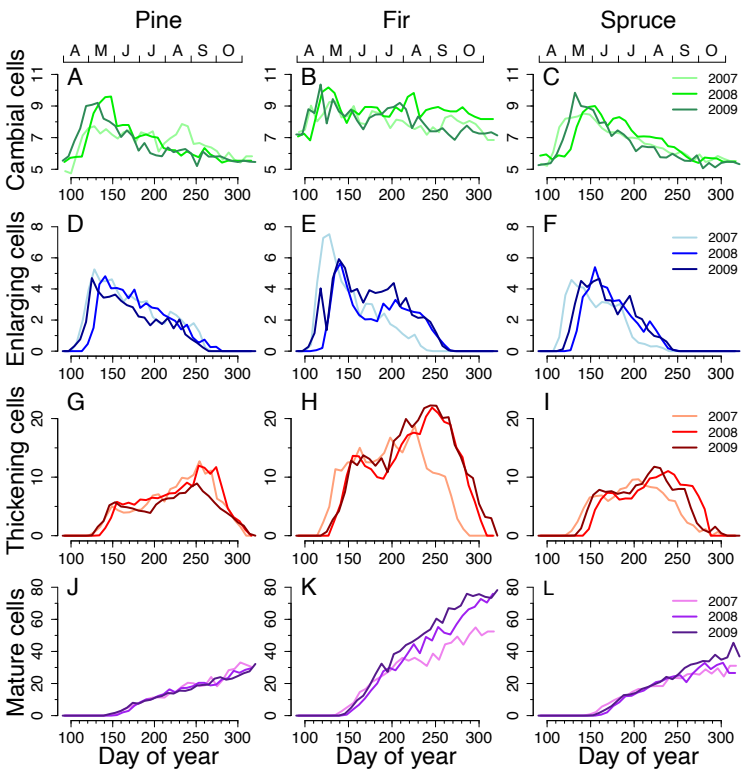
3.5 | Cell count data preparation and manipulation

3.5.1 | Introduction

Raw data in wood formation monitoring studies generally consist in the number of differentiating xylem cells in the enlargement (nE), wall-thickening (nW), and mature zone (nM), counted along (at least) three radial files, plus the number of cells in the previous ring (nPR), for each tree at each sampling date [Fig. 3.6]. Wood formation critical dates can be directly computed using these raw data. However, when describing tree-ring formation dynamics, standardisation may be used before fitting growth models, in order to reduce the noise in the dataset {Rossi et al. 2003}. Indeed, ring width and cell number vary all around the tree circumference, and consequently, among the different wood samples taken week after week at different places around the stem. Therefore, the difference in the number of cells between two samples is not only related to the sampling date (the signal of interest), but also to their positions —this circumferential variability is generally considered as "noise" in wood-formation-monitoring studies.

In this section, we show how to manipulate and standardise wood-formation-monitoring raw data in order to maximize the signal and reduce the noise before proceeding to further manipulations.

Data manipulation is done using CAVIAR, an R package for checking, displaying and processing wood-formation-monitoring data {Rathgeber et al. 2011a, 2018}.



12 — Figure 3.6 | Number of cells in the cambial, enlargement, thickening, and mature zones of wood formation for Scots pine, silver fir, and Norway spruce grown in Grandfontaine.

Lines represent the mean of five trees per species per year. The upper x-axis represents the months. Figure from Cuny et al. {2013}.

3.5.2 | Cell count data aggregation

First of all, raw cell count data are averaged by tree. Then, the "instantaneous" cell counts (nE, nW and NM) are used to compute two cumulative counts (nWM and nEWM) by adding the cell counted in the different zones to the increasing number of mature cells.

nWM,i = nW,i + nM,i [Equation 3.1]

nEWM,i = nE,i + nWM,i [Equation 3.2]

With i the sampling date.

In CAVIAR, a didicated function automatically aggregates (using the means or the medians) the quantitative variables (nC, nE, nW, nM and NPR) over three (or more) radial files, for all the trees and sampling dates available in the dataset. Moreover, it computes the two additional cumulative counts (nWM and nEWM), and output all the values in a new data frame [Table 3.1].

Field	Variable	Article and CAVIAR plots	CAVIAR logs	Unit
Counting	Number of cells in the cambial zone	n_C	CZ	Nb of cell
	Number of cells in the enlargement zone	n_E	EZ	Nb of cells
	Number of cells in the wall-thickening zone	n_W	WZ	Nb of cells
	Number of cells in the mature zone	n_M	MZ	Nb of cells
	Number of cells in the wall-thickening and mature zone	n_{WM}	WMZ	Nb of cells
	Number of cells in the enlargement, wall-thickening and mature zone	n_{EWM}	EWMZ	Nb of cells
	Number of cells in the previous ring	n_{PR}	PR	Nb of cells
	Total final number of cells in the current ring—Ring Cell Number	*RCN*	RCN	Nb of cells
	Number of cells in the dormant cambium, before the current growing season—cambium Initial Cell Number	*ICN*	ICN	Nb of cells
	Number of cells in the dormant cambium, after the current growing season—cambium Final Cell Number	*FCN*	FCN	Nb of cells
Phenology	Beginning of enlargement phenophase	b_E	bE	Day of year
	Beginning of wall-thickening phenophase	b_W	bW	Day of year
	Beginning of mature phenophase	b_M	bM	Day of year
	Cessation of enlargement phenophase	c_E	cE	Day of year
	Cessation of wall-thickening phenophase	c_W	cW	Day of year
	Duration of enlargement phenophase	d_E	dE	Days
	Duration of wall-thickening phenophase	d_W	dW	Days
	Total duration of xylogenesis (i.e., wood formation)	d_X	dX	Days
Dynamics	Asymptote of the Gompertz function		A	Nb of cells
	Beta parameter of the Gompertz function		b	Dimensionless
	Kappa parameter of the Gompertz function		k	Dimensionless
	Time at which the Gompertz function reach 5% of the total final number of cells (α)	t_b	tb	Day of year
	Time at which the Gompertz function reach 95% of the total final number of cells (α)	t_c	tc	Day of year
	Period of time elapsed between t_b and t_c, and during which the main part of growth (90%) is performed	d_g	dg	Nb of days
	Mean growth rate computed between t_b and t_c	g_m	gm	Cell/day
	Maximal growth rate	g_x	gx	Cell/day
	Date of occurrence of the maximal growth rate	t_x	tx	Day of year

Table 3.1 | List of the principal variables used in this chapter and the following ones, along with their abbreviations in the manuscript and in CAVIAR logs and plots. Table from Rathgeber et al. {2018}.

3.5.3 | Cell count data standardisation

Following Rossi et al. {2003}, cell count data can be standardised by the total number of cells (or alternatively by the total width) of the previous tree ring(s). According to the relative position of the sample on the stem, the number of cells in each developmental zone (E, W and M), and for each sample, can be standardised using the number of cells in the previous ring as follow:

n'j,i = nj,i x nPR / nPR,i

Where nj,i is the number of cell in the developmental zone i for the sample i; nPR,i is the number of cells in the previous ring for the sample i; nPR is the mean number of cells in the previous ring; and finally n'j,i is the standardised number of cell.
This standardisation process can be easily applied with CAVIAR, using a dedicated function, which automatically standardises averaged and cumulated cell counts for the whole dataset.

3.5.4 | Final ring cell number computation

When working on wood formation dynamics, it is very important to estimate how many xylem cells were produced by the cambium in the radial direction during the studied growing season.
The total annual production of the cambium can be quantified by measuring the tree-ring width —as it is done classically in dendrochronology, or by counting the number of xylem cells along a selection of several radial files (3–30) on an anatomical section —as it is generally done in wood anatomy. Both methods convey the same information as the two measurements are highly correlated (Vaganov et al. 2006).
However, because of the variability of cambial activity all around the stem, counting the number of cells for few radial files in the very last sample where xylem has fully matured, will not provide the best estimate of the final ring cell number all around the stem.
Therefore, we developped function in CAVIAR, which automatically estimates the final total ring cell number (RCN) by pulling together data from all the samples collected after the cessation of the cell enlargement phenophase —i.e. when all the cells of the current year have be produced. This process yields the best estimates for RCN. These robust estimates of RCN can then be used to fix the asymptote of the Gompertz model during the fitting process (see next section for more details).
Additionally, this function automatically estimates two other important features: (1) the initial cambial cell number (ICN), the number of cell composing the dormant cambium before cambial activity has started; and (2) the final cambial cell number (FCN), the number of cell composing the dormant cambium after cambial activity has ceased.
Depending on the selected option, RCN, ICN and FCN are computed using the median (by default) or the mean, along with the associated measure of variation (standard deviation and standard error for the mean; median absolute deviation, median absolute error, and normalised median absolute error for the median).

3.6 | Conclusion and perspectives

3.6.1 | Optimization of the sampling depth

Wood formation monitoring is very time consuming. Going every week on the field to sample growing trees require much organisation, dedication, and time — About one month (30 sampling days) of field work to monitors 15 trees for example in a temperate forest over a growing season. Processing all the collected samples in the lab require also organisation, dedication, and time — Also about one month of lab work to prepare anatomical sections for 15 trees monitored over a growing season of about 30 weeks. Finally, observing all the prepared anatomical slides under the

microscope require also organisation, dedication, and time — Also about one month of work under the microscope to count or measure xylem on about 500 anatomical sections.
So, how many trees must be monitored to accurately describe cambial phenology, tree-rings formation dynamics, and cell differentiation kinetics, with the minimal effort is a very important question in this field.
In a work to come we will address this question using a dataset containing data from 4 stands of 15 larch trees spread along an altitudinal gradient and monitored over one year in the South of the French Alps. 15-trees groups will be randomly re-sampled applying the bootstrap approach for quantifying the loss of information while the number of sampled trees is decreasing.

3.6.2 | To standardize or not to standardize?

Standardization process is also a time-consuming step, particularly because it requires the measurements of previous year tree-ring width or total cell number.
Also, if we apply standardization to be beneficial to the data, we observed that in certain circumstances that it could be detrimental.
In an article to come, we will provide tools (e.g., signal-to-noise ratio, expressed population signal, Gompertz model efficiency) to properly assess the effect of standardization on wood-formation-monitoring data in order to decide whether it should be applied or not.

3.6.3 | Synthesis and future development of the GloboXylo database

The GloboXylo initiative, which aims to collect, store and use global wood formation monitoring data to identify large-scale biogeographic and ecological trends in xylogenesis, can significantly contribute to the development of this new discipline. Data collected in the GloboXylo database are currently available upon request. We aim to improve the visibility of GloboXylo by building an online relational database.

3.6.4 | Synthesis and future development of the R package CAVIAR

In this chapter we presented CAVIAR, an R package specifically dedicated to the checking, displaying and processing of wood-formation-monitoring data [Fig. 3.7].
First, we showed how to import formatted wood-formation-monitoring data into R.
Then, we proposed a visual 5-step procedure designed to meticulously check raw data. This procedure should help authors to better master the raw data they produce, and consequently improve the quality of the available datasets.
We also demonstrated how to manipulate and standardize raw data in order to maximize the growth signal and reduce the noise in the dataset before producing elaborated data. CAVIAR provides robust and efficient functions to compute (based on logistic regressions), display and analyse (based on bootstrap tests) wood formation phenology critical dates and durations.
CAVIAR provides also robust and efficient algorithms to model (based on Gompertz functions), display and analyse (based on bootstrap tests) intra-annual

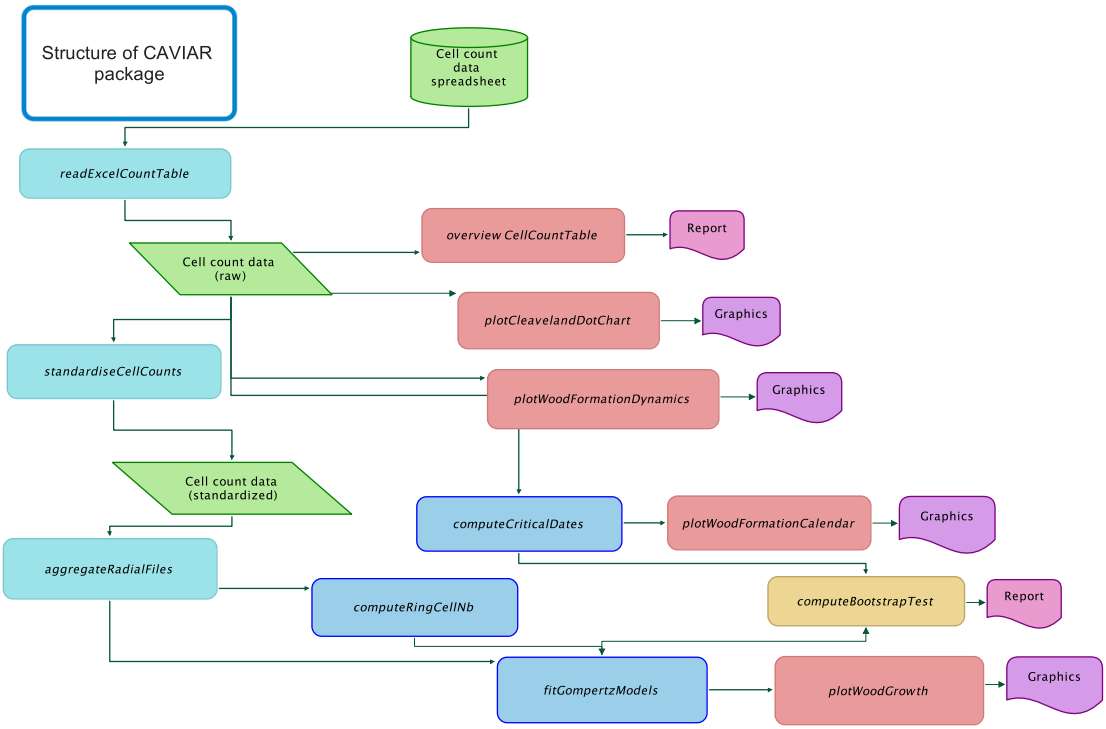
dynamics of xylogenesis. For temperate and cold forest trees, logistic regressions provide the best estimates of tree-ring formation critical dates, while Gompertz models very well capture wood formation dynamics during the main part of the growing season.

We believe that the current version of CAVIAR represents a 'classic standard' in wood-formation-monitoring data processing. Indeed, for the first time, robust and efficient algorithms allowing performance of the main classical data treatments (e.g., raw data verification, data standardization, wood formation critical dates and durations computation, and wood formation dynamics mod- elling) are gathered together in the same coherent package.

The CAVIAR package is available as an add-on package in R. Interested users can download and install R from the Comprehensive R Archive Network (CRAN) website: http://cran. r-project.org/. The package is also available for download and installation via the terminal (R CMD INSTALL CAVIAR) on the first author's webpage: https://www6.nancy.inra.fr/silva/Equipes-de-recherche/EcoSILVA/Personnels/Pages-personnelles/RATHGEBER-Cyrille.

In the third version of CAVIAR we plan to develop new algorithms based on generalized additive models to upgrade wood formation phenology and dynamics functions and develop new functions devoted to cell differentiation kinetics {Cuny et al. 2013, 2014}. This should improve the robustness and accuracy of CAVIAR in case of missing or scarce data (e.g., bi-weekly or monthly sampling), and extend its scope further to new environments (e.g., Mediterranean and tropical areas) and new species (e.g., angiosperms exhibiting ring- or diffuse-porous wood).

Moreover, a smooth connexion between CAVIAR and GloboXylo would be without any doubt a wonderfull tool to make new discoveries concerning wood formation dynamics.



13 — Figure 3.7 | Work flow chart of the CAVIAR R package.

Green cylinder and parallelograms indicate data. Light blue rectangles indicate functions for manipulating primary data; deep blue rectangles, functions for producing elaborated data; yellow ones, function for analysing elaborated data; and finally, red rectangles, functions for outputting results. Light purple forms indicate text report; deep purple forms, graphic outputs. Figure from Rathgeber et al. {2018}.

3.6.5 | Conclusion

Thanks to recent advances in wood sample collection, microcore preparation, and anatomical section observation {Rossi et al., 2006a, 2006b}; as well as in raw data manipulation, data visualisation and elaborated data analyses {Rathgeber et al., 2011a, 2018}; wood-formation-monitoring studies produce now abundant and high quality data, which can be used to better understand the process of tree-ring formation dynamics and its relationships with tree physiological state and development stages, as well as with environmental factors {e.g., Cuny at al., 2014, 2016, 2018}.

To conclude, we believe that the GloboXylo initiative and the CAVIAR package will both help to strengthen tree-ring formation science, contributing to standardize its methodologies, improve further the quality of its data and reinforce the significance of its results.

3.7 | References

This reference list is incomplete and not properly formatted

Bates DM, Chambers JM (1992) Nonlinear models. In: Chambers J, Hastie T (eds) Statistical models in S. Wadsworth and Brooks/Cole, Pacific Grove, CA, pp 421–453.

Bunn AG (2008) A dendrochronology program library in R (dplR). Dendrochronologia 26:115–124.

Bunn AG (2010) Statistical and visual crossdating in R using the dplR library. Dendrochronologia 28:251–258.

CamareroJJ, Guerrero-Campo J, Gutiérrez E (1998) Tree-ring growth and structure of Pinus uncinata and Pinus sylvestris in the Central Spanish Pyrenees. Arctic Alp Res 30:1–10.

Cufar K, Prislan P, Griçar J (2008) Cambial activity and wood formation in beech (Fagus sylvatica) during the 2006 growth season. Wood Res 53:1–12.

Cuny H, Rathgeber CBK, Lebourgeois F, Fortin M, Fournier M (2012) Life strategies in intra-annual dynamics of wood formation: example of three conifer species in a temperate forest in north-east France. Tree Physiol 32:612–625.

Cuny H, Rathgeber CBK, Kiessé TS, Hartmann FP, Barbeito I, Fournier M (2013) Generalized additive models reveal the intrinsic complexity of wood formation dynamics. J Exp Bot 64:1983–1994.

Cuny H, Rathgeber CBK, Frank D, Fonti P, Fournier M (2014) Kinetics of tracheid development explain conifer tree-ring structure. New Phytol 203:1231–1241.

Cuny H, Rathgeber CBK, Frank D et al. (2015) Woody biomass produc- tion lags stem-girth increase by over one month in coniferous forests. Nat Plants 1:15160.

Denne MP, Dodd RS (1981) The environmental control of xylem differ- entiation. In: Barnett JR (ed) Xylem cell development. Castle House Publications, Tunbridge Wells, pp 236–255.

Deslauriers A, Morin H, Bégin Y (2003) Cellular phenology of annual ring formation of Abies balsamea in the Quebec boreal forest (Canada). Can J For Res 33:190–200.

Evert RF (2006) Esau's plant anatomy. JohnWiley & Sons, Hoboken, NJ. Gompertz B (1825) On the nature of the function expressive of the law of human mortality, and on a new mode of determining the value of life contingencies. Philos Trans Royal Soc London 115: 513–583.

Fick SE, Hijmans RJ. 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. International Journal of Climatology 37: 4302–4315.

Griçar J, Rathgeber CBK, Fonti P (2011) Monitoring seasonal dynamics of wood formation. Dendrochronologia 29:123–125.

Grissino-Mayer HD (1993) An updated list of species used in tree-ring research. Tree-Ring Bull 53:17–43.
Grissino-Mayer HD (2001) Evaluating crossdating accuracy: a manual and tutorial for the Computer Program COFECHA. Tree-Ring Res 57: 205–221. Hyndman RJ, Koehler AB (2006) Another look at measures of forecast accuracy. Int J Forecast 22:679–688.
Harris I, Jones PD, Osborn TJ, Lister DH. 2013. Updated high-resolution grids of monthly climatic observations - the CRU TS3.10 Dataset. International Journal of Climatology 34: 623–642.
Mayer DG, Butler DG (1993) Statistical validation. Ecol Modell 68: 21–32. Piñeiro G, Perelman S, Guerschman JP, Paruelo JM (2008) How to evaluate models: observed vs. predicted or predicted vs. observed? Ecol Modell 216:316–322.
Prislan P ,Cufar K , Koch G , Schmitt U, Griçar J (2013) Reviewofcellular and subcellular changes in the cambium. IAWA J 34:391–407.
R Development Core Team (2015) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.
Rathgeber CBK, Longuetaud F, Mothe F, Cuny H, Le Moguédec G (2011a) Phenology of wood formation: data processing, analysis and visualisation using R (package CAVIAR). Dendrochronologia 29: 139–149.
Rathgeber CBK, Rossi S, Bontemps JD (2011b) Cambial activity related to tree size in a mature silver-fir plantation. Ann Bot (Lond) 108: 429–438.
Rathgeber CBK, Cuny H, Fonti P (2016) Biological basis of tree-ring formation: a crash course. Front Plant Sci 7:46.
Rossi S, Deslauriers A, Morin H (2003) Application of the Gompertz equation for the study of xylem cell development. Dendrochronologia 21:33–39.
Rossi S, Anfodillo T, Menardi R (2006a) Trephor: a new tool for sampling microcores from tree stems. IAWA J 27:89–97.
Rossi S, Deslauriers A, Anfodillo T (2006b) Assessment of cambial activ- ity and xylogenesis by microsampling tree species: an example at the Alpine timberline. IAWA J 27:383–394.
Rossi S, Deslauriers A, Anfodillo T, Morin H, Saracino A, Motta R, Borghetti M (2006c) Conifers in cold environments synchronize maximum growth rate of tree-ring formation with day length. New Phytol 170:301–310.
Rossi S, Anfodillo T, Cufar K et al. (2016) Pattern of xylem phenology in conifers of cold ecosystems at the Northern Hemisphere. Glob Chang Biol 22:3804–3813.
Vaganov EA, Hughes MK, Shashkin AV (2006) Growth dynamics of conifer tree rings: images of past and future environments. In: Ecological studies. Springer, Berlin, Heidelberg, New York, 183.
Wilson BF, Wodzicki TJ, Zahner R (1966) Differentiation of cambial deri- vatives: proposed terminology. Forest Sci 12:438–440.
Wilson JW (1970) The growing tree. The University of Massachusetts Press, Amherst, MA.
Zang C, Biondi F (2014) treeclim: an R package for the numerical cali- bration of proxy-climate relationships. Ecography 38:431–436.
Zeide B (1980) Ranking of forest growth factors. Environ Exp Bot 20: 421–427.
Zeide B (1993) Analysis of growth equations. Forest Sci 39:594–616. Zeide B (2003) The U-approach to forest modeling. Can J For Res 33: 480–489.
Zuur AF, Ieno EN, Elphick CS (2010) A protocol for data exploration to avoid common statistical problems. Methods Ecol Evol 1:3–14.

CHAPTER 4 | PHENOLOGY OF CAMBIAL ACTIVITY AND TREE-RING FORMATION

4.0 | Synthesis

4.0.1 | Related papers

Rathgeber CBK, Longuetaud F, Mothe F, Cuny H, Le Moguédec G. 2011a. Phenology of wood formation: Data processing, analysis and visualisation using R (package CAVIAR). *Dendrochronologia* 29: 139–149.

Rathgeber CBK, Rossi S, Bontemps J-D. 2011b. Cambial activity related to tree size in a mature silver-fir plantation. *Annals of Botany* 108: 429–438.

Cuny, H., Rathgeber, C.B.K., Lebourgeois, F., Fortin, M., Fournier, M., 2012. Life strategies in intra-annual dynamics of wood formation: example of three conifer species in a temperate forest in north-east France. *Tree Physiology* 32, 612–625. doi:10.1093/treephys/tps039

Michelot, A., Simard, S., Rathgeber, C.B.K., Dufrêne, E., Damesin, C., 2012. Comparing the intra-annual wood formation of three European species (Fagus sylvatica, Quercus petraea and Pinus sylvestris) as related to leaf phenology and non-structural carbohydrate dynamics. *Tree Physiology* 32, 1033–1045. doi:10.1093/treephys/tps052

Delpierre N, Vitasse Y, Chuine I, Guillemot J, Bazot S, Rutishauser T, Rathgeber CBK. 2015. Temperate and boreal forest tree phenology: from organ-scale processes to terrestrial ecosystem models. *Annals of Forest Science* 73: 5–25.

Rossi, S., Anfodillo, T., Čufar, K., Cuny, H., Deslauriers, A., Fonti, P., Frank, D., Griçar, J., Gruber, A., Huang, J.-G., Jyske, T., Kašpar, J., King, G., Krause, C., Liang, E., Mäkinen, H., Morin, H., Nöjd, P., Oberhuber, W., Prislan, P., Rathgeber, CBK., Saracino, A., Swidrak, I., Treml, V., 2016. Pattern of xylem phenology in conifers of cold ecosystems at the Northern Hemisphere. *Global Change Biology* 22, 3804–3813. doi:10.1111/gcb.13317

Rathgeber CBK, Santenoise P, Cuny H. 2018. CAVIAR: an R package for checking, displaying and processing wood-formation-monitoring data (R Tognetti, Ed.). *Tree physiology* 38: 1246–1260.

Delpierre, N., Lireux, S., Hartig, F., Camarero, J.J., Cheaib, A., Čufar, K., Cuny, H., Deslauriers, A., Fonti, P., Griçar, J., Huang, J.-G., Krause, C., Liu, G., de Luis, M., Mäkinen, H., Martinez del Castillo, E., Morin, H., Nöjd, P., Oberhuber, W., Prislan, P., Rossi, S., Saderi, S., Treml, V., Vavrick, H., Rathgeber, C.B.K., 2018. Chilling and forcing temperatures interact to predict the onset of wood formation in Northern Hemisphere conifers. *Global Change Biology* gcb.14539–17. doi:10.1111/gcb.14539

Saderi S, Rathgeber CBK, Rozenberg P, Fournier M. Wood formation phenology of larch trees growing along a 1,000 m elevation gradient in the French Southern Alps. accepted in *Annals of Forest Science*.

Pérez-de-Lis G, Rathgeber CBK, Andrianantenaina AN, Fernández de Uña L. Tree-ring structure and leaf traits determine the coordination between tree organ phenology. In preparation.

4.0.2 | Summary

In the first section of this chapter, we presented our approach to describe and quantify accurately tree-ring formation phenology using the CAVIAR R package (which is currently based on logistic regressions).
In the second section, we explored the variability of tree-ring formation phenology according to tree age, size and social status, tree species, and site conditions. We showed that tree characteristics have as much influence as site and climatic conditions in determining the wood formation calendar.
In the third section, we synthesized the most recent knowledge on the influence of environmental cues on the phenology of temperate and boreal forest trees showing that temperatures are the main driver of the onsets of cambial activity and wood formation, while photoperiod determine their cessations, which could be occasionally hasten by water shortage or cold spell.
In the fourth section, we addressed the phenology of tree leaves and reproductive structures, fine roots, and wood, and we also consider carbon and nitrogen reserves as well as the relationships between organs, which are all essential to understand the functioning of trees.
In the fifth section, we showed through a functional approach based on a meta-analysis of wood and leaf phenology data, that tree-ring structure and leaf traits determine the coordination of tree organ 'phenologies' according to plant functional type life strategies.
Finally, in the sixth section, we presented a new process-based model approach, and we apply it to data from four conifer species covering a wide range of temperature and photoperiod conditions in the Northern Hemisphere to show that forcing and chilling temperatures (influenced by photoperiod) determine the onsets of xylem formation.

4.0.3 | Key words:

Wood phenology, Vegetation period, Elevation gradients, Phenological models, Photoperiod, Forcing and chilling temperatures

4.1 | Introduction

4.1.1 | Definition and History of phenology

Humans have long been interested in the seasonality and recurrences of natural events. Aside from the fundamental need to identify the most favourable periods for sowing and harvesting, which likely dates back to the beginning of agriculture (over 10,000 years ago), observations of some sticking seasonal events of nature's calendar have evolved as social rituals and have become elements of the culture of several peoples around the world (e.g., the famous cherry blossom festival celebrated in Japan for over 1200 years or autumn "leaf-peeping" in Japan and North America).
Phenology is the study of periodic plant and animal life cycle events and how these are influenced by seasonal and inter-annual variations in their environment. The word 'phenology' is derived from the ancient Greek 'φαίνω' (phainō), which means "to show, to bring to light, make to appear »; and indicates that phenology has been

principally concerned with the dates of first occurrence of biological events in their annual cycle.
The timings of a number of important natural events, such as grapevine harvesting in France and cherry blossoming in Japan, that have been recorded for centuries (e;g., in diaries, agricultural calendars, and poems) have been collated into historical chronicles and used to infer past climatic conditions {Chuine et al. 2004; Aono and Kazui 2008}. In the eighteenth century, the collection of phenological data developed as a pastime for certain educated people and then as a matter of scientific interest {Sparks and Carey 1995}. Réaumur {1735} reported what is likely the first quantified evaluation of the influence of temperature on the occurrence of phenophases and introduced the notion of "heat sum" which, 280 years later, remains a basic component of current phenological models. Another seminal work is from Linné {1751, cited in Dahl and Langvall 2008}, who initiated a coherent network of observations of both plant phenophases and local weather in Sweden. The proper birth of phenology as a scientific discipline occurred in the nineteenth century, with both the coining of the term "phenology" by the Belgian scientist Charles Morren {in 1853; Demarée and Rutishauser 2011} and the establishment of several phenological networks throughout Europe {see Koch 2008}, which unfortunately, in the majority of cases, were not perpetuated {but see Schaber and Badeck 2005}. In the 1950s, large-scale phenological surveys were revived under both the initiative of national weather services in several European countries and the constitution of the international phenological gardens network {Menzel 2013}. In combination with more recently established professional and citizen science networks, these surveys remain the main sources of ground-based phenological data.

4.1.2 | Importance of phenology for trees

A long evolutionary history has resulted in the precise timing of tree annual developmental cycles, minimizing the damage associated with abiotic stresses while maximizing tree reproductive output. In temperate and boreal trees, the main adaptation is realized as a so-called dormancy period in autumn and winter, which prevents organ development during adverse climatic conditions. The timing of phenophases in trees is known to affect their biological functions and ecological interactions at several organizational scales. There is ample evidence that year-to-year variations in leaf phenology modulate ecosystem carbon, water, and energy balances {Goulden et al. 1996; Barr et al. 2004; Delpierre et al. 2009b; Wilson and Baldocchi 2000}. At the community scale, the timing of leafing is known to condition the fitness of insect herbivores that feed on trees {Tikkanen and Julkunen-Tiitto 2003}, with potential consequences at upper levels of the trophic web {Visser and Holleman 2001; Visser et al. 2006; Donnelly et al. 2011}. Comparatively, considerably less effort has been made on assessing the impact of phenology on the functioning of individual plants. It is commonly observed that understory adult trees and seedlings "avoid" shading from the overstory as a result of phenological shifts (i.e., hastening of spring phenophases), which is thought to improve the understory carbon balance {Jolly et al. 2004, Augspurger and Bartlett 2003; Vitasse 2013}.
The phenology of leaves has been well studied, and the timing of leaf phenology has proven to be crucial in controlling the acquisition of carbon, the loss of water,

and the internal nutrient cycling in trees. In contrast, the phenology of other organs, e.g, wood, fine roots, and reproductive structures, has received less attention, even though it also influences overall plant functioning and fitness. An example is the influence of the differential timing of wood and leaf growth in sessile oak (a ring-porous species) and European beech (a diffuse-porous species) on the amplitude of the draw-down of carbohydrate reserves observed in spring {Barbaroux and Bréda 2002}. Similarly, fine-root phenology is expected to directly influence mineral acquisition {Nord and Lynch 2009}, with potential impacts on individual fitness. Finally, phenology has been shown to be a key determinant of tree fitness and tree species distribution {Chuine 2010}.

4.1.3 | The phenology of tree leaves and other tree organs in vegetation models

It is striking that the vast majority of published studies on the phenology of forest trees refer in fact to the seasonality of leaves. This discrepancy is partly attributed to the fact that the phenology of leaves is more visible, and therefore easier to monitor than the phenology of other organs (e.g., wood, fine roots, reproductive structures). In addition to the growing number of observational reports on leaf phenology, a number of studies published during the previous 20 years have been dedicated to the modeling of leaf phenology {Hänninen and Kramer 2007; Chuine et al. 2013}, resulting in the development of models that have been incorporated, with moderate success {see Richardson et al. 2012}, into dynamic global vegetation models (DGVMs). In contrast, as a consequence of the scarcity of papers documenting the phenology of non-leafy organs in forest trees, the underlying organ-specific phenological processes are often poorly represented in models. To our knowledge, for example, only one paper addresses the question of process-based modeling of wood phenology {Delpierre et al. 2019}.

In this context, the phenology of non-leafy organs (i.e., wood, fine roots, reproductive structures) has often been ignored in DGVMs and, when represented, only defined relatively to leaves, despite an increasing amount of evidences suggesting that there is not simple, direct link between the phenologies of the different organs of the different tree species. Moreover, the phenologies of non-leafy organs clearly deserve proper characterization, as there is increasing evidences that the growth of tree organs does not solely depend on the fueling of carbon from photosynthesis but is rather an active developmental process determined by seasonal variations in the ability of the organ to grow {Fatichi et al. 2014; Dietze et al. 2014}.

4.1.4 | Aim of the chapter

A few articles have recently reviewed the environmental determinism of leaf phenology in temperate and boreal trees {Badeck et al. 2004; Hänninen and Tanino 2011; Chuine et al. 2013; Hanes et al. 2013; Richardson et al. 2013}, the phenology of flowering in temperate plants {Tooke and Battey 2010}, the phenology of non-structural carbon allocation in trees {Dietze et al. 2014}, and the coordination of the 'phenologies of the different tree organs. A large comprehensive review focusing on wood phenology is still missing.

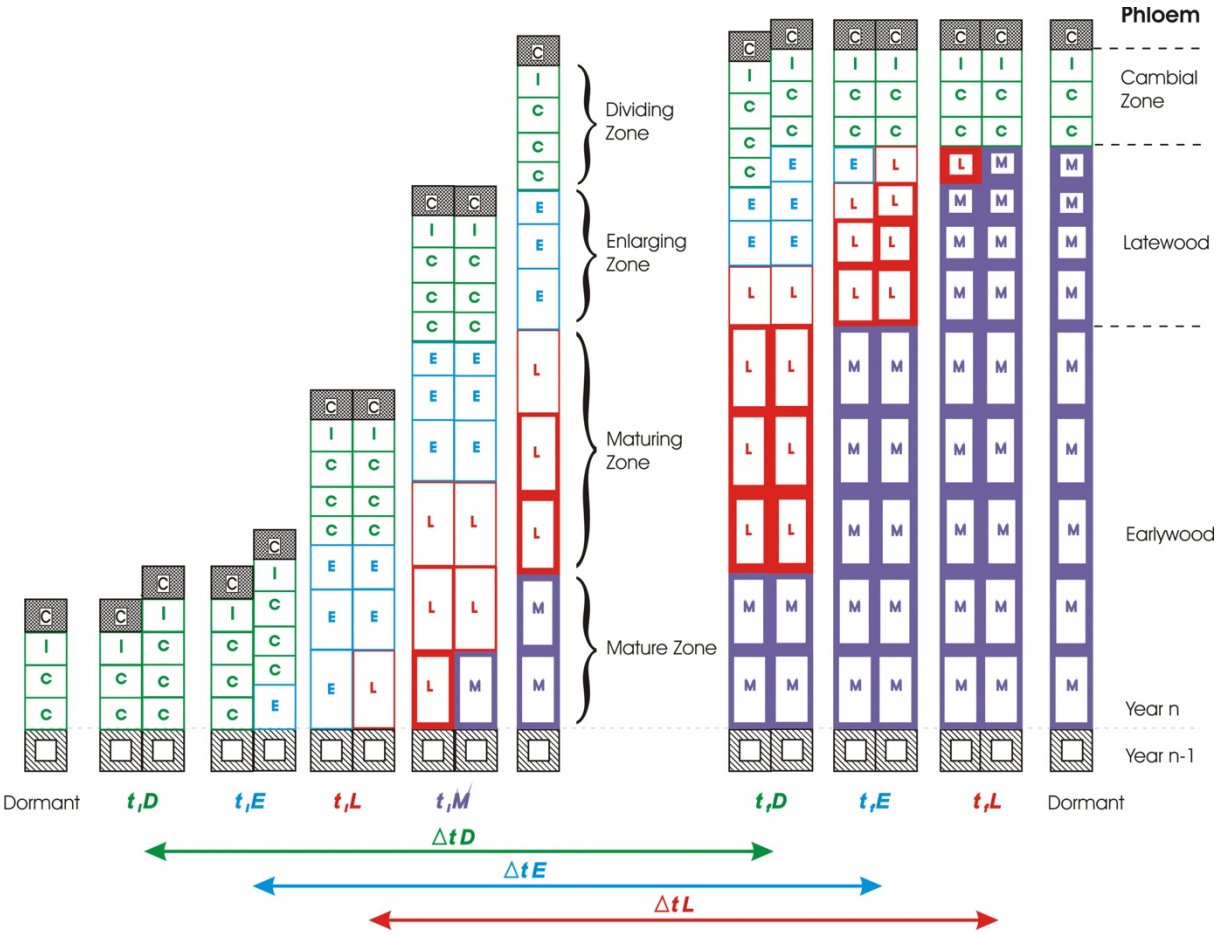
In this chapter, we aim to enlarge the scope of these studies, and following {Delpierre et al. 2015} propose to review our general understanding of the whole tree phenology in temperate and boreal forest, putting a special emphasis on the recent works published on wood phenology.
In the first section, we present our approach to describe and quantify accurately tree-ring formation phenology. In the second section we explore the variability of tree-ring formation phenology according to tree size and social status, tree species, and site conditions. In the third section we synthesize the most recent knowledge on the influence of environmental cues on the phenology of temperate and boreal forest trees. We address the phenology of tree leaves and reproductive structures, fine roots, and wood, and we also consider carbon and nitrogen reserves as well as the relationships between organs, which are all essential to understand the functioning of trees. In the fourth section we develop a plant functional approach based on a meta-analysis of wood and leaf phenology data, to show that tree-ring structure and leaf traits determine the coordination of tree organ 'phenologies' according to species life strategies. Finally, in the fifth section, we present a new process-based model of the onset of xylem formation and we apply it to data from four conifer species (black spruce, European larch, Scots pine, and Norway spruce) covering a wide range of temperature and photoperiod conditions in the Northern Hemisphere.

4.2 | Describing and quantifying the phenology of tree-ring formation

4.2.1 | Definition of wood formation critical dates

In a recent paper {Rathgeber et al. 2011a}, we proposed objective definitions of the five main critical dates of tree-ring formation, based on sound cellular observations. The five main critical dates we propose to focus on are: (1) the onset of the enlargement period (bE); (2) the onset of the wall thickening period (bW); (3) the appearance of the first mature tracheids (bM); (4) the cessation of the enlargement period (cE); and finally, (5) the cessation of the wall thickening and lignification period (cW).
These critical dates are defined based on the presence-absence of xylem cells in a given differentiation phase [Fig. 4.1]. At the beginning of the growing season, the cell-enlargement phenophase is declared started at the date at which more than 50 % of the counted radial files present enlarging cells {Rathgeber et al. 2011a}. The same rule applies for the wall-thickening, and mature phenophase. At the end of the growing season, the cell-enlargement phenophase is declared terminated at the date at which less than 50 % of the counted radial files present enlarging cells. Based on the five previous critical dates, the duration of three wood formation phenophases are also defined. The duration of the enlargement period is: dE = cE – bE; while the duration of the wall-thickening period is: dW = cW – bW; and the total duration of wood formation (i.e. the duration of xylogenesis) is: dX = cW – bE.
The onset, cessation and duration of cambial activity are also very important pieces of information. However, we argue that it is not possible to define these dates accurately using only cell counts {Rathgeber et al. 2011a, Prislan et al. 2013}, we

believe that additional anatomical observations are needed. For this reason, we didn't include yet the computation of the onset and cessation dates of cambial activity into CAVIAR.



14 — Figure 4.1 | Drawing illustrating the definitions of critical dates and durations thanks to the representation of two schematic xylem radial files that are developing throughout the growing season.
Radial file cells are classified according to their differentiation stage in cambial cells (C), enlarging cells (E), maturing cells (W), and mature cells (M). bD, bE, bW, and bM stands for the beginning of dividing, enlarging, maturing (secondary wall thickening and lignification) and mature phases respectively; while dD, dE and dW stands for the end of dividing, enlarging, maturing and mature phases respectively; and dD, dE, dW and stands for the durations of dividing, enlarging and maturing phases respectively, while dX stands for the total duration of tree-ring formation. Figure modified from Rathgeber et al. {2011a}.

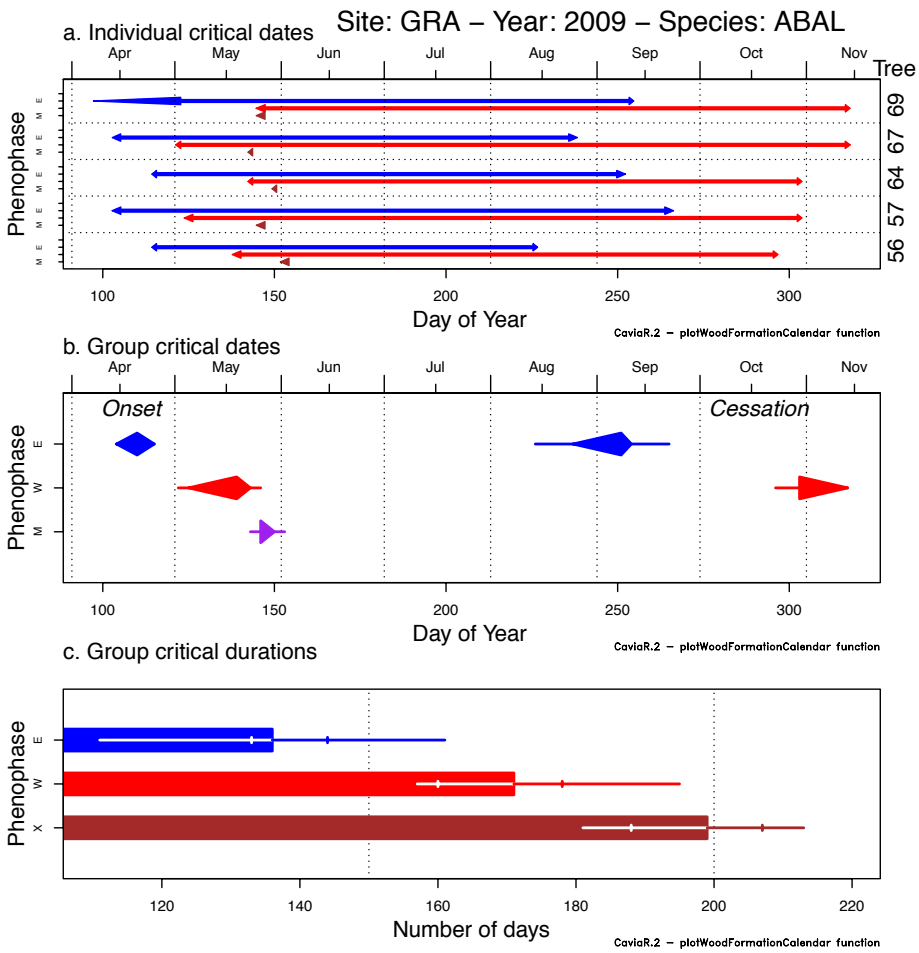
4.2.2 | Computation of tree-ring formation critical dates with CAVIAR

Tree-ring formation critical dates can be assessed using a dedicated function of the R package CAVIAR, which applies the definitions presented here above to cell count raw data. For each tree of a dataset and each year of monitoring, this function provides the five presented critical dates: bE, bW, bM, cE and cW ; along with their confidence intervals {Rathgeber et al. 2011a}. Critical dates and confidence intervals are computed using logistic regressions and outputted in a

convenient data frame. The main dates correspond to the 50 % probability of a phenophase to have started or ended, while the associated (95 %) confidence interval was taken as the time elapsed between the dates defined by the 2.5 % and 97.5 % probabilities. Optional verification plots allow checking the consistency of each estimate {see Rathgeber et al. 2011; 2018 for more details}.

4.2.3 | Visualisation of tree-ring formation critical dates with CAVIAR

Computed critical dates can then be plotted using another dedicate function of the R package CAVIAR in order to check the quality of the estimates at the tree level, as well as their consistency at the group level. A synthetic chart of wood formation critical dates and durations can then be plotted for a coherent group of trees using the same function in order to display the wood formation calendar for this group [Fig. 4.2]. Wood formation calendars can then be compared between groups of trees representing different e.g. species, years, or sites, for visual insights of the changes in the phenological patterns.

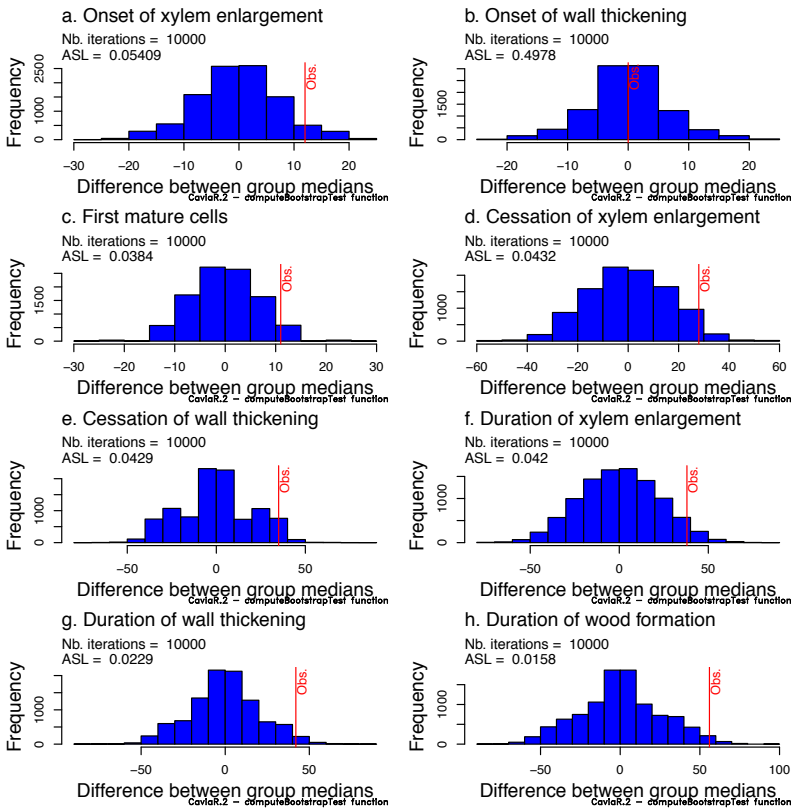


15 — Figure 4.2 | Example of presentation plots produced by the plotWoodFormationCalendar function of the R package CAVIAR.
Option level = "Tree", "Group.Dates", and "Group.Durations", for a, b, and c respectively, using data from five silver firs monitored in 2009 in the Vosges Mountains {Cuny et al. 2012}. The first plot (a) represents individual critical dates and durations for each tree of the group and each phenophase: E for enlargement (in blue), W for cell wall thickening

(in red), and M for mature (in purple). The lengths of the arrowheads are proportional to the confidence intervals associated to the estimated dates. The second plot (b) is a synthetic wood formation calendar showing critical dates for the group of trees. Crossed diamond shape signs show minimal, second quartile, median, third quartile, and group maximum values —note that when the group is composed of five trees (as in the GRA dataset), all the data are represented. The third plot (c) represents a synthetic view of the wood formation durations (E for enlargement (in blue), W for cell wall thickening (in red), and X for xylogenesis (in brown)) for a group of trees. The horizontal bars show the medians, while the horizontal lines and the vertical tick marks stand for the minimal, second quartile, third quartile, and group maximum values. Figure from Rathgeber et al. {2018}.

4.2.4 | Statistical analysis of tree-ring formation critical dates with CAVIAR

The comparisons of wood formation critical dates and durations between groups of trees can be carried out using bootstrap permutation tests, which have proven to be particularly well adapted for this type of data {Rathgeber et al. 2011a}.
Bootstrap permutation tests can be performed using a dedicated function of the R package CAVIAR, which allows considering individual confidence intervals for more accurate comparison between groups [Fig. 4.3].
Note that when performing multiple comparisons, Bonferroni correction must be applied to maintain the family-wise error rate at a chosen level a, while computing the multiple pair-wise comparisons, by testing each of the n individual tests at a significance level of a/n {see Rathgeber et al. 2011a for more details}.



16 — Figure 4.3 | Example of presentation plots produced by the computeBootstrapTest function using data from the original GRA dataset and

illustrating the comparison between fir and spruce wood formation calendar in 2009.
Plots based on one tailed two-sample bootstrap tests of significance on medians (n = 5). Figure from Rathgeber et al. {2018}.

4.3 | Exploring the variability of tree-ring formation phenology

4.3.1 | Variability of tree-ring formation phenology with tree characteristics

Introduction

Our knowledge about the influences of environmental factors on tree growth is principally based on the study of dominant trees {Fritts, 1976; Schweingruber, 1996}. However, intermediate and suppressed trees represent a large proportion of the stems in a stand and have an important contribution in wood production {Oliver and Larson, 1996}. Many functional processes are closely related to tree dimensions, and thus undergo changes as tree age and size increase {Martinez-Vilalta et al., 2007; Vanderklein et al., 2007}. The extent to which the relationships between growth and environmental factors depend on age (genetic control) and/or size (physiological control) is difficult to determine. Recent investigations suggest that size is the most important factor {Mencuccini et al., 2005; Penuelas, 2005; Munne-Bosch, 2007}.

Within a stand, trees of different sizes and social status compete differently for light, water and other resources {Oliver and Larson, 1996}, and may thus respond differently to environmental stresses {Linares et al., 2009, 2010; Mérian and Lebourgeois, 2011}. De Luis et al. {2009} have reported greater differences between sizes than between species in terms of sensitivity to climate. Although dominant trees obviously have larger stems because of their higher annual growth, the causes of the formation of wider rings are still not understood in detail. In particular, it is not known if variation in radial growth among trees of different social status results from variations in the length of the growing period or in the rate of cell production.

Recently, several studies have investigated the intra-annual dynamics of wood formation in conifers, describing the timings, durations and rates of cell production and differentiation through the vegetation period {Deslauriers et al., 2003; Rossi et al., 2006b}, and relating tree-ring formation with intrinsic {Lachaud et al., 1999; Savidge, 2001; Wodzicki, 2001} or extrinsic factors {Wodzicki, 1971; Horacek et al., 1999; Rossi et al., 2007}. Most of these studies focused on the influence of (a) climatic factors {Oribe and Kubo 1997; Abe et al., 2003; Gricar et al., 2006; Rossi et al., 2008b} or (b) hormones {Lachaud, 1989; Sundberg et al., 1991; Savidge, 1996}. However, very few studies have considered the influence of biotic interactions, such as tree-to-tree competition {Grotta et al., 2005; Linares et al., 2009}, or intrinsic factors, such as tree age or vitality {Rossi et al., 2008a, 2009b}.

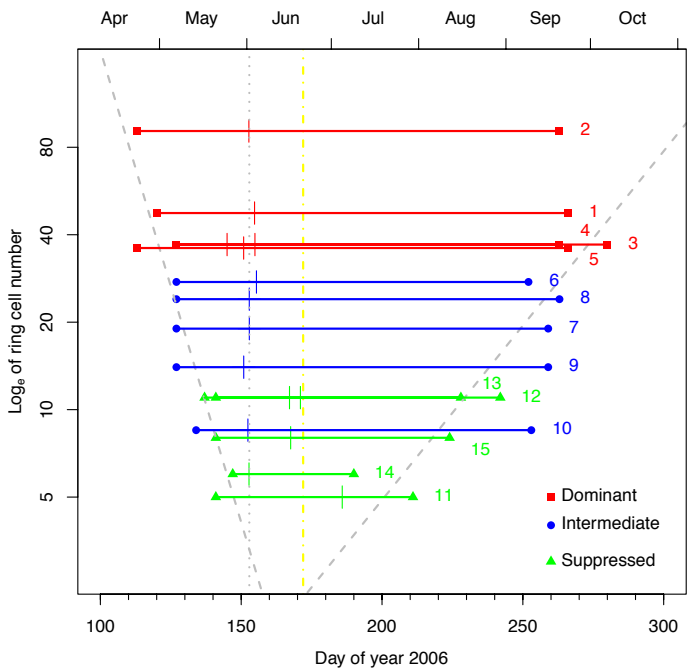
Rossi et al. {2008a} observed different timings of xylem formation between adult and old trees, which could explain the observed age-dependent climate sensitivity {Carrer and Urbinati, 2004}. However, the experimental design of their study did not make it possible to discriminate between the effects of age and size.

Summary of material, method and results

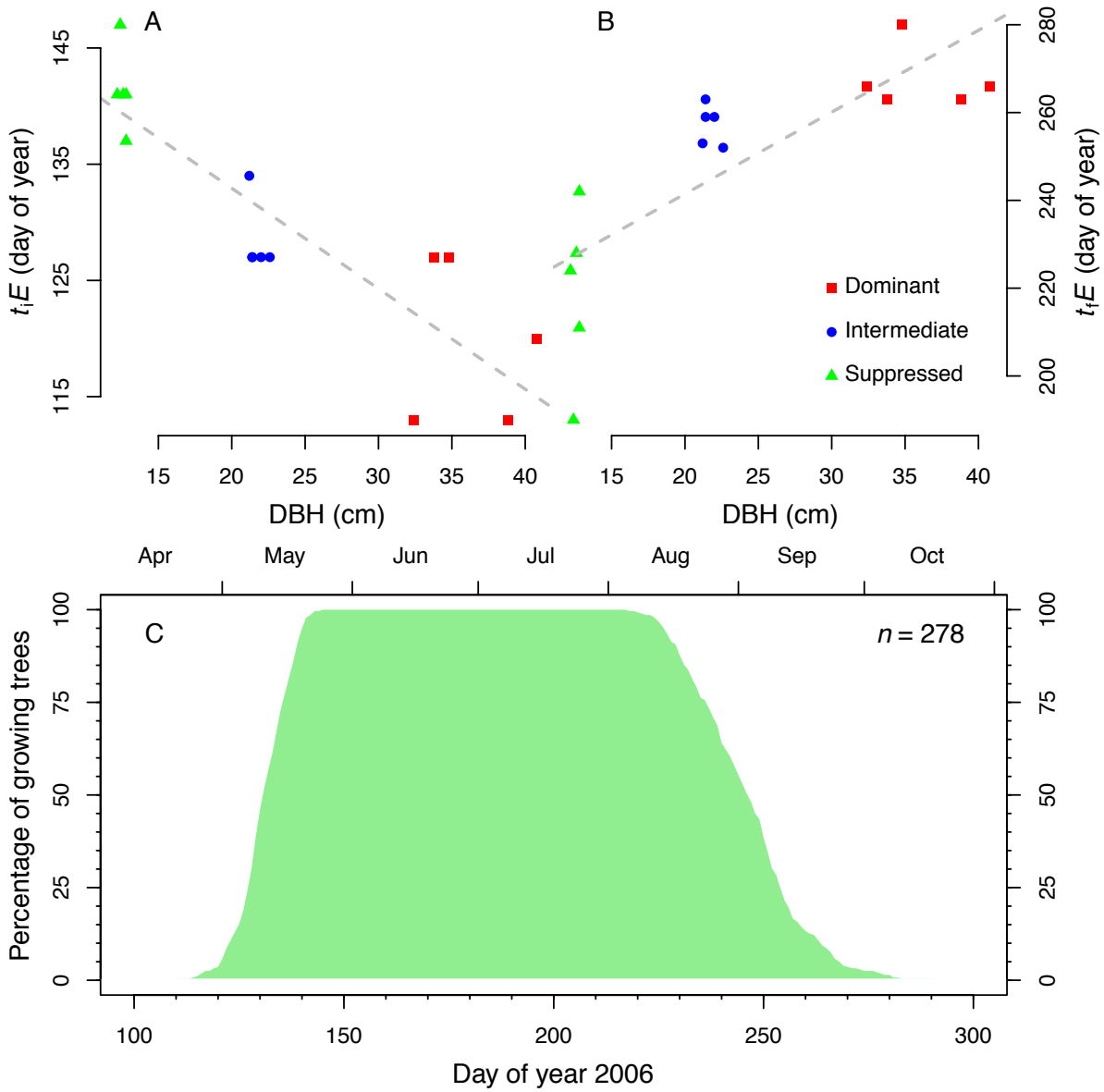
In 2006, we monitored weekly cambial activity for three crown classes in a 40-year-old silver-fir (*Abies alba* Mill.) plantation near Nancy (France). Timings, duration and rate of tracheid production were assessed from anatomical observations of the developing xylem.

The main objective of this study was to understand how the timings, duration and rate of xylem cell production – the most important features of tree-ring formation – change according to tree size, while the other main influencing factors (site conditions, climate, species and age) are fixed. Our general hypothesis was that the timings and duration of cambial activity are principally under the control of environmental factors external to the stand. Following this idea, we tested the hypothesis that all the trees have similar timings (initiation, culmination and cessation) of cambial phenology because they all follow the same external environmental forcing.

In this study we found that cambial activity started earlier, stopped later and lasted longer in dominant trees than in intermediate and suppressed ones [Fig. 4.4]. The onset of cambial activity was estimated to have taken 3 weeks to spread to 90% of the trees in the stand, while the cessation needed 6 weeks [Fig. 4.5]. Moreover, growth duration was better correlated to tree height.



17 — Figure 4.4 | Timing of cambial activity for 15 silver firs (Abies alba Mill.) monitored in 2006 in Amance forest (48.748N, 6.328E; 270m a.s.l).
Beginning (data points on the left), end (data points on the right) and duration (horizontal lines) of the enlargement phase, as well as the date of occurrence of the maximal production rate (tp, vertical bars). Grey dashed lines represent the regression lines. The grey dotted line represents the median for tp, and the yellow line represents the summer solstice. Figure from Rathgeber et al. {2011b}.



18 — Figure 4.5 | Relationships between (A) the beginning (tE,i) and (B) the end (tE,f) of the xylem enlargement phase and the diameter at breast height (dbh) of the monitored trees.
(C) proportion of trees actively growing in the inventoried stand in 2006. Dashed lines illustrate the linear regressions. Figure from Rathgeber et al. {2011b}.

Discussion

On the basis of the evidence that, in temperate and boreal regions, cambial activity is under the control of temperature at the beginning of the growing season {Rossi et al., 2007, 2008b; Seo et al., 2008; Linares et al., 2009; Begum et al., 2010}, the hypothesis that it starts at the same date for all the trees of a stand was formulated. The results compelled us to reject this hypothesis because they showed that trees from the same species and the same age, grown in the same stand under the same climatic conditions, started to grow at different dates according to their crown classes. Thus, the widely used assumption that temperature controls growth resumption in spring without interaction with the individual characteristics of trees {Linares et al., 2009} should be partially revised.

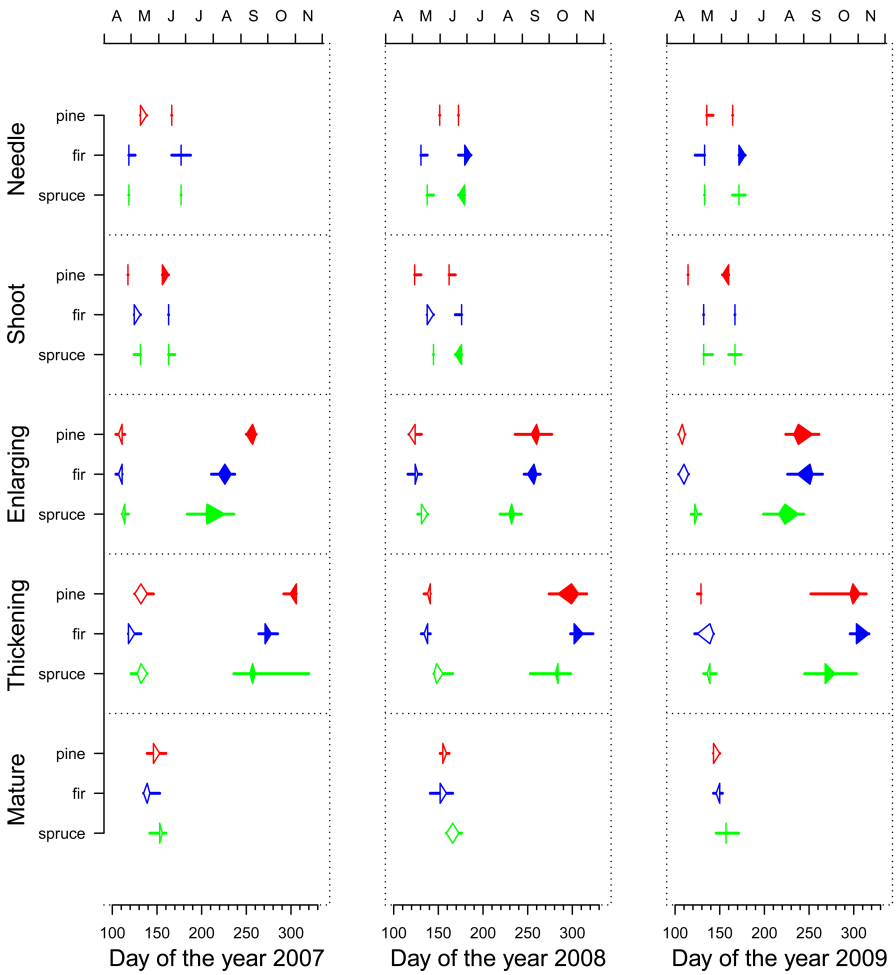
The hypothesis that cambial activity stops at the same date for all the trees of the stand at the end of the growing season was also refuted by the present results, as the dominant trees stopped growing later than the intermediate and suppressed ones. The present findings confirm other studies on conifer species {Kozlowski and Peterson, 1962; Grotta et al., 2005; Linares et al., 2009}, which shows that suppressed trees have a shorter growing season compared to dominant trees.
The present results have shown a strong relationship between the beginning, end and duration of cambial activity and the diameter of a tree. In 2006, in the studied stand, the onset of cambial activity was estimated to take nearly 3 weeks to spread to most of the trees (from 5% of the biggest trees to 5 % of the smallest ones), while the cessation of cambial activity needed 6 weeks, i.e. double the time. The duration of cambial activity was estimated to vary from 3 months for the smallest trees of the stand, to 4 months for the average trees, and to 5 months for the biggest ones. Thus, the range of variations observed within the stand between dominant and suppressed trees was as wide as that observed between temperate and boreal (dominant) trees {Rossi et al., 2008b}. Such large differences in the length of the growing period should lead to important differences in many other aspects of life, functioning and adaptation capabilities of trees.
As a result, different climate – growth relationships are expected between dominant and suppressed trees {Pichler and Oberhuber, 2007; De Luis et al., 2009; Linares et al., 2010}. For example, extreme climatic events occurring at the beginning or at the end of the growing season (e.g. late or early frost) may only affect the biggest trees, while other events, occurring during the main period of growth (e.g. drought), should affect all the trees of the stand (but not necessarily with the same intensity). This could partially explain the observations made by Liu and Muller {1993} that, during frost years, dominant and co-dominant trees exhibited significantly greater reduction of radial growth (15%) than suppressed ones (3%). This body of results suggests that concerning intra-annual dynamics of wood formation at least, but more probably concerning forest production in general, dominant trees are not representative of the response of the stand to the environmental factors.

4.3.2 | Variability of tree-ring formation phenology between trees from different species

Comparison of the phenology of three contrasted conifer species on the same site

In our Grandfontaine study site located at mid-altitude in a temperate coniferous forest, cambial activity began between late April and early May, a few days before the onset of needle unfolding and shoot elongation [Fig. 4.6]. While needle unfolding and shoot elongation lasted only 1–1.5 months, tracheids accumulated over 3–4.5 months and matured over 5–6 months, so that the major part of wood formation occurred after the production of needles and shoots {Rossi et al. 2009}. Very similar timings of tracheid production and differentiation have been described in temperate forests in central Europe, for Scots pine in Poland {Wodzicki 1971, 2001} and for Norway spruce and silver fir in Slovenia {Gricar 2007}.

Based on the theory that pioneer species adopt riskier life strategies than late-successional species {Korner 2006, Korner and Basler 2010}, we expected an earlier onset of growth in pines. Primary growth actually began earlier in Scots pine, but surprisingly cambial activity began at the same time for pines and firs. The greater age and the lower height of pines (see Table 1 in Cuny et al. {2012}, for more details) may explain this unexpected result. Indeed, Rossi et al. {2008b} reported that cambial activity began later as trees aged, while Rathgeber et al. {2011b} found that, at the same age, smaller silver firs begin cambial activity later than taller ones. Therefore, because the selected pines were older and smaller than the firs and spruces, we probably overestimated their onset and underestimated their cessation and durations of wood formation. Moreover, by establishing the wood-formation calendar of similar-age (≈40 years old) trees grown in the same stand, Rathgeber et al. {2011a} found that cambial activity began 20 days earlier for Scots pine than for silver fir.



19 — Figure 4.6 | Needle unfolding, shoot elongation and wood formation calendar.

Onset and cessation of needle unfolding and shoot elongation, along with critical dates of wood formation for pines, firs and spruces. Critical dates of wood formation are the onset and cessation of the enlarging and thickening phases along with the onset of the mature phase. Note: for each date, the five trees are represented by diamond-crossed-by-a-line marks; the left end of the line represents the first tree to begin or finish, the left end of the diamond the second tree, the middle of the diamond the third tree, the right end of

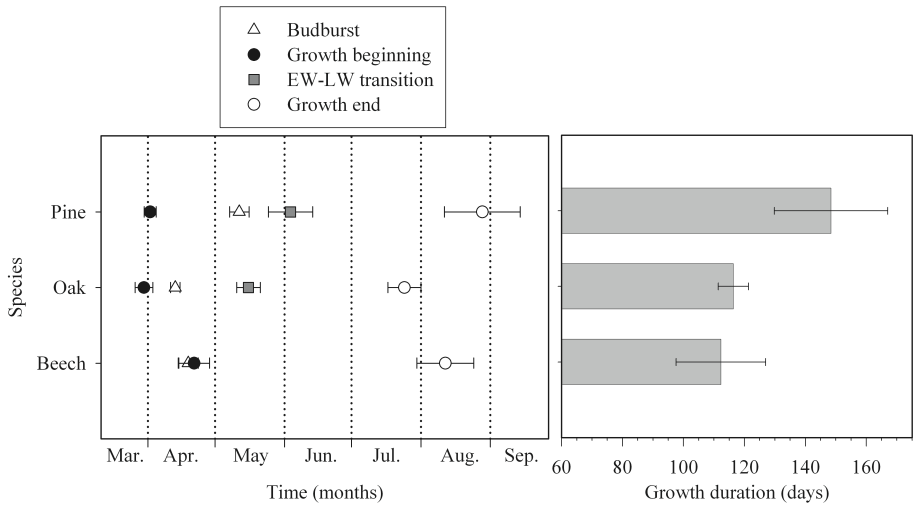
the diamond the fourth tree and the right end of the line the fifth tree. Figure from Cuny et al. {2012}.

Comparison of the phenology of three species exhibiting contrasted tree-ring structure on the same site

There have been few studies concerning intra-annual wood formation in lowland temperate forests with high time resolution, especially for the comparison between deciduous and coniferous species. The main objective of this study was to determine how the timing, duration and rate of radial growth change between species as related to leaf phenology and the dynamics of non-structural carbohydrates (NSC) under the same climatic conditions.

We studied three species exhibiting contrasted tree-ring structures and phenological habits: European beech (*Fagus sylvatica*) a deciduous angiosperm presenting diffuse porous tree-rings, sessile oak (*Quercus petraea*) a deciduous angiosperm presenting ring porous tree-rings, and Scots pine (*Pinus sylvestris*) an ever-green gymnosperm. During the 2009 growing season, wood formation and leaf phenology were monitored weekly. The non-structural carbon (NSC) content was also measured in the eight last rings of stem cores taken in April, June and August 2009.

The leaf phenology, NSC storage and intra-annual growth were clearly different between species, highlighting their contrasting carbon allocation. Beech growth began just after budburst [Fig. 4.7], with a maximal growth rate when the leaves were mature and variations in the NSC content were low. Thus, beech radial growth seemed highly dependent on leaf photosynthesis. For oak, earlywood quickly developed before budburst, which probably led to the starch decrease quantified in the stem from April to June. For pine, growth began before the needles unfolding and the lack of NSC decrease during the growing season suggested that the substrates for radial growth were new assimilates coming from old needles.



20 — Figure 4.7 | Dates of budburst, growth beginning, earlywood–latewood (EW–LW) transition and growth end (left), as well as growth duration (right) for beech, oak and pine in 2009 in Fontainebleau.

The horizontal bars correspond to the 95% confidence limits of the mean (N = 5 trees per species). Figure from Michelot et al. {2012}.

4.3.3 | Variability of tree-ring formation phenology along elevation gradients

Introduction

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 °C / 100 m {Korner 2007}. Thus, monitoring wood formation along elevation gradients 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 a 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 °C for the onset of cambial activity and duration of tree-ring formation. 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}. Moreover, 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 Mohave 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 that in lower latitudes {Jackson 2009}. 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, King et al. {2013} and Nardin et al. {2015} reported the absence of clear genetic structure in the forest stands spread along our elevation gradients. 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}.

The main objective of this section is to unravel the relationships between environmental factors and the phenology of wood formation along elevation

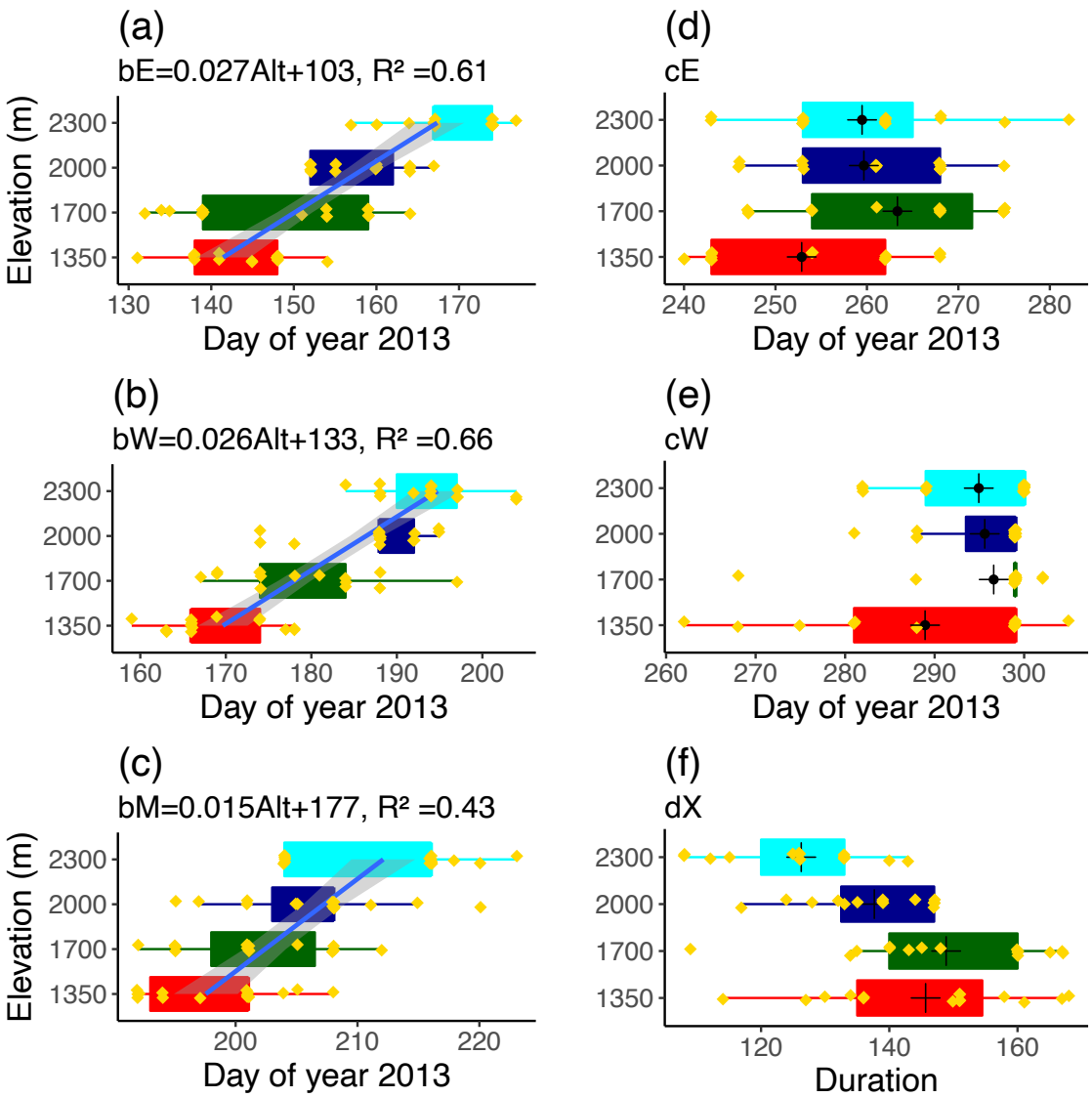
gradients. We assumed that temperature is the main driver of larch tree wood formation phenology. Then, we tested the following hypothesis: (1) at the beginning of the growing season, we expect a delay in the onset of wood formation phenophases, with critical dates increasing linearly from the bottom 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 bottom to the top 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.

Summary of material, method and results

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 1,000 m (at 1350, 1700, 2000 and 2300 m) located in the locality of the Mélézin (hereafter MLZ), in the French Southern Alps.

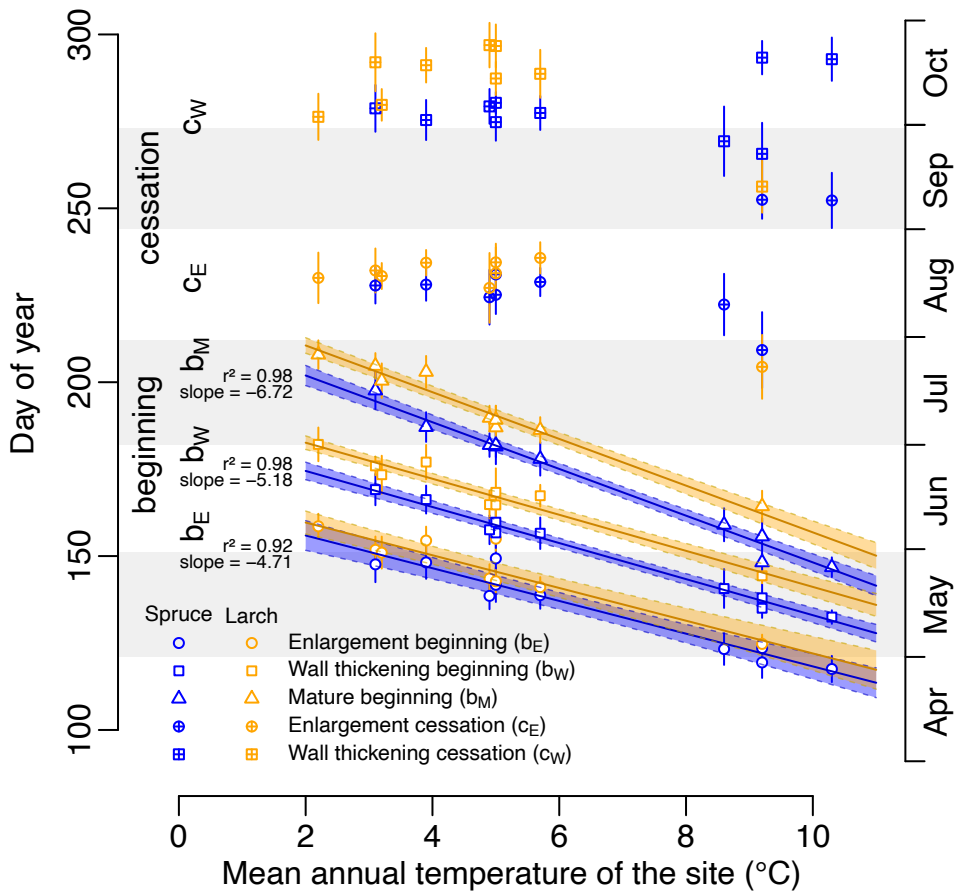
We also used observations from the Donon (DNN) and the Lötschental (LST) elevation gradient, compiling data for Norway spruce (*Picea abies*) and Larch (*Larix decidua*) across an 8°C thermal gradient from 800 to 2,200 m a. s.l. in central Europe.

Cambial activity started around mid-May at the lowest site (1,350 m) in MLZ and around mid-June at the highest one (2,300 m), showing a delay of 2.7 days per 100 m [Fig. 4.8]. The onset of wall thickening and mature phenophases followed the same linear trend with a delay of 2.5 and 1.5 days per 100 m, respectively. 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 lasts. 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.



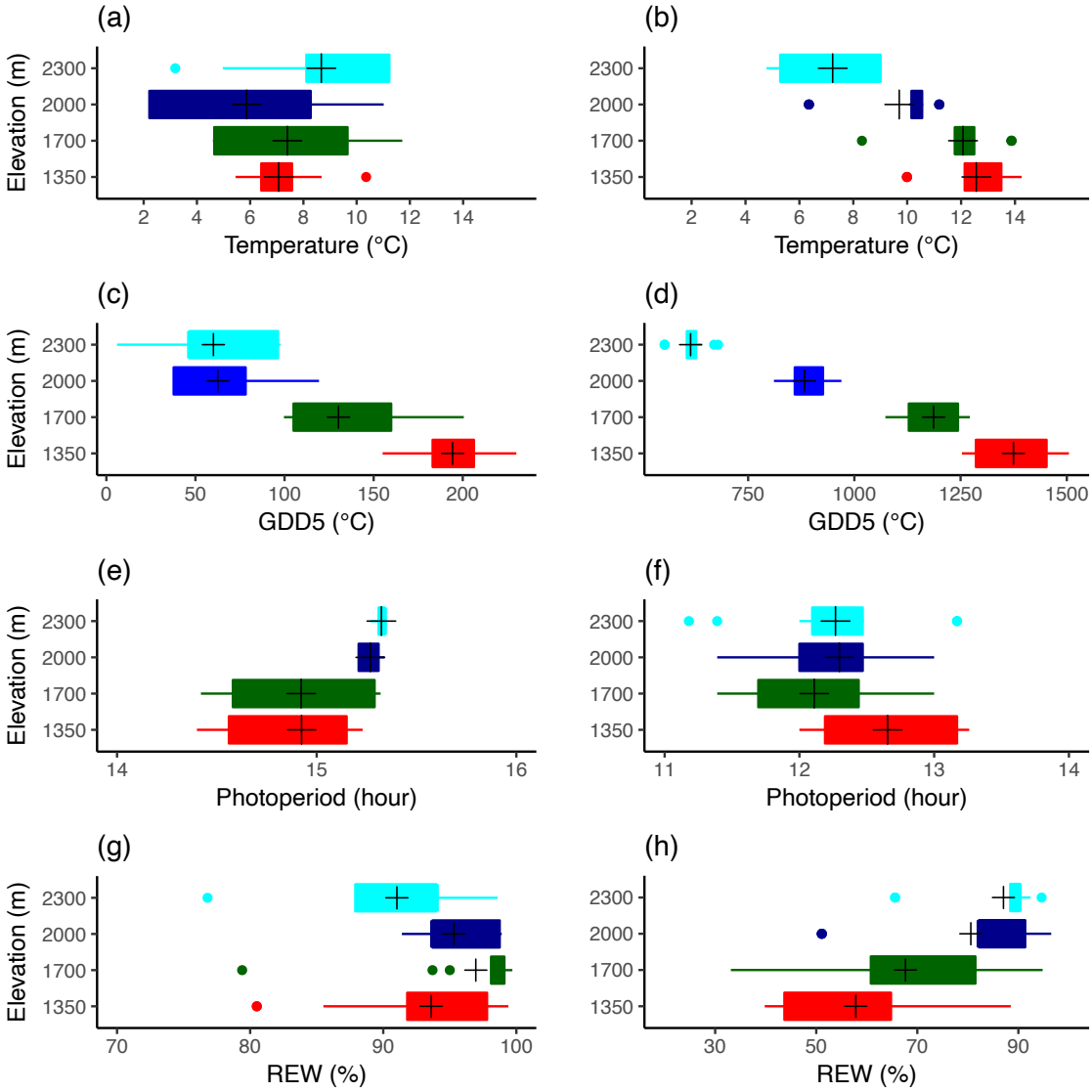
21 — Figure 4.8 | Phenology of wood formation along the MLZ elevation gradient in the South-East Alps.
(a) onset of enlargement period (bE); (b) onset of wall thickening period (bW); (c) appearance of the first mature cells (bM); (d) cessation of enlargement period (cE); (e) cessation of wall thickening period (cW); (f) wood formation duration (dX). Figure from Saderi et al. {accepted in Annals of Forest Science}.

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 [Fig. 4.9]. 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 [Fig. 4.10].



22 — Figure 4.9 | Phenology of wood formation for Larch and Spruce along the LST + DNN elevation gradient.

Timing of xylem tissue formation along the thermal gradient with the beginnings of cell enlargement (bE), wall thickening (bW) and mature (bM) periods and the cessations of cell enlargement (cE) and wall thickening (cW) periods. Each point represents a site average for one species, whereas bars symbolize the associated standard deviation. Orange and blue lines represent the relationships between air temperature and the different phenological dates for larch and spruce, respectively. Coloured areas around lines represent the 95% confidence intervals. Figure from Cuny et al. {2018}.



23 — Figure 4.10 | Change in the environmental factors along the MLZ 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. Figure from Saderi et al. {accepted in Annals of Forest Science}.

Discussion

'Natural experiment' of wood formation monitoring along a 6 K temperature gradient

In mountain regions, the beauty of the progressive leaf appearance in spring along elevational gradients has always fascinated humans beyond any scientific scope. However, this phenomenon is not only fascinating but is an essential driver of ecosystem structure and functioning. In particular it regulates carbon, water, and energy exchanges between the biosphere and the atmosphere, influencing the entire Earth's climate system and is a major determinant of plant fitness. An attempt to generalize how phenology of plants and animals varies across geographical space covering climatic gradients (latitude, longitude, and altitude)

was conducted almost one century ago by the American entomologist, Andrew Delmar Hopkins, and is summarized in the so-called "Hopkins' bioclimatic law" {Hopkins 1918, 1920, 1938}. In a recent paper, Vitasse et al. {2018} show that the elevation-induced phenological shift (EPS) has significantly declined from 34 days per 1,000 m conforming to Hopkins' bioclimatic law in 1960, to 22 d·1,000 m−1 in 2016, i.e., −35%. The stronger phenological advance at higher elevations, responsible for the reduction in EPS, is most likely to be connected to stronger warming during late spring as well as to warmer winter temperatures. Authors conclude that future climate warming may further reduce the EPS with consequences for the structure and function of mountain forest ecosystems, in particular through changes in plant–animal interactions, but the actual impact of such ongoing change is today largely unknown. The EPS value of 22 days assed by this study in 2016 over a large dataset of leaf phenology is very close the the 27 days we found for wood phenology on our MLZ elevation gradient.

One of the difficulties while working with 'natural experiments' is to sample comparable individuals (e.g., same age, size, 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 (see previous sections). 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 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, 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 1,000 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 suggest 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.

Spring temperatures 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} and Cuny et al. {2018} working also with larch trees on a comparable gradient 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. All these evidences point out to temperatures 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 stem 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 2008b} since we showed that the average temperature during the seven 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 don't 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 photoperiod 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 in Begum et al. {2013}), after the chilling requirement has been fulfilled during endodormancy (referred to as the "rest phase" in Little and Bonga {1974}; 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. Additionally, the role of other factors such as soil temperature could be explored since some authors suggest that an increase in the soil temperature is

needed for the resumption of cambial activity {Lupi et al. 2012; Jyske et al. 2012; Treml 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 they are either under the control of the same environmental factors (temperatures), or more probably they follow each other in a pre-determined 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}.

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 lasts. 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. 2015}. 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 hours. 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 favour of a control of the cessation of wood formation by photoperiod is provided by the fact that trees at higher latitudes finished their growth earlier in autumn compare to trees at lower latitudes {Jackson 2009; Jyske et al. 2014; Rossi et al. 2014}.
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 to the other stand of our gradient, the bottom stand experienced the longest period of water deficit, the highest drought intensity, as well as 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.

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 four months), and the longest ones at the 1700 m site (about five month), 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. Another example is the length decrease of the growing season from 5 to 2 months in Norway spruces from lower elevation to the Alpine treeline {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 altitude 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 zero degree Celsius, the metabolism of trees decreases in order to maintain the minimum physiological activity and restrict the water uptake and root functioning {Korner 2003}.

The observed delay of 5 days / °C and the projected 2.5 °C 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 to 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; Prislan 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}.

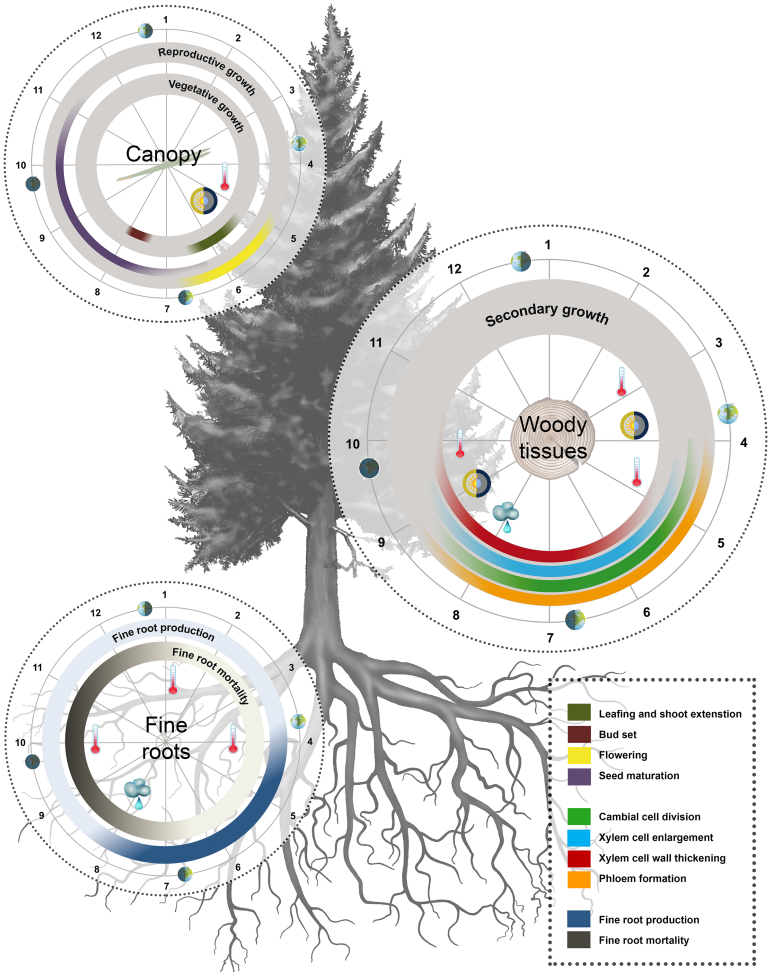
Moreover, our results suggested that the cessation of cambial activity is more related to the photoperiod, but that water stress can hasten the termination of the growing season at lower elevations. The more intense evaporation associated with the ongoing climatic warming will limit even more water availability 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.

4.4 | The seasonal cycles of the different tree components in relation with the environment

4.4.1 | Introduction

A long evolutionary history has shaped the ability of temperate and boreal trees to cope with freezing winter temperatures. In autumn, leaves of deciduous trees escape winter freezing via a senescence process that leads to leaf abscission. All overwintering tree tissues, which include leaves in evergreen species, undergo a process of cold acclimation to cope with winter freezing {Yoshie and Sakai 1982}. The primary and secondary meristems further enter dormancy, a resting state under which no growth or tissue maturation is observed {Mellerowicz et al. 1992; Arora et al. 2003}.

We begin this section by reviewing the current knowledge on the environmental controls of the dormancy/activity cycle of tree meristems (for leaves and reproductive structures, stem wood and fine roots). We then examine their impacts on carbon and nitrogen reserves, which are key to the integrated functioning of trees. We conclude by describing an integrated view at the tree scale, addressing the links between the individual phenologies of tree organs [Fig. 4.11].



24 — Figure 4.11 | Overview of primary and secondary growth along with their main environmental cues.

The annual course of primary growth in the canopy (upper left) is shown for both vegetative and reproductive events. The annual course of secondary growth in the stem comprises the phenology of xylem enlargement and wall thickening, cambial activity, and phloem formation. Main periods of fine root production and mortality indicate the annual course of primary growth in roots. Relevant environmental cues affecting tree phenology are shown the for canopy, stem and roots. Figure from Rathgeber et al. {in preparation b}, prepared by G. Perez-de-Lis.

4.4.2 | The phenology of leaves and reproductive structures

Seasonal cycle of buds

Among the overwintering tissues of temperate and boreal trees, buds are essential because they contain primordia of the future leaves or needles. The regulation and maintenance of bud dormancy is a key to understand the phenological responses of trees to climate change. The developmental processes involved in bud dormancy are complex and interact with environmental cues to optimize the timing of bud-burst in order to avoid late spring frost. In this section, we focus on the environmental cues that affect the phenology of the primary aerial meristems of temperate and boreal trees (budburst, flowering, fruit and cone maturation, and leaf senescence) and refer to detailed reviews from Wareing and Saunders {1971}, Arora et al. {2003}, Horvath et al. {2003}, and Cooke et al. {2012} for the hormonal and molecular changes involved in these phenophases.

New buds (either vegetative, reproductive, or compound) are formed from late summer to early autumn, depending on the species and the prevailing climate. Bud-set (i.e., the formation of a mature dormant bud) of boreal trees tends to occur earlier than in temperate trees, and within species, populations from higher latitudes or elevations exhibit earlier bud-set {Alberto et al. 2013}. Once formed, buds undergo three main phases of dormancy from late summer – early autumn to the following spring, namely, para-dormancy, endo-dormancy, and eco-dormancy. During paradormancy (from late summer to early autumn), bud cell growth is repressed by distant organs within the branch (leaves or other buds) via the influence of hormones, such as auxins {Horvath et al. 2003}. During endodormancy (from early autumn to mid-winter), bud cell growth is inhibited by internal factors that are still not completely understood, but that are suppressed by low temperatures, hereafter called chilling temperatures. A study conducted on downy birch (*Betula pubescens*) suggests that the connections between bud meristematic cells are progressively re-established via plasmodesma channels after a certain time of exposure to chilling temperatures {Rinne et al. 2001}, possibly explaining the transition between the endodormancy and ecodormancy phases. During ecodormancy (from mid-winter to mid-spring), bud cell growth is only inhibited by unfavourable external environmental conditions. The end of bud endodormancy classically corresponds to the so-called dormancy break. Budburst (end of spring) marks then the beginning of the active phase of bud development.

The separation of the dormancy period into three distinct phases is conceptually practical and has therefore been incorporated into the construction of process-based models. However, this definition, originally proposed by Lang et al. {1987}, is currently under scrutiny because it appears that these three phases are not strictly separated in time, and interactions among them have been reported {Cooke et al. 2012}. However, this conceptual model, originally proposed by Lang et al

{1987}, is currently under revision because it seems that: (1) the three phases may not be strictly separated in time; and (2) interactions between them are possible {Cooke et al. 2012}.

Influence of environmental factors on Bud-burst

Overall, in temperate and boreal forest trees, bud development is mainly under the control of temperature and photoperiod. Whereas the influence of temperature has been well documented in recent decades, especially with respect to the timing of budburst {e.g., Linkosalo et al. 2006; Menzel et al. 2006; Linkosalo et al. 2009}, the understanding of the influence of photoperiod still generates intense debate within the scientific community {e.g., Körner and Basler 2010 and the reply in Chuine et al. 2010; Laube et al. 2014}. Temperature has contrasting effects on bud development depending on the dormancy state (e.g., endodormancy vs. ecodormancy). Indeed, it has been assumed since the beginning of the twentieth century that, for the majority of temperate and boreal tree species, a certain duration of chilling temperatures ranging between 0 and 5 °C is responsible for the release of endodormancy {Coville 1920; Sarvas 1974; Hänninen 1990; Caffarra et al. 2011b}. However, the range of chilling temperatures required for an efficient release of endodormancy has not yet been clearly identified {Dantec et al. 2014}. Recent advances suggest that this range of chilling temperature is likely to be species-specific {Laube et al. 2014} and may cover a broader spectrum than commonly assumed {Harrington et al. 2010}.

Once endodormancy is released, warm temperatures have a clear positive effect on bud development during ecodormancy, with a warmer spring leading to an earlier budburst {Sarvas 1972; Sarvas 1974; Campbell and Sugano 1975; Lang et al. 1987; Caffarra et al. 2011b}. The influence of warm spring temperatures is particularly obvious in mountainous areas, where tree populations flush gradually later toward higher elevations {Vitasse et al. 2009}, with only a small fraction of the variability explained by the genetic differentiation among populations {Vitasse et al. 2013}.

Finally, several studies have suggested that the influence of a warm temperature in early spring depends on the state of bud endodormancy and, in certain species (such as European beech and Norway spruce), on the photoperiod {Heide 1993; Myking and Heide 1995; Caffarra et al. 2011b; Vitasse and Basler 2013; Basler and Körner 2014; Laube et al. 2014}. However, the effect of the photoperiod on the resumption of bud cell division and growth during ecodormancy varies widely among species and has been reported to clearly affect the timing of budburst in only one third of the temperate and boreal tree species for which this factor has been explicitly tested in experiments (18 out of 55 tree species, mainly temperate trees) {Way and Montgomery 2014}. Anyway, chilling requirement and photoperiod control are both regarded as the most prominent mechanisms to prevent overly precocious leaf, needle, or flower development, allowing tree's efficient adaptation to potential frost injuries.

Influence of environmental factors on bud-set

Bud-set must be initiated in late summer or early autumn before the occurrence of the first frost, as a prerequisite to the initiation of cold acclimation. The timing of bud-set closely follows the cessation of primary shoot growth {Gyllenstrand et al.

2007; Rohde et al. 2011}. It is often assumed that the autumnal cessation of primary meristem activity and bud-set are fully controlled by the photoperiod in the majority of boreal and temperate tree species {Tanino et al. 2010; Way 2011}. The response of the plant to the relative lengths of light and darkness is called photoperiodism. Phytochromes are the leaf pigments which are used by the plant to detect periods of light and darkness. Phytochromes exist in two forms, an active form and an inactive form. During the day, when leaves absorb red light (~660 nm), the inactive form of phytochrome is converted into the active form. During the night, the active form will gradually revert to the inactive form in the absence of light (darkness reversion). Additionally, the active form of phytochrome is also broken down into the inactive form when it absorbs far red light (~725 nm). Because sunlight contains more red light than moonlight, the active form is predominant during the day. Similarly, as the active form is reverted in darkness, the inactive form is predominant during the night. Only the active form of phytochrome is capable of triggering phenological events, however its action differs depending on plant types {Arana et al. 2017; Seaton et al. 2018}.
However, recent experimental studies have demonstrated that temperature additionally modulates the timing of bud-set and the cessation of primary growth in a number of temperate and boreal tree species, with both temperature and photoperiod acting in an interactive, non-monotonous manner {e.g., Salminen and Jalkanen 2007; Rohde et al. 2011; Heide 2011}.

Influence of environmental factors on the phenology of the reproductive structures

The seasonal development of reproductive structures has received considerably less attention in forest trees than in crop trees {Sedgley and Griffin 1989; Srivastava 2002}. The ontogenetic sequence from the apparition of flowers (in angiosperms) or strobili (in gymnosperms) to the time of fruit ripening or seed maturation involves a number of stages (e.g., pollination, fertilization, the growth and differentiation of the embryo) {Pallardy 2008} that we group here under the term of fruit (or cone) maturation. The seasonality of fruit (cone) maturation (in terms of size and mass) is relatively well documented — see for example Sharp and Spargue {1967} and Fujii {1993} for temperate deciduous oaks, or Linder and Troeng {1981} and Koppel et al. {1987} for boreal conifers. However, the information regarding the environmental dependence of these processes is scarce. Temperature has been identified as the main cue for both cone maturation {Mutke et al. 2003} and seed maturation {Meunier et al. 2007} in conifers.

Influence of environmental factors on leaf senescence

The timing of leaf senescence has received considerably less attention than leaf unfolding in forest trees, likely because it affects tree productivity to a relatively smaller extent because the meteorological and light conditions in autumn are substantially less optimal for photosynthesis than the conditions in spring {Vitasse et al. 2009; Richardson et al. 2012}. Leaf senescence is also relatively more difficult to monitor because, in contrast to budburst (which occurs in a few days and involves morphological changes that are easy to detect), leaf senescence is a slow, continuous ensemble of processes, visually progressing from the leaf coloration

induced by the degradation of chlorophylls to, ultimately, leaf fall. The recent development of satellite-based and ground-based imagery {Hmimina et al. 2013; Keenan et al. 2014} is expected to increase the level of research effort on the leaf senescence phase. Temperature and photoperiod are regularly mentioned as the most influential environmental cues in triggering leaf senescence, but their respective contributions have not been fully elucidated {see Estrella and Menzel 2006}. Several stresses, such as drought {Hwang et al. 2014; Estiarte and Peñuelas 2014}, water logging, and mineral deficiencies {Smart 1994}, can also hasten leaf senescence, primary growth cessation, and bud-set.

Finally, it is important to consider that the different phenophases considered here (e.g., bud-set, budburst, leaf maturation, and leaf senescence) are all interconnected. A change in the timing of one phase can potentially affect the timing of the subsequent phases {Hänninen and Kramer 2007; Fu et al. 2014}. Hence, the environmental cues driving the timing of each phase are also indirectly involved in the timing of subsequent phases.

4.4.3 | The seasonal cycle of root production, growth and shedding

The seasonal cycles of fine roots

Adult tree roots are organized as structured systems of several branching orders. There is clearly not a single class of "fine roots" that can be easily identified on the basis of morphological criteria such as diameter, but rather a continuum of sizes {Pregitzer 2002}, functions {Guo et al. 2008}, and lifespans {King et al. 2002} across branching orders.

In this section, we focus on describing the phenology of the finest, "ephemeral" roots because these roots constitute the most dynamic part of the root system {Eissenstat et al. 2013}. These ephemeral roots are typically less than 1 mm in diameter, non-woody, and mycorrhizal and play the main role in the absorption of water and nutrients {Guo et al. 2008}. However, the reality of field observations is that the phenological cycles of production and mortality are studied for ensembles of root branching orders that usually consider fine roots as the roots below a given diameter threshold of 1 or 2 mm.

The extent to which the primary growth of fine roots in trees is indeterminate (or not) remains unknown {Shishkova et al. 2008}. However, mini-rhizotron studies have demonstrated clearly that growth and mortality may occur simultaneously in the root system. However, growth and mortality are generally out of phase, creating a distinct pattern for the overall phenology of the root systems. Characteristic features for temperate and boreal trees are a burst of fine root growth in spring and early summer and significant mortality in late summer and autumn {Pregitzer et al. 2000; Joslin et al. 2001; see also McCormack et al. 2014}. This seasonal pattern of fine root production and mortality is strikingly similar among deciduous and evergreen tree species growing under the same climate {Quan et al. 2010}.

Influence of the environmental factors on the seasonal cycle of fine roots

Soil temperature has been shown to regulate fine root elongation in forest trees. Fine root elongation does not occur below a +2 to +4 °C threshold {Alvarez-Uria and Körner 2007; Schenker et al. 2014}, with species-specific optima between +20 and +30 °C {Lyr and Hoffmann 1967; Dougherty et al. 1994}. This temperature cue is likely dominant in the pattern of root formation, driving: (1) the spring – early-summer burst; and (2) the autumn strong winding down or cessation, irrespective of the tree functional group or leaf phenology {Teskey and Hinckley 1981; Kuhns et al. 1985; Hendrick and Pregitzer 1992; Burke and Raynal 1994; Puhe 2003; Konôpka et al. 2005; McCormack et al. 2014}. A few studies have suggested that fine root growth can continue at a minimal rate during winter in evergreen trees {Dougherty et al. 1994; Puhe 2003}, a feature that is clearly not systematic {Noguchi et al. 2005; Quan et al. 2010; McCormack et al. 2014}. Fine root growth may also be reduced {Joslin et al. 2001} or interrupted under drought conditions {Teskey and Hinckley 1981; Kuhns et al. 1985; Konôpka et al. 2005}, as defined by a species-specific soil water potential threshold {Joslin et al. 2001}.

In addition to interspecific differences {Leuschner et al. 2001} and methodological aspects {Gaul et al. 2008}, different sensitivities of fine root growth and mortality to soil temperature and water deficit likely explain the variations among the responses to drought observed in field studies, which have reported both increases {Joslin and Wolfe 1998; Leuschner et al. 2001} and decreases {Konôpka et al. 2005} in living root biomass.

Interestingly, overwinter mortality is minimal, resulting in the maintenance of a substantial fine root stock during winter {Hendrick and Pregitzer 1992; Konôpka et al. 2005}.

Phenology of wood formation in higher-order roots

The secondary growth of higher-order roots has seldom been studied {Chaffey 2013}, but is subjected to the same cascade of phenological events as stems or branch wood. The timing, however, differs and generally follows an extended calendar, with an earlier timing of onset and later cessation of the differentiation period compared to stem wood {Thibeault-Martel et al. 2008}.

4.4.4 | The seasonal cycles of carbon and nitrogen reserves

Carbon and nitrogen reserves in trees

Carbon (C) and nitrogen (N) reserves are key actors of the seasonal variability of temperate and boreal tree functioning. Carbon reserves are primarily formed from non-structural compounds, including starch and soluble sugars, as well as lipids in a number of species {Hoch 2015}. Nitrogen reserves are composed of non-structural compounds, including the so-called vegetative storage proteins, amino acids {Staswick 1994}, and a range of metabolically active proteins, such as Rubisco {Millard et al. 2007}.

C and N reserves are distributed throughout the tree. A gradient from low concentrations of reserve compounds in the stem to higher concentrations in distal organs (e.g., branches, leaves, coarse roots) is partially counterbalanced by the size of the compartments {Bazot et al. 2013}. Hence, the stem remains a quantitatively

important store of C {Barbaroux et al. 2003} and N reserves {Valenzuela Nuñez et al. 2010}.
In this section, we focus on the seasonal variations of C and N reserves, acknowledging the existence of diurnal variations in transient reserves forms of C (e.g., starch) in leaves {Zeeman et al. 2007} and N (mainly amino-acids) in roots {Geßler et al. 2002}. Traditionally, the storage of C and N reserves has been viewed as a passive process according to which C and N are allocated to reserve stores after other demands (e.g., organ growth, maintenance respiration) have been met. Consequently, the seasonal dynamics of C and N reserves have been primarily described according to the phenology of tree organs. The timing of resource acquisition obviously varies with tree functional type, imposing notable differences on the seasonal dynamics of reserves.

The seasonal cycle of C and N reserves in evergreen tree species

The seasonal dynamics of C reserves show that starch concentration peaks prior to budburst {Fischer and Höll 1991; Fischer and Höll 1992; Hoch et al. 2003}, with only slight variations in C storage throughout the growing season, regardless of the considered form (including lipids) {Hoch et al. 2003}. In contrast to deciduous trees, carbohydrate stores remain relatively constant in evergreen species throughout winter {Fischer and Höll 1991, 1992}, whereas lipids may accumulate {Sudachkova et al. 2004}. Indeed, conifers can perform photosynthesis during winter when the air temperature is above −2 to −3°C {Bergh et al. 1998}. Therefore, sprouting buds are supplied with C originating from the previous cohorts of needle {Ursino et al. 1968}.
A remobilization of N from older shoots {Salifu and Timmer 2003} or the needles from the previous year {Millard and Proe 1992} provides the N necessary for the growth of newly formed tissues. Fluctuations of N reserves in evergreen species have not been well studied for the rest of the year but appear to be less variable than those of C reserves {Millard and Grelet 2010}.

The seasonal cycle of C and N reserves in deciduous tree species

The growth of new organs (e.g., buds, leaves, wood, fine roots) induce a massive, short-distance mobilization of reserves in deciduous trees {Bazot et al. 2013}. This mobilization typically results in a decrease in concentrations of C reserves in the majority of tree organs {Barbaroux et al. 2003}. New leaves and photosynthetic twigs can act as a C source a few days after budburst, allowing a rapid increase in C reserves {Damesin and Lelarge 2003}, which culminate at leaf senescence {Barbaroux and Bréda 2002}. The amount of C reserves typically decreases during winter as a result of fueling maintenance processes in perennial organs {Barbaroux and Bréda 2002}.
The seasonal pattern of N reserves is less continuous than that of C reserves in deciduous trees. After the spring mobilization of N reserves to grow leaves {Millard and Grelet 2010; El Zein et al. 2011; Bazot et al. 2013}, N reserves remain at low levels throughout the leafy season {Bazot et al. 2013}, likely reflecting the balance between N requirements (for growth and maintenance) and supplies from the current root absorption. N storage mainly take place in autumn since it is strongly related to the reabsorption of nutrients occurring during the leaf senescence

processes. We have less informations on what is happening during winter, but the level of variation of N reserves seems to be reduced compared to C reserves {Gomez and Faurobert 2002; Valenzuela Nuñez et al. 2011; El Zein et al. 2011; Gilson et al. 2014}.

Concluding remarks

The extent to which the formation of C reserves is an active process is a current matter of discussion {e.g., Hoch 2015; Palacio et al. 2014; Dietze et al. 2014}. There is evidence that even under severe stress conditions, C reserves are never fully depleted {Gruber et al. 2012; Hartmann et al. 2013; Klein et al. 2014} and that reserve storage may, in certain cases, be favored at the expense of growth {Wiley et al. 2013; Saffell et al. 2014}. Recent studies have demonstrated that, within a given growing season, the formation of C reserves occurs earlier in mature trees compared to young trees in experiments conducted under the same pedoclimatic conditions and leaf phenology {Gilson et al. 2014}. The extent to which this pattern results from a differential regulation of active C storage in mature trees versus young trees or is simply the result of differences in the seasonal C balance has not yet been determined (e.g., through modeling). Identifying the nature and influence of the internal and environmental factors that drive reserve storage processes is an open field for future research. Seasonal reserve storage is likely to be highly regulated (biochemically and genetically), but the mechanisms involved remain to be elucidated.

4.4.5 | The phenology of cambium, phloem and xylem

Wood formation phenology overview

The resting cambium over winter time

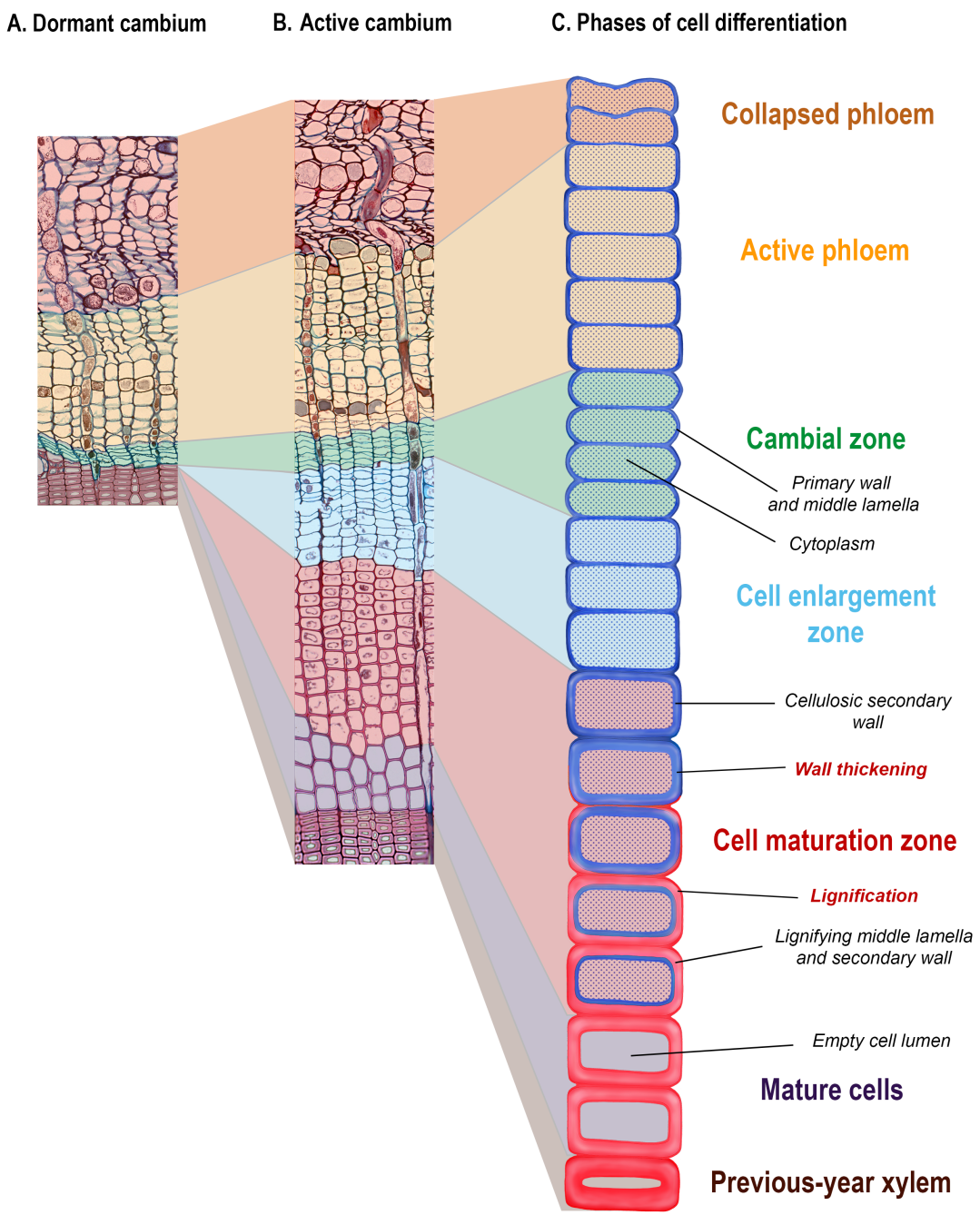
Cambial activity follows the cycle of the seasons. In extra-tropical regions, the cambium is dormant during winter and active during summer, while in tropical regions it may rest during the dry season and be active during the wet season {Denne and Dodd 1981}. The inactive cambium is composed of a few layers of dormant cells (c. 3-6) [Figure 4.12A], while the active cambium is composed of numerous dividing cells (c. 6-18) {Prislan et al. 2013}.

The reactivation of the cambium in spring time

At the beginning of the growing season in spring, cambium is reactivated and start dividing to produce new phloem cells first and new xylem cells second [Figure 4.12B]. Generally, a few over-wintering phloem cells also start to enlarge at about the same time marking the beginning of stem radial growth and phloem formation {Prislan et al. 2019}. A couple of days or weeks after the start of cambial cell divisions, newly created phloem and xylem cells begin radial enlargement, becoming thus part of the developing phloem and xylem, respectively. The appearance of the first enlarging xylem cells marks the onset of xylem radial growth and wood formation {Wilson 1970}.

A couple of weeks after the start of their enlargement, these first xylem cells enter the thickening phase where they build their secondary walls. Because secondary walls hold most of the biomass, the appearance of the first thickening cells can be

seen as the effective beginning of carbon sequestration into wood {Cuny et al. 2015}.
Finally, a couple of months after their birth, xylem cells undergo programmed cell death and reach their final mature and functional state.



25 — Figure 4.12 | Transverse sections showing xylem, phloem and the cambial zone at dormancy (A) and during the active period (B), along with a schematic view of a developing radial file (C).
Different background colours indicate main zones of xylem differentiation and mature xylem, functional and collapsed phloem, and the cambial zone. Figure from Rathgeber et al. {in preparation b}, prepared by G. Perez-de-Lis.

The structure of the developing xylem over summer time

During the growing season, new xylem cells, resulting from cambial cell division, are disposed along radial files, and successively undergo their differentiation program according to their identity and their place in the radial file [Figure 4.12C]. At the tissue level, the succession in time of cells belonging to the different stages of differentiation is well coordinated between all the radial files, creating a characteristic spatial pattern composed of strip-like developmental zones. Once established, this organization remains rather stable throughout the growing season {Vaganov et al., 2006}. Provided that no environmental stress comes into play, cambial activity and xylem radial growth rate generally peak around the summer solstice, when the photoperiod is maximal {Rossi et al. 2016}. This period also generally marks the transition between early- and late-phloem and wood {Cuny et al., 2014; Prislan et al. 2019}.

The cessation of cambial activity and phloem and xylem formation in Autumn

At the end of the growing season, in autumn —or even earlier if water or temperature conditions are not favorable anymore— cambial activity stops, soon followed by cell enlargement cessation (flagging the end of stem radial growth). However, the completion of wood formation (marking the end of carbon sequestration) occurs a couple of months later {Cuny et al., 2015}. Indeed, lignification is a slow process, so the last xylem cells need up to two months for ending cell wall maturation and reaching maturity {Cuny & Rathgeber, 2016}.

Synthesis

The key phenological events in the process of wood formation are the beginning and end of cambial cell division or xylem cell expansion (both are used as proxies for the onset and cessation of cambial activity), the beginning of secondary cell-wall deposition, and the end of cell-wall lignification (used as a proxy for the cessation of wood formation) {Rathgeber et al. 2011a}. Recently, it has been demonstrated that this sequence of events is structurally fixed {Lupi et al. 2010; Rossi et al. 2012} and that the delays between the beginning and the end of successive wood phenophases are highly conserved among temperate and boreal tree species {Rossi et al. 2013}. Considering that the xylem cells produced by cambial division must undergo long differentiation processes, an earlier onset or a higher rate of cambial activity, presumably associated with a higher number of cells produced along the growing season, would result in a later cessation of wood formation {Rathgeber et al. 2011b; Rossi et al. 2012; Kalliokoski et al. 2013}.

Influence of environmental factors

Despite these strong structural constraints, environmental factors are also known to have significant influences on the timing of wood formation {Denne and Dodd 1981}. Both external and internal factors control xylogenesis {Wilson 1984; Aloni 2013} and are likely involved in the regulation of its timing {Lachaud et al. 1999}. Among these factors, temperature is expected to be a major cue for the phenology of xylogenesis, considering its influence on cell structure {Begum et al. 2012} and on basic processes, such as the modulation of the division rate of cambium initials

{Begum et al. 2013} and the regulation of gene expression related to active auxin transport {Schrader et al. 2003}.
To date, the majority of research efforts on the phenology of wood formation have been conducted in conifers, mainly in high-altitude or northern locations. There are comparatively fewer data on deciduous angiosperms in the recent literature, and among them, the majority relate to diffuse-porous (as opposed to ring-porous) species. The results of these studies indicate a primary role of temperature as an environmental cue for xylogenesis. In natural stands, the onset of cambial activity occurs within a relatively narrow range of daily minimum air temperature (+2 to +7°C in 80% of the data {Rossi et al. 2008}, resulting in altitudinal gradients of cambium resumption {Moser et al. 2010; Oladi et al. 2010; Prislan et al. 2013}.
Moreover, 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; Begum et al. 2013}, after the chilling requirement has been fulfilled during endodormancy {referred to as the "rest phase" in Little and Bonga 1974; Begum et al. 2013}. However, the influence of temperature on cambium activity remains equivocal. Indeed, the cessation of the division of cambial initials in late summer or autumn occurs at considerably milder temperatures than those for spring resumption {between +5°C and +13°C for gymnosperms, Rossi et al. 2008}, but cessation can be hastened by artificial cooling {Gričar et al. 2007}. This equivocal role of temperature results in the absence of clear altitudinal gradients in the timing of the cessation of cambial activity {Moser et al. 2010; Oladi et al. 2010; Prislan 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. Soil drought, for instance, has been suggested to hasten the cessation of cambial activity {Dünisch and Bauch 1994; Gričar and Čufar 2008}, though this result is not systematically observed {Gruber et al. 2010}. Day length, which likely modulates the rate of cambial division {Rossi et al. 2006; Cuny et al. 2012}, may also act as a cue for the cessation of cambial activity, but this hypothesis remains to be tested.
Finally, the ending of xylem differentiation, which marks the end of annual wood formation, occurs at similar temperatures to the prevailing temperatures during the onset of cambium cell division {Rossi et al. 2008}. The ending of xylem differentiation generally occurs earlier at higher altitudes {Moser et al. 2010} and can be hastened by drought {Gruber et al. 2010}.

4.5 | Tree-ring structure and leaf traits determine the coordination between tree organ phenology

4.5.1 | Introduction

In the previous sections we saw how the synchronization of the activities of organs is vital and shapes the seasonal variability in the assimilation and use of resources (e.g., growth, maintenance, reproduction, storage) at the tree scale. We also saw that the 'phenologies' of the different organs of a tree are sensitive to

environmental factors and are related together through hormonal regulations (e.g., auxin concentration), physiological constraints (e.g., water potential, carbon availability), and functional links (e.g., water transport, photo-assimilates distribution and storage). In this section, we would like to go one step further and show how the tight coordination between the 'phenologies' of the different organs of a tree species is related to its anatomical tree-ring structure and leaf traits, creating a coherent life strategy that allows this species to thrive in its environment.

Clear evidences of distinct sequences in the phenologies of wood and leaves have been reported in deciduous angiosperms {Lachaud and Bonnemain 1981; Suzuki et al. 1996}. These references document distinct patterns among tree functional types with regard to the relative onsets of cambium division and leaf appearance.

In this section, we review studies combining the monitoring of leaf and xylem phenology in order to shed light on the role played by leaf traits and tree-ring structure on the coordination between primary and secondary growth. We also investigate the interactions between C sources and sinks at the tree level. For this purpose, we gather species exhibiting similar leaf traits (evergreen or deciduous) and tree-ring structure (gymnosperm soft-wood, or angiosperm diffuse or ring porous hard-wood) together to form distinct plant functional types.

The objective of this section is to use the current knowledge on tree phenology to: (1) test the pertinence of the different plant functional types (PFTs) according to their respective calendars of wood and leaf critical dates; (2) elucidate the extent to which tree phenological responses to environmental changes are linked to the PFTs; and (3) discuss how the PFT approach can be used to improve tree, and specially wood, phenology models.

4.5.2 | Short presentation of material and methods

Despite tree phenology (both for primary and secondary growth) has become a prolific research field in the last decades, only a small portion have integrated phenological events simultaneously occurring in different organs of a tree, such as leaves and wood. Here, we focused on about 30 studies dealing with the links between leaf and wood phenology, given that relationships with other tree organs (e.g., phloem, roots, reproductive tissus) have been even more rarely addressed {Delpierre et al. 2015}. Our meta-analysis cover both deciduous and evergreen species, as well as the three main tree-ring structure (diffuse porous, ring porous and conifer). However, not all the possible combinations were equally represented, and almost no studies were found for evergreen or semi-deciduous angiosperms (but see Dié et al. {2012} for a study on an evergreen ring-porous hardwood species: the teak (*Tectona grandis)*}. Because of the higher number of available studies, our knowledge on deciduous angiosperms in temperate regions and evergreen conifers in boreal regions are clearly prevalent with respect to the other cases. Interestingly, there are almost no studies from the Mediterranean and Tropical areas, even if several studies were carried out along the Temperate-Mediterranean ecotone.

4.5.3 | Results and discussion

Evergreen gymnosperms: the case study of Scots pine in continental temperate forests

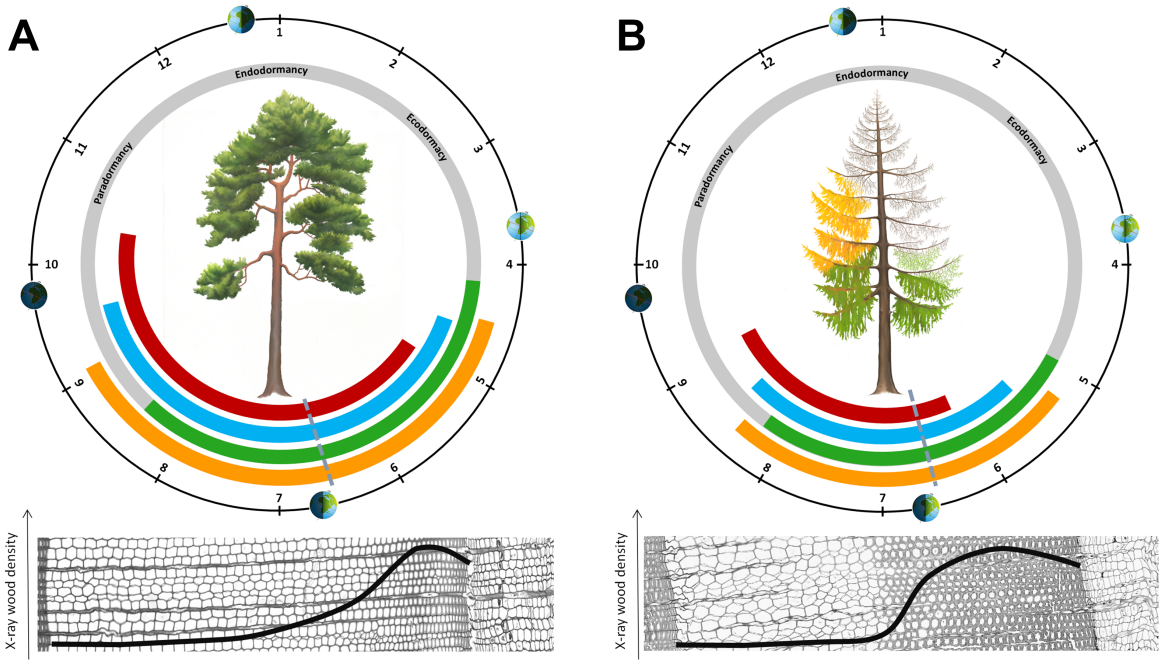
Evergreen soft-wood gymnosperms, hereafter called EG-SWs —and represented here by Scots pine (*Pinus sylvestris*.) in continental temperate forests— is a plant functional type (PFT) gathering numerous pioneer trees species, which are able to grow under very contrasted climates: from Mediterranean to Boreal regions, and from lowland to sub-alpine forests.

During winter, EG-SWs still bear several cohorts of needles from the previous years [Fig. 4.13A]. Their xylem and phloem, which exhibit strong heterogeneity between early- and late-part are generally frozen in winter because of low temperatures. While the living phloem cells are functional for only one year, the dead xylem cells, on the other hand, remains functional for several years.

In spring, EG-SWs start cambial activity early, producing new phloem cells first and new xylem cells second, using fresh photo-assimilates produced by old needles from previous years. Budburts and needle unfolding occurs a couple of weeks later, at about the same time as shoot elongation {Rossi et al. 2009; Gruber et al. 2010; Cuny et al. 2012; Michelot et al. 2012; Huang et al. 2014}.

During summer, the cambium produces phloem and xylem cells at a moderate rate using new assimilates from old and new leaves. The transition between the production of early- and late-wood cells occurs at about the same time than the termination of needle and shoot lengthening (ibidem).

At the beginning of the autumn, cambial activity stops producing phloem and xylem cells. Then leaves falls progressively and wood maturation stops (ibidem).



26 — Figure 4.13 | Aboveground tree phenology in temperate conifers.
On the top, the seasonal cycle of growth and dormancy is shown for (A) the evergreen Scots pine (*Pinus sylvestris*) and (B) the deciduous European larch (*Larix decidua*). The phenology of secondary growth is represented by cambial activity (green), xylem cell enlargement (blue), xylem cell thickening (red) and phloem formation (orange). In the

bottom, a characteristic X-ray microdensity profile for each species is shown over their respective tree-ring anatomical structure. From Perez-de-Lis et al. {in preparation}.

Deciduous gymnosperms: the case study of larch in sub-alpine forests

Deciduous soft-wood gymnosperms, hereafter called DS-SWs —here represented by larch (*Larix decider*) in the subalpine forests— is a PFT gathering few trees species growing under very cold and often also dry climate (e.g., sub-alpine zones, cold continental climates).

During winter DS-SWs are needless [Fig. 4.13B]. Their xylem and phloem, which exhibit strong heterogeneity between early- and late-part are generally frozen in winter because of very low temperatures. While the phloem cells are functional for only one growing season, the xylem cells, on the other hand, remains functional for several years.

In spring, DS-SWs start cambial activity early, starting producing new early-phloem cells first and new early-xylem cells second, using NSC reserves stored the previous years. Needle unfolding occurs rapidly afterwards, while shoot elongation starts a couple of weeks later {Rossi et al. 2009}.

During summer, the cambium keep producing phloem and xylem cells using fresh assimilates from new leaves, but switch from early- to late-phloem and xylem cells. The change from early- to late-wood is associated by several authors with the end of needle growth {e.g., Larson et al. 1969; Rossi et al. 2007}. One possible explanation could be that, after needles stop to grow, the carbon provided to them could be redirected to the cambium for xylem and phloem production (ibidem). This hypothesis however is based on the common misconception that late-wood production uses more carbon than early-wood production {but see Cuny et al. 2015}. A more convincing hypothesis could be that the expansion of the differentiating xylem and phloem cells is much less stimulated after needle growth stops, because mature needles produce much less auxins that the growing ones {Dufour et al. 2010}.

At the beginning of the autumn, cambial activity stops producing phloem and xylem cells. Then leaves falls progressively and wood maturation stops {Moser et al. 2010}.

Deciduous ring-porous Angiosperms: the case study of pedunculate oak in 'dry' oceanic forests

Deciduous hard-wood ring-porous Angiosperms hereafter called DS-RP —here represented by pedunculate oak (*Quercus robur*) in lowland oceanic forests— is a PFT gathering numerous trees species growing under mild temperate climate (e.g., mild oceanic or continental climate in lowland areas).

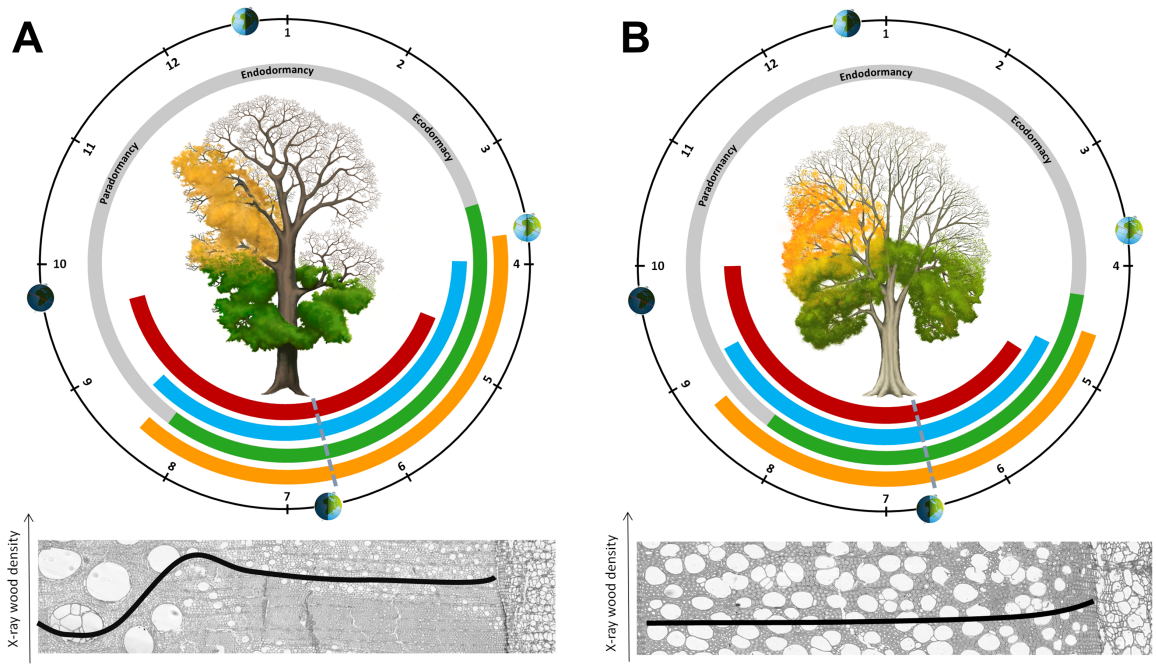
During winter DS-RP are leafless [Fig. 4.14A]. DS-RP species are the most vulnerable to freezing-induced embolism {Cochard and Tyree 1990}. Their xylem (and phloem) exhibits a strong heterogeneity with large vessels in the early-wood and small vessels and fibers in the late-wood. Even if the climatic conditions are not too harsh during winter, early-wood large vessels are prone to freeze. Once frozen, large vessels cannot be repaired, so they are functional for only one growing season, as phloem. In fact large vessels, may even be functional for only the first part of the growing season if a dry spell occurs during summer for example,

because they are also prone to cavitation under water stress. On the other hand, it seems that small vessels and fibers may escape freezing or can be repaired, and thus can be functional for a couple of years.

In spring, DS-RP resume xylogenesis first. Large super-efficient early-wood vessels are rapidly grown using NSC reserves from previous years to be ready to supply raw sap to new leaves. Bud-burst, leaves unfolding and shoot elongation can then follow {Michelot et al. 2012; Pérez-de-Lis et al. 2016; Fernández-de-Uña et al. 2017}. In certain ring-porous species (e.g., deciduous oaks), the onset of cambial division is the first step of spring resumption {Gričar 2010; González-González et al. 2013}, whereas in others (e.g., *Fraxinus excelsior*), overwintering vessels start to enlarge before budburst {Sass- Klaassen et al. 2011} but cambial division resume after budburst {Frankenstein et al. 2005}.

During summer, the cambium produces phloem and xylem cells using fresh assimilates from new leaves, but switch from early- to late-phloem and xylem cells. If a dry spell occurs during summer, large vessels are not able to sustain the water potential decrease and cavitate. Those air-blocked vessels are then filled with thylosis {Pérez-de-Lis et al. 2016}.

At the beginning of the autumn, cambial activity stops producing phloem and xylem cells. Then leaves falls progressively and wood maturation stops {Gričar et al. 2017; Lavrič et al. 2017}.



27 — Figure 4.14 | Aboveground tree phenology in temperate broadleaved trees.

On the top, the seasonal cycle of growth and dormancy is shown for (A) the ring-porous Pedunculate oak (*Quercus robur*) and (B) the diffuse porous European beech (*Fagus sylvatica*). The phenology of secondary growth is represented by cambial activity (green), xylem cell enlargement (blue), xylem cell thickening (red) and phloem formation (orange). In the bottom, a characteristic X-ray microdensity profile for each species is shown over their respective tree-ring anatomical structure. From Perez-de-Lis et al. {in preparation}.

Deciduous diffuse-porous Angiosperms: the case study of European beech in humid continental forests

Deciduous hard-wood diffuse-porous Angiosperms hereafter called DS-DPs —here represented by European beech (*Fagus sylvatica*) in continental low elevation forests— is a PFT, gathering numerous tree species growing under cool and humid temperate climate (e.g., mild oceanic or continental climate at low elevations).
During winter DS-DPs are leafless [Fig. 4.14B]. Their xylem structure are quite homogeneous with no clear change between early-and late-wood, but small vessels all along the ring. Either because the climatic conditions they face during winter are not too cold or because they are able to repair them, diffuse porous species vessels usually remain functional for several years. Phloem, however is still functional only one year. However, late-phloem from the previous year is stil functional at the beginning of the growing season.
In spring, DS-DPs resume cambial activity, starting producing new phloem cells first and new xylem cells second (and also expanding over-wintering sieve tubes), at about the same time or just few days after bud burst {Suzuki et al. 1996; Čufar et al. 2008; Michelot et al. 2012; Prislan et al. 2013, 2019} — though delays of several weeks have been observed in some species: e.g., 8 weeks in *Carpinus betulus* {Delaporte 2010}. DS-DPs grow the rapidly numerous small vessels and fibers using mainly recent assimilates from young leaves {Michelot et al. 2012; Prislan et al. 2013}.
During summer, the cambium produces phloem and xylem cells at a very high rate using new assimilates from mature leaves {Michelot et al. 2012; Prislan et al. 2013}.
At the beginning of the autumn, cambial activity stops producing phloem and xylem cells. Then leaves falls down progressively and wood maturation stops. An interesting new theory (Campioli pers. com.) postulates that, if at the beginning of the growing season growing leaves are controlling wood formation phenology, it is the other round at the end of the growing season: leaf senescence being under the control of wood maturation. The mechanism behind this control, however, remains to be found. During summer time or autumn, if the climatic conditions deteriorate too much, vessels may be blocked by thylosis, but some of them can be repaired afterwards. The width of the sapwood area (the wood which is able to conduct water) is highly variable in DS-DPs going from 10 to 100 rings for example.

4.5.4 | Regulation of the coordination of organ 'phenologies' at tree scale

The internal factors involved in the phenology of the organs are numerous and have been well studied, though large gaps in knowledge remain. Phytohormones and sugars are among the prominent internal factors that regulate the phenology of organs {e.g., Horvath et al. 2003}. The phytohormones produced in a given organ can regulate the phenology of distant organs. For instance, during the paradormancy of buds (from bud-set to endodormancy), bud development is likely inhibited by the auxins produced in neighboring leaves {Horvath et al. 2003}. Similarly, auxins produced in expanding buds influence the rate of cambial divisions in the stem {Little and Bonga 1974; Lachaud et al. 1999}. These examples

indicate that the phenology of a given organ may, to a certain extent, be influenced by processes in distant organs and raise the question of the autonomy of an organ's phenology.

Differences in the delays between the spring phenophases of wood and leaves are also observed from year to year {Takahashi et al. 2013} and among individuals within species. From this ensemble of results, it is difficult to infer the influence of auxin produced in swelling or elongating buds on the timing of the onset of cambium division. The presence of auxins in overwintering tissues may decouple the onset of cambium division from the timing of bud elongation, as observed from stem heating experiments {Oribe et al. 2003; Begum et al. 2010}. However, it is likely that the auxins produced in elongating buds are necessary for sustaining a high division rate in the newly activated cambium {Dufour and Morin 2010}. Temperature is a major cue for all of these processes because it controls both the break from the period of ecodormancy in buds (hence, the production of auxins) and the sensitivity of cambium to auxins {e.g., Lachaud et al. 1999; Fonti et al. 2007; Begum et al. 2013}. The particular example of the timing of leaf and wood phenology in spring demonstrates that the influence of distance-signaling varies among species and, most importantly, is itself modulated by environmental conditions.

Further research is clearly required to evaluate the relative influence of environmental and internal controls in determining the phenologies of individual organs. Future investigations should, for instance, clarify the respective roles of environmental and internal controls on the timing of fine root elongation, which is typically observed to start before budburst {Lyr and Hoffmann 1967; Teskey and Hinckley 1981; Kuhns et al. 1985; Burke and Raynal 1994} in deciduous species and may lag several months (1 to 3) ahead of budburst in conifers {Puhe 2003; Konôpka et al. 2005}. Further research is also required to clarify the putative role of distance-signaling molecules originating from roots in affecting the timing of budburst {Greer et al. 2005; De Barba et al. 2015} because recent results indicate that budburst can occur simultaneously in both a donor tree and its twig cuttings that have already been separated from the root system {Vitasse and Basler 2014}.

4.5.5 | Conclusion and perspectives

In this section, we showed how the tight coordination between the 'phenologies' of the different organs of a tree species is related to its anatomical tree-ring structure and leaf traits, creating a coherent life strategy that allows this species to meet its developmental, physiological and environmental requirements and thrive in its environment.

Because tree-ring structure and leaf traits are well-known and easy to observe, while tree phenology is still not well-know and labor intensive (think about wood but also root phenology), understanding the functional links between phenology and leaf and wood structure will allow us to apply the knowledge we hardly learn on some particular species to other species of the same plant functional type. This approach could be used to improve tree phenology representation in dynamic global vegetation models, which in turn can be used to better understand the relationships between soil, forest and atmosphere in terms of heat, water, carbon or even Nitrogen fluxes.

4.6 | Modelling tree-ring formation phenology

4.6.1 | Introduction

The seasonality of physiological processes is an essential component of the dynamic global vegetation models (DGVM) {Delpierre et al., 2012; Kramer, 1995}, but is usually poorly represented being mostly confined to the simulation of leaf onset and leaf loss {Delpierre, Vitasse, et al., 2016}. In such models, the phenology of non-leaf organs or tissues (e.g., wood) is simulated according to leaf phenology or using generic, non-organ-specific temperature functions for modulating the allocation of carbon {Delpierre, Vitasse, et al., 2016; but see Schiestl-Aalto et al. 2015}. This reflects the state of our knowledge on the phenology of trees, which is far more developed for leaves as compared with other organs or tissues {Delpierre, Vitasse, et al., 2016; Ford, Harrington, Bansal, Gould, & St. Clair, 2016}.

It is difficult to quantify how strongly this knowledge gap affects the predictive ability of DGVMs, but it certainly jeopardizes their biological realism {Guillemot et al., 2017; Friend et al. 2019}. For example, it has been demonstrated in evergreen conifers that the spring resumption of cambium activity generally occurs before budburst {Cuny, Rathgeber, Lebourgeois, Fortin, & Fournier, 2012; Gruber, Strobl, Veit, & Oberhuber, 2010; Huang, Deslauriers, & Rossi, 2014; Michelot, Simard, Rathgeber, Dufrêne, & Damesin, 2012; Rossi et al., 2009}. Moreover, several studies have shown that, independent from leaf phenology, the duration of the wood growing season per se is a major determinant of wood production {Delpierre, Berveiller, Granda, & Dufrêne, 2016; Lempereur et al., 2015}, so that an earlier onset of cambium activity or a later cessation may result in a higher cell production {Lupi, Morin, Deslau- riers, & Rossi, 2010; Mäkinen, Jyske, & Nöjd, 2018}. Consequently, there is a clear need for the development of wood phenology modules for inclusion into DGVMs.

In order to develop wood phenology modules for DGVMs, we first have to understand the causal climatic drivers of wood phenology. In the temperate and boreal regions of the Northern Hemisphere, the formation of wood is seasonal and occurs from late spring to early autumn {Rossi et al., 2016, 2008}. In spring, cambial mother cells start dividing, producing new derivatives of phloem outward and xylem inward {Rathgeber et al. 2016}. As a base model for this cycle, several authors have proposed that, just as for buds, the spring resumption of cambium activity is the outcome of a three-phase dormancy period {Begum et al., 2018; Begum, Nakaba, Yamagishi, Oribe, & Funada, 2013; Ford et al., 2016; Little & Bonga, 1974; Rensing & Samuels, 2004}. According to this model, cambium activity is prevented by tree's internal factors (e.g., physiological state, developmental signals) during the paradormancy phase. During endodormancy phase cambium is still resting but start to record environmental signal. And finally during the ecodormancy phase the cambium is waiting for external conditions to become favorable so it can resumes activity.

The main candidate as external factor driving the resumption of cambium activity in temperate and boreal ecosystems is the spring temperature as demonstrated in the previous section and reviewed in Begum et al., {2018} and Delpierre, Vitasse, et al., {2016}. Based on these evidences, previous studies have developed different model formulations based on spring temperature to predict the timing of cambial resumption.

A first class of models uses a temperature threshold for predicting the onset of cambial activity in conifers from cold biomes {Deslauriers, Rossi, Anfodillo, & Saracino, 2008; Rossi et al., 2007, 2008, 2011}. Although these threshold models are able to correctly identify likely periods of cambial activity, they predictive power to determine the beginning or the end of the growing season is low {for detailed analysis see Delpierre et al. 2019}. A recent simplification of this statistical approach made use of empirical models (linear regression using spring-averaged temperature) to predict the timing of cambial resumption of boreal and temperate conifer trees {Rossi et al., 2016}.

Another model class of models uses heat-sums for predicting the onset of cambial activity in conifers, also from cold biomes {Giagli, Gricar, Vavrcik, & Gryc, 2016; Schmitt, Jalkanen, & Eck- stein, 2004; Seo, Eckstein, Jalkanen, Rickebusch, & Schmitt, 2008; Swidrak, Gruber, Kofler, & Oberhuber, 2011}. The underlying hypothesis is that the cambium resumes its activity after a sufficient exposure to heat. Thus, heat-sum models mimic the progress of cambium through the ecodormancy phase, making the implicit hypothesis that the endo- and eco-dormancy phases are strictly sequential, and that endodormancy stops at the date when heat accumulation starts {Delpierre, Vitasse, et al., 2016}.

However, there is no consensus concerning the day or period of year from which the cambium becomes sensitive to forcing temperatures. For practical reasons, some studies choose January 1 or spring equinox {Giagli et al., 2016; Schmitt et al., 2004}, whereas others start degree-days accumulation when trees have experienced a daily mean temperature above +5°C for at least five consecutive days {Seo et al., 2008}. Moreover, heat-sum models usually fail in identifying a species-specific heat-sum threshold above which cambium would systematically be active {Giagli et al., 2016; Moser et al., 2010}, which is indicative of their low biological realism and predictive ability.

More recently, chilling-influenced heat-sum models have been shown able to predict spring cambial reactivation in Douglas fir {Ford et al., 2016}. Similar to heat-sum models, those models were originally designed for describing the progress of primary meristems (e.g., leaf or flower buds) from dormancy to budburst. The basic hypothesis here is that the cambium requires a lower accumulation of forcing temperatures during the ecodormancy phase when it has been exposed to higher levels of cold temperatures (the so-called chilling temperatures) during the endodormancy phase {Cannell & Smith, 1983; Lit- tle & Bonga, 1974}. It has been proposed recently that the endodormancy phase may precede or be concomitant to the ecodormancy phase {Chuine, Garcia de Cortazar-Atauri, Kramer, & Hänninen, 2013}. The underlying physiological basis of such chilling-influenced heat-sum models is not fully understood {Rinne, Kaikuranta, & Van Der Schoot, 2001; Singh, Svystun, AlDahmash, Jönsson, & Bhalerao, 2017}.

Though previous studies evaluated the ability of the three above-mentioned model classes separately in simulating the date of the resumption of cambium activity in spring, there has been no comparison of those models merits on the same data set. In Delpierre et al. {2019}, we use of a large number of field observation data collected over Europe and Canada (using the GLOBOXYLO initiative database) to conduct for the first time a systematic evaluation of the causal factors affecting the breaking of cambial dormancy and to propose an improved model of cambial spring resumption. Specifically, by identifying which type of models receives most support

from the observed data, we aim to evaluate: (a) if the resumption of cambium activity of Northern Hemisphere conifers in spring is more likely caused by the crossing of a given temperature threshold or by an accumulation of heat ("do threshold models outperform heat-sum models?"); and (b) if observation data support the existence of a separate endodormancy phase that can be broken by chilling exposure ("do chilling-influenced heat-sum models fit the data better?"). Our hypotheses are that (a) threshold models are fine for identifying a thermal probability of cambium activity but have low predictive ability since the daily variability of temperature superimposed to the seasonal variations cannot serve as a reliable environmental cue for trees; (b) that over large geographical gradients, models incorporating both the effects of chilling and forcing temperatures are better able to describe the variability of the onset of tree-ring formation (since over large climate zones, multiple climate limitations interact).

4.6.2 | Summary of material, methods and results

Study sites

The selected study sites were extracted from the GLOBOXYLO initiative database, a data set gathering wood formation and meteorological information collected over the past 15 years from several research teams all over the world. The selected data concern the four most observed coniferous species: *Larix decidua* (LADE), *Pinus sylvestris* (PISY), *Picea abies* (PCAB) and *Picea mariana* (PCMA), covering a wide range of temperature and photoperiod conditions in the Northern Hemisphere (from 40.0°N to 67.5°N in latitude, from 79.2°W to 29.4°E in longitude, and from 30 m to 2,150 m in elevation). Specifically, the data set includes the beginning of wood formation (bE) from 2001 to 2013 over 46 study sites for a total of 220 site-years, representing 1,105 tree-site-year observations. All sampled trees were dominant individuals. The average (± SD) tree age was 124 ± 70 years, with a diameter at breast height (DBH) of 44 ± 30 cm and a tree height of 21 ± 8 m.

Temperature and photoperiod data

Mean daily temperatures have been collected at the study sites. However, local weather stations were usually not installed before the start of the wood formation monitoring. To be able to consider in our models, weather conditions also before the monitoring period, we used, for European sites, the WATCH gridded meteorological data set (grid-resolution =0.5°) {Weedon et al., 2014} to extrapolate those missing data, after establishing linear regression between the local and corresponding WATCH temperature data (correlation between overlapping local and WATCH temperature time series was $0.95 < r < 0.99$), and removing the (low) biases of WATCH data. For Canadian sites, that is, for *Picea mariana*, we did not extrapolate the temperature time series. Day length was calculated daily as a function of latitude, using astronomical formulae.

Overview of the phenological models we tested

We compared three types of ecophysiological models and one empirical model in their ability to predict the date of the onset of xylem cell enlargement period (bE) in the four species of interest. The three types of model are as follows: (a) threshold models, (b) heat-sum models, (3) chilling-influenced heat-sum models. Since the

patterns of xylem formation have been strongly related to mean temperatures over large geographical gradients {Rossi et al., 2016}, we used an empirical model relating bE to early season (January– June) average temperature as a benchmark for ecophysiological models.
For all ecophysiological models we tested, we used photoperiod thresholds to delineate the start and end of the endo- and eco-dormancy periods, different to earlier phenological modelling studies, which usually considered temperature accumulation to start at a given day of year (e.g., usually January 1 in most phenological studies considering heat-sum models) {Linkosalo, Carter, Hakkinen, & Hari, 2000; Seo et al., 2008}. This choice was motivated by the fact that our study covers a large latitudinal gradient over which a given calendar day (not perceptible by trees per se) may correspond to a large variation in photoperiod (an environmental signal which is perceptible by trees).

Temperature and photoperiod threshold models

In this class of models, we assumed that bE occurs when a given temperature and/or photoperiod threshold has/have been crossed. A first formulation of this model (henceforth referred to as Tt model) is as follows:
[Delpierre 2019 Equation 1]
where bE is the beginning of the xylem enlargement period (DoY), d is a day of year (DoY), T is the daily average temperature, and T* is a temperature threshold (°C). In this first sub-type of model (the temperature threshold models: TTMs), we assume that the temperature threshold cannot be exceeded before the winter solstice of the previous year (i.e., DoY 355 of the previous year, or DoY −10 of current year).
In the second sub-type of models: the temperature and photoperiod threshold models (TPTMs), we assume bE occurs when the thresholds of both temperature and photoperiod have been exceeded. The model writes:
[Delpierre 2019 Equation 2]
where P is the daily photoperiod (hours) and P* is a photoperiod threshold (hours).

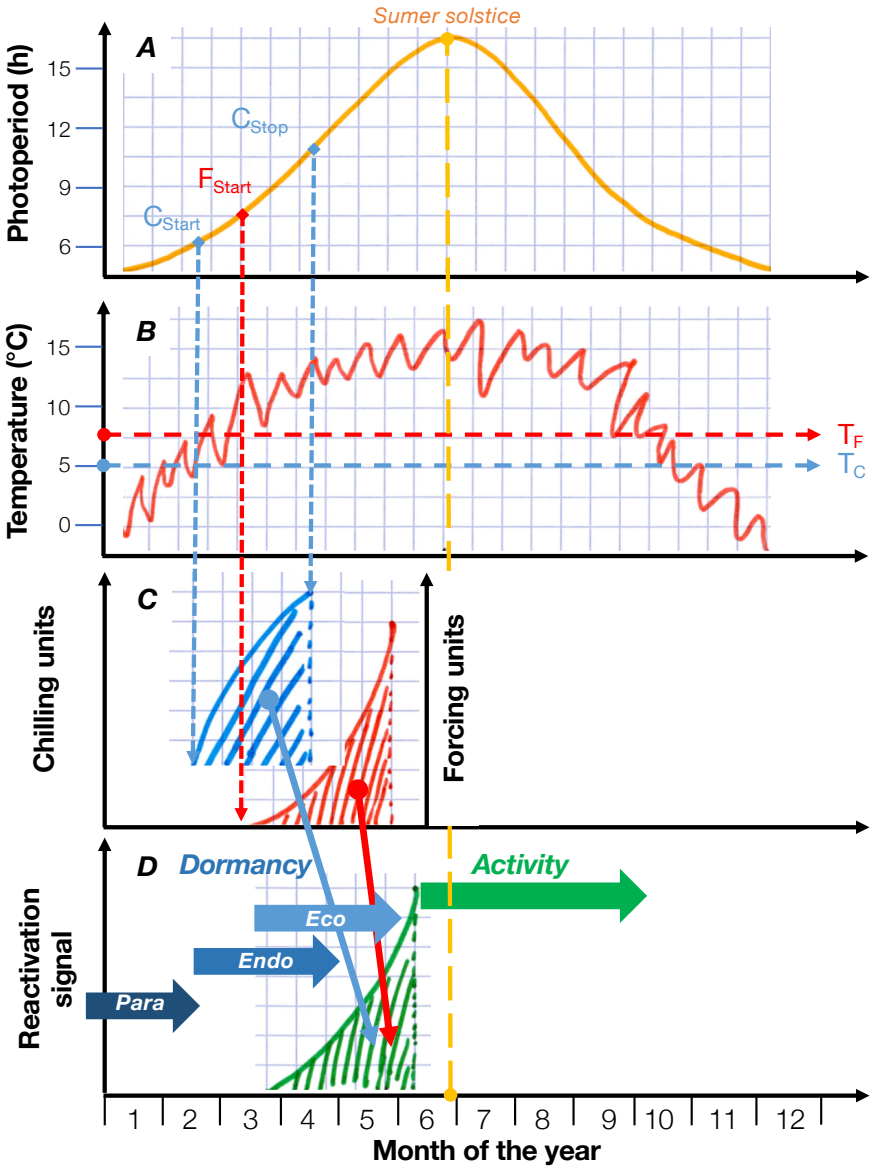
Heat-sum models

In the heat-sum models (HSMs), we assumed that bE occurs after a sufficient exposure of the cambium to heat. In practice, a degree-days accumulation is calculated by summing 'forcing temperatures' above a certain 'base temperature' threshold of typically + 5°C (or more, but rarely less) {but see Antonucci et al., 2015; Li et al., 2017}, from a given date fixed a priori, to the date of onset of cambial activity. The model writes:
[Delpierre 2019 Equation 3 & 4]
where Tbase is a temperature threshold above which forcing temperatures are accumulated, F(d) is the heat-sum at day d (degree-days) and F* is the forcing units requirement at which bE occurs (degree-days). In this model, the accumulation of forcing temperature starts at a given photoperiod threshold DLFstart (hours), occurring after the winter solstice of the previous year such that:
[Delpierre 2019 Equation 5]

This model simulates the progress of cambium through the ecodormancy period and makes the implicit hypothesis that the preceding endodormancy period ends at Fstart.

Chilling-influenced heat-sum models

In the chilling-influenced heat-sum models (CHSMs), the progress of cambium through the endo and ecodormancy phases is explicit, and bE occurs at the end of the ecodormancy phase [Fig. 4.15]. During endodormancy, cambium division is inhibited by tree internal factors, the effects of which are counteracted by low temperatures. Following the approach proposed by Cannell & Smith {1983} for bud meristems, this hypothesis translates into an accumulation of chilling temperatures, quantified as a number of chilling units (Ctot, in chill units C.U.). Ctot is calculated on a daily basis from Cstart (DoY), up to the Cstop date as follows:
[Delpierre 2019 Equation 6]
where the daily rate of chilling (Rc) can be calculated as a linear function of temperature:
[Delpierre 2019 Equation 7]
Besides the accumulation of chilling, the model assumes that the progression of the cambium towards bE during ecodormancy is favoured by the accumulation of forcing temperatures F(d), as described in Equations (3) and (4). The CHS model postulates that, as the accumulation of chilling proceeds, the requirement for forcing temperatures decreases, such that the critical sum of forcing F* is defined daily, and linearly depends on Ctot: [Delpierre 2019 Equation 8] where g is the slope of the relation between required forcing units and chilling accumulation (degree-days per C.U.), and h is the forcing units requirement in the absence of chilling (degree-days).
In this model, both the period of cambium sensitivity to chilling temperatures (delimited by days of year Cstart and Cstop, Equation [6]) and the start of forcing temperature accumulation (on day of year Fstart, Equation [4]) are parameterized as photoperiods (through parameters DLCstart, DLCend and DLFstart, respectively; see Equation (5) for the correspondence of, e.g., day of year Fstart with photope- riod DLFstart). We set the parameter bounds such that DLCstart (DLCend) cannot occur earlier than the autumn equinox (winter solstice) of previous year. Letting the model inference procedure free to find the most likely photoperiod limits for chilling and forcing accumulation within a large range (from autumn equinox of the pre- vious year up to summer solstice of the current year), our model may represent several temporal combinations of the chilling and forcing temperature accumulation functions, corresponding to different hypotheses of the interplay between the endo and ecodormancy phases (i.e., sequential or parallel) {see Chuine et al., 2013}.



28 — Figure 4.15 | Schematic representation of the chilling influenced heat sum model.
(A) Seasonal course of the photoperiod, C_{start} and C_{stop} indicate the beginning and end of the accumulation of chilling temperatures, F_{start} indicates the beginning of the accumulation of forcing temperature. (B) Seasonal course of the temperatures and T_f indicate the temperature threshold for the accumulation of chilling and forcing respectively. (C) Accumulation of chilling and forcing units over the calendar year. (D) Accumulation of the reactivation signal over the calendar year, along with the phenological state of the cambium (para-, endo-, and eco-dormancy, and activity).

Empirical relation with spring average temperature

This empirical model {analogous to Rossi et al., 2016} assumes that bE can be related to spring temperature via a linear regression.

4.6.3 | Results and discussion

Chilling influenced heat-sum models better fit the onset of wood formation data

The purpose of this study was to improve our understanding of the phenology of wood formation, and in particular to unravel the causal triggers for the spring onset of xylem growth in coniferous species. To this end, we evaluated the ability of three families of ecophysiological models and one empirical model to predict the start of the enlargement period of the xylem cells. Our results demonstrate that models based on temperature sums perform better than those based on temperature and photoperiod thresholds. Moreover, our results clearly support the chilling-influenced heat-sum model (CHSMs), explicitly considering the processes of chilling and forcing temperature accumulation, for the prediction of the spring onset of wood formation. Beside its high posterior probability compared to the other models, the CHSM also predicted the spring onset of xylem formation with good accuracy. Its RMSE on the validation data, averaging 7.7 days (Table 3), is close to the temporal resolution of micro-core sampling from the trees (i.e., 7 days), and similar to the typical prediction accuracy of budburst (i.e., primary meristems), when deployed over continental gradients {e.g., Basler, 2016}. The clear support for a CHSMs of spring xylem phenology is different from what is reported in budburst model comparisons. For the latter, heat-sums and chilling-influenced heat-sum models do not usually differ in their performances {Basler, 2016; Vitasse et al., 2011}.

Influences of forcing, chilling and photoperiod

The identification of the CHSMs as receiving most support from the inference procedure suggests that both forcing and chilling temperatures play a role in determining the spring resumption date of xylem formation. To our knowledge, there is no direct evidence in the literature of a modulation of the date of onset of xylem cell formation in trees exposed to various chilling temperatures during winter and/or spring. Stem heating experiments showed that an artificial resumption of cambial activity can be triggered during late winter, but not in early winter {Begum, Nakaba, Oribe, Kubo, & Funada, 2010}. This observation supports the existence of an endodormancy phase, during which the cambium activity is repressed by unknown tree internal factors {Delpierre, Vitasse, et al., 2016, but see Singh et al., 2017, for a review of dormancy processes in primary meristems}. However, it does not prove, nor does it quantify the role of chilling temperatures in hastening the reactivation of xylem formation in spring. Thus, there is a clear need for quantifying the actual role of chilling temperatures in modulating the spring resumption of xylem formation, in line with pioneer works regarding buds and seeds {see Sarvas, 1974, reviewed in Hänninen, 2016}, which have recently been actualized {e.g., Flynn & Wolkovich, 2018}.

We delineated the time periods for the accumulation of chilling or forcing temperatures with photoperiod limits, instead of day of year (DoY) as usually done in phenological modelling over large latitudinal gradients {see, e.g., Olsson & Jönsson, 2014; Basler, 2016}. If the use of DoY is perfectly sound in local studies (i.e., for which the relation between DoY and photoperiod is unequivocal), it is questionable in studies spanning continental scales since plants sense time from

variations in the photoperiodic signal. Across a latitudinal gradient, a given photoperiod is reached at different DoYs (except the 12-hr photoperiod occurring at spring equinox (March 20) across the entire gradient). This resulted in large differences in our southern vs. northern study sites as regards the timing of the chilling accumulation for PCAB and the duration of both chilling and forcing accumulation in PISY, two species spanning large latitudinal gradients in our data set. Whether such variations of the actual dates of cambium sensitivity to temperatures are realistic remains to be determined. This could experimentally be done by comparing the sensitivity of cambium to chilling in genetically identical plants placed in various photoperiod conditions, either in climate chambers (as done for budburst, e.g., Basler & Körner, {2014}) or in natural conditions (e.g., International Phenological Gardens, Chmielewski & Rötzer {2001}). In the CHSMs, the threshold temperatures promoting the progress of the ecodormancy phase (forcing temperature threshold, Tf, ranging from −2.9°C to +3.4°C) were comparable to values generally used in the modelling of budburst (typically 0°C or +5°C, Hänninen, {2016}), based on experimental results (from −5°C to +1°C in, {Heide 1993}). On the other hand, the values of threshold chilling temperatures (Tc) determined by the parameter inference procedure span a larger range (from −5.6°C to +6.1°C) and appear quite low in the cases of PCAB (−5.6°C) and LADE (−1.1°C) as compared to the values either determined experimentally in buds and seeds {for which Sarvas, 1974, reports −3°C as a lower limit for chilling effectiveness} or considered by expert judgement {0°C to +4.5°C in Coville, 1920; +2°C to +4°C for cambium in Little & Bonga, 1974} as effective for chilling.

From a larger perspective, the questions about the plausibility of parameter values we inferred are further linked with the range of environmental conditions in which the bE data were obtained. Indeed, inferring model parameters from data acquired from trees growing under natural conditions, inevitably exposed to multiple interacting environmental factors (think about the strong concurrent latitudinal, temperature and photoperiod gradient), is not equivalent to inferring them from a controlled experiment where the environmental conditions can be at least partially be disentangled {Verdier et al., 2014}, and their biological interpretability is necessarily less generic. However, we noticed that our species-specific parameterizations of the CHSMs were able to reproduce the locally observed between-species difference in bE at those sites where two species of interest co-occur, giving credit to the overall plausibility of the inferred parameters.

The mechanistic foundation of phenology models

In this study, we used model formulations initially developed for simulating the occurrence of budburst, assuming similar environmental controls of the phenology of primary and secondary meristems {Delpierre, Vitasse, et al., 2016}. However, even for budburst, those models lack an indisputable biological support {Clark, Salk, Melillo, & Mohan, 2014; Delpierre, Vitasse, et al., 2016}. New model formulations for the phenology of budburst appear in the literature from time to time, considering more complex interactions of chilling and forcing temperatures in interaction with photoperiod {e.g., Blümel & Chmielewski, 2012; Caffarra, Donnelly, & Chuine, 2011}. Similar to the necessary effort to calibrate and compare those continuous-state budburst models to continuous data (for instance by measuring the release of plasmodesmata closure by callose, which is an indicator

of bud endodormancy {Singh et al., 2017}), a biologically indisputable modelling of spring cambial activity will require the evaluation of those models with continuous seasonal markers of cambial cells activity (i.e., cytoplasmic changes in cambial cells such as presence and form of microtubules, vacuoles, lipid droplets, plastids and other cell organelles or metabolite content {Begum et al., 2012; Chaffey & Barlow, 2002; Prislan et al., 2013; Rensing & Samuels, 2004}.

Even if the CHSM has no clear mechanistic foundation, we remind that the exposure to chilling temperature promotes soluble sugars accumulation from starch conversion, especially sucrose (along with raffinose, stachyose and other metabolites {Sakai & Larcher, 1987; Strimbeck, Schaberg, Fossdal, Schröder, & Kjellsen, 2015}) that remain high until spring dehardening. Since cell production is limited by local sucrose availability {Deslauriers, Huang, Balducci, Beaulieu, & Rossi, 2016}, exposure to chilling temperatures may constitute a local pool of sucrose readily available for cell production when temperatures become favourable for mitosis and/or cell expansion. In case of low chilling, this local sucrose pool would be low, and carbon-fuelling for cell formation would rely more on the resumption of photosynthesis, which responds to forcing temperature accumulation {Mäkelä, Hari, Berninger, Hänninen, & Nikinmaa, 2004; Pelkonen & Hari, 1980}. This mechanistic hypothesis is coherent with the general behaviour of the CHSM (the required forcing accumulation decreases with increasing chilling exposure), and would explain why we infer in some species low temperature thresholds for chilling accumulation (−1.1°C in LADE, −5.6°C in PCAB). Indeed, the rate of starch to sugar conversion has been shown to be maximum at temperatures from −3°C to −5°C and continued down to −15°C in Salix sachalinensis twigs {Sakai, 1966}.

Influence of phytohormones

Moreover, the successful use of model structures designed and used to predict budburst to simulate the resumption of cambial spring activity raises the question of the coordination and interaction of the phenologies of tree organs {Delpierre, Vitasse, et al., 2016}. Phytohormones can play a significant role, with, for example, auxins produced in expanding buds influencing the rate of stem cambial divisions {see review of Sorce, Giovannelli, Sebastiani, & Anfodillo, 2013}. Yet, the important role of auxin is also interconnected with cytokinin in the vascular cambium. Although auxin peaks in the middle of cambium and cytokinin in the middle of phloem, the latter acts as a positive regulator of cell division in the vascular cambium by increasing the number of cambial cell {Immanen et al., 2016} because of its crucial role on the cell division cycle {Schaller, Street, & Kieber, 2014}. Thus, the resumption of xylem formation in spring is at least partially independent from auxin-producing buds, as clearly demonstrated in stem heating experiments (where xylem formation resumes artificially whereas buds remain dormant {Begum et al., 2010; Gričar et al., 2006; Oribe, Funada, & Kubo, 2003}, and from the observed earlier timing of enlargement of new xylem cells, as compared to bud elongation in the evergreen coniferous trees studied here {Antonucci et al., 2015; Cuny et al., 2012; Huang et al., 2014; Michelot et al., 2012}. The presence of auxins in overwintering tissues (Egierszdorff, 1981) and of a local pool of sucrose may decouple the onset of cambium division and xylem enlargement from the timing of bud elongation, as observed from stem heating experiments; along with the

presence of signal-transduction chains involving phytochromes (proteins acting as photoreceptors, i.e., able to sense modifications of the photoperiod) in the cambium {Petterle, Karlberg, & Bhalerao, 2013}, this suggests that the cambium may well respond to variations of environmental conditions independently from buds. This hypothesis is supported by interannual variability in the delays between the spring phenophases of wood and leaves in both gymnosperms {Cuny et al., 2012} and angiosperms {Takahashi, Okada, & Nobuchi, 2013}.

Unexplained variability

The chilling-influenced heat-sum model produced mostly unbiased results when the data were aggregated at the site-year or at the site scale, pointing to its overall accurate capacity to simulate the spring resumption of xylem formation in coniferous species. Yet, one of our bias-detection methods suggested that the model underestimated the range of tree individual and annual anomalies. Though our models rely on environmental (temperature and photoperiod) data collected at the tree population scale, we conducted the parameter inference with the most basic level of information available (i.e., at the individual tree level). It is clear that part of the model bias that is detected at the individual scale is related to the model structural incapacity to simulate the variety of individual tree responses to the same environmental conditions that are observed in a population {Delpierre, Guillemot, Dufrêne, Cecchini, & Nicolas, 2017} and can actually be quite large (e.g., the within-population SD of observed bE dates for a given year is 5 days on average). Bias in the predictions of annual bE anomalies may further originate from the simplicity of the model structure, which probably does not represent the whole range of environmental interactions resulting in the spring onset of xylem formation.

Influence of local adaptation

A study aiming at simulating the date of budburst of *Betula pendula* and *Picea abies* individuals from central to Northern Europe (i.e., a bioclimatic scale comparable to the one considered in our work) reported a lower performance over validation data as compared to our results for bE (with prediction RMSE of 8.9 and 9.1 days, respectively, for their best heat-sum model), along with a non-homogeneous bias over the continent, suggesting a role for the local adaptation of trees phenological traits {Olsson & Jönsson, 2014}. It is not clear whether the latitudinal bias observed in Olsson and Jönsson {2014} originates from local adaptation that has been evidenced several times for budburst {see, e.g., Chuine, Mignot, & Belmonte, 2000; Osada et al., 2018; Vitasse, Delzon, Bresson, Michalet, & Kre- mer, 2009; von Wuehlisch, Krusche, & Muhs, 1995} or is related to the uncertainty of budburst observations recorded through local phenological protocols. The data we use in our work are less prone to such problems since the observations were collected and processed according to a common protocol across the entire study zone {Rathgeber et al., 2011; Rossi et al., 2016}. To this respect, we conclude from the absence of bias in the prediction of site average dates of bE that local adaptation is, if any, of marginal influence in determining bE {Perrin, Rossi, & Isabel, 2017} as compared to the plasticity of bE driven by varying temperature and photoperiod conditions.

These results echo our results on elevation gradients, where we didn't find any significant genetic structuration of the populations, and promote the idea of a global analysis crossing altitudinal and latitudinal gradient in order to disentangle the effect of photoperiod and temperature on wood phenology. *Picea abies*, but also *Pinus silvestris*, and may be *Larix decidua* + *siberica* are good potential candidates for such analysis.

Impact of climatic changes

This study is the first comparative assessment of ecophysiological models aiming at simulating the spring resumption of xylem formation in trees. We demonstrated that chilling-influenced heat-sum models are best supported by the data for the four coniferous species studied. Thus, analogous to what is commonly observed for buds, we state that winter–spring temperatures exert ambivalent effects on the spring onset of wood formation (bE) (i.e., on the one hand, warmer temperatures tend to hasten the occurrence of bE through the accumulation of forcing temperature, but on the other hand, they are associated to less chilling, imposing a higher forcing temperature sum to trigger wood formation). Previous results from Rossi et al. {2011} suggested that spring warming would result in a continuous trend to earlier bE in the next decades. Our results question these predictions, since warming reduces the number of chilling days. This is probably the cause of the recently evidenced reduced sensitivity of spring leaf phenology to warm temperatures {Fu et al., 2015}, which we also forecast to happen for wood formation (note that the length of the wood phenology time series is still too short to test this hypothesis).

Perspectives

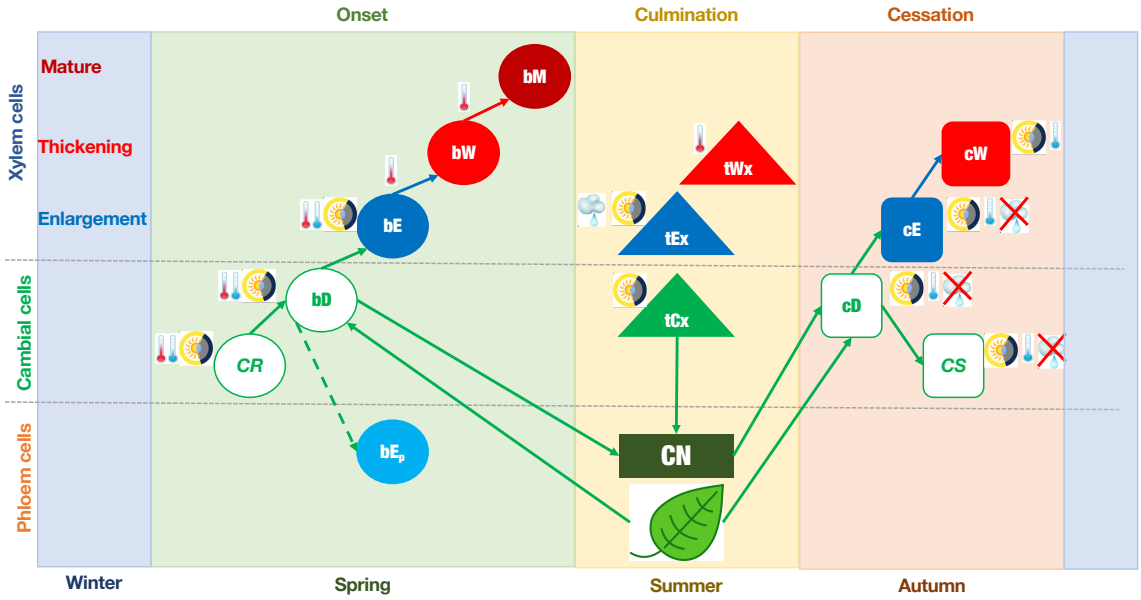
Our work paves the way for the development of ecophysiological models simulating the whole phenological sequence of wood formation. We expect the CHSM to be included as a component of schemes representing the whole seasonal cycle of wood formation, into which subsequent wood formation phenophases would partially depend on the occurrence of bE {Hänninen & Kramer, 2007; Lupi et al., 2010}. Such a model is also urgently needed in ecosystem models of the carbon cycle {Delpierre, Vitasse, et al., 2016} which are undergoing core changes in their representation of wood growth {Guillemot et al., 2017; Schiestl-Aalto et al., 2015}.

4.7 | Conclusion and perspectives

4.7.1 | Synthesis

In the first section of this chapter, we presented our approach to describe and quantify accurately tree-ring formation phenology using the CAVIAR R package.
In the second section, we explored the variability of tree-ring formation phenology according to tree age, size and social status, tree species, and site conditions. We showed that tree characteristics have as much influence as site and climatic conditions in determining the wood formation calendar.
In the third section, we synthesized the most recent knowledge on the influence of environmental cues on the phenology of temperate and boreal forest trees showing that temperatures are the main driver of the onsets of cambial activity and wood

formation, while photoperiod determine their cessations, which could be occasionally hasten by water shortage or cold spell [Fig. 4.16].
In the fourth section, we addressed the phenology of tree leaves and reproductive structures, fine roots, and wood, and we also consider carbon and nitrogen reserves as well as the relationships between organs, which are all essential to understand the functioning of trees.
In the fifth section, we showed through a functional approach based on a meta-analysis of wood and leaf phenology data, that tree-ring structure and leaf traits determine the coordination of tree organ 'phenologies' according to plant functional type life strategies.
Finally, in the sixth section, we presented a new process-based model approach, and we apply it to data from four conifer species covering a wide range of temperature and photoperiod conditions in the Northern Hemisphere to show that forcing and chilling temperatures (influenced by photoperiod) determine the onsets of xylem formation.



29 — Figure 4.16 | Conceptual model of cambium and wood phenology.
With CR: cambium reactivation (not observed); bD, bE, bE_p, bW, bM: beginning of expansion, wall thickening and mature phenophase for xylem and phloem; CN: total cell number in the current xylem ring; cD, cE, cW: cessation of expansion, wall thickening and mature phenophase; CS: cambium set. Weather icons indicate the influence of temperatures (hot and cold), photoperiod and water availability on the different phenological events.

4.7.2 | Perspectives

This chapter has highlighted several knowledge gaps concerning the phenology of wood formation and the associated functioning of the trees and we would like to propose some research avenues that could fill these gaps in the future.
The first promising research avenue is the development of the GloboXylo database in order to include more conifer species, but also angiosperm species and more climatic conditions for the different tree species. Collecting large dataset will allow us to explore the space-time variability in order to find global ecological trends.

Moreover, this will allow us to develop, parametrize and test process-based models of wood formation phenology. And we showed in this chapter that this is the best way to understand the influence of the environmental factors on wood formation phenology. We have submitted a new PhD thesis project on this subject in 2019 together with Nicolas Delpierre (Université Paris-Sud).

All studies on wood formation encounter the difficulty of correctly defining the beginning and end of cambial activity. Indeed, if the beginning of xylem formation is clearly defined by the appearance of the first thickening xylem cells, the beginning of cambial activity is often determined using the number of cambial cells only, and we have shown that this criterion is poor. However, it is the resumption of cambial activity that is the first event in the phenological chain, and therefore probably the most influential. It is therefore important to find reliable anatomical criteria to define when the cambium is active, or when the cambial divisions resume, and to document these new critical dates for the greatest number of species and situations.

This work on better defining cambial activity resumption would benefit from a work on the dynamics of phloem formation. Phloem formation represent the other side of the coin, and is even more important for the functioning of a tree, than xylem formation. However, it is still often discarded because observations are still challenging. However, Prislan et al. 2019, showed that the formation of the phloem can be describe very accurately for beech using a combination of light microscopy as well as scanning and transition electron microscopy. It would be very interesting to develop such work on Hesse site, for example. This could be the subject of another PhD thesis project, in collaboration with Daniel Epron (Lorraine University).

4.8 | References

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Antonucci, S., Rossi, S., Deslauriers, A., Lombardi, F., Marchetti, M., & Tognetti, R. (2015). Synchronisms and correlations of spring phenology between apical and lateral meristems in two boreal conifers. Tree Physiology, 35(10), 1086–1094. https://doi.org/10.1093/treephys/tpv077

Basler, D. (2016). Evaluating phenological models for the prediction of leaf-out dates in six temperate tree species across central Europe. Agricultural and Forest Meteorology, 217, 10–21. https://doi.org/10. 1016/j.agrformet.2015.11.007

Basler, D., & Körner, C. (2014). Photoperiod and temperature responses of bud swelling and bud burst in four temperate forest tree species. Tree Physiology, 34(4), 377–388. https://doi.org/10.1093/treephys/ tpu021

Begum, S., Nakaba, S., Oribe, Y., Kubo, T., & Funada, R. (2010). Cambial sensitivity to rising temperatures by natural condition and artificial heating from late winter to early spring in the evergreen conifer Cryptomeria japonica. Trees, 24(1), 43–52. https://doi.org/10.1007/s00468-009-0377-1

Begum, S., Nakaba, S., Yamagishi, Y., Oribe, Y., & Funada, R. (2013). Reg- ulation of cambial activity in relation to environmental conditions: Understanding the role of temperature in wood formation of trees. Physiologia Plantarum, 147(1), 46–54. https://doi.org/10.1111/j.1399-3054.2012.01663.x

Begum, S., Kudo, K., Rahman, M. H., Nakaba, S., Yamagishi, Y., Nabe-shima, E., ... Funada, R. (2018). Climate change and the regulation of wood formation in trees by temperature. Trees – Structure and Func- tion, 32(1), 3–15. https://doi.org/10.1007/s00468-017-1587-6

Begum, S., Shibagaki, M., Furusawa, O., Nakaba, S., Yamagishi, Y., Yoshi- moto, J., ... Funada, R. (2012). Cold stability of microtubules in wood-forming tissues of conifers during seasons of active and dor- mant cambium. Planta, 235(1), 165–179. https://doi.org/10.1007/ s00425-011-1500-2

Blümel, K., & Chmielewski, F. M. (2012). Shortcomings of classical pheno- logical forcing models and a way to overcome them. Agricultural and Forest Meteorology, 164, 10–19. https://doi.org/10.1016/j.agrformet. 2012.05.001

Caffarra, A., Donnelly, A., & Chuine, I. (2011). Modelling the timing of Betula pubescens budburst. II. Integrating complex effects of pho- toperiod into process-based models. Climate Research, 46(2), 159– 170. https://doi.org/10.3354/cr00983

Cannell, M. G. R., & Smith, R. I. (1983). Thermal time, chill days and pre- diction of budburst in Picea sitchensis. The Journal of Applied Ecology, 20(3), 951–963. https://doi.org/10.2307/2403139

Chaffey, N., & Barlow, P. (2002). Myosin, microtubules, and microfila- ments: Co-operation between cytoskeletal components during cam- bial cell division and secondary vascular differentiation in trees. Planta, 214(4), 526–536. https://doi.org/10.1007/s004250100652

Chmielewski, F. M., & Rötzer, T. (2001). Response of tree phenology to climate change across Europe. Agricultural and Forest Meteorology, 108(2), 101–112. https://doi.org/10.1016/S0168-1923(01)00233-7

Chuine, I., Garcia de Cortazar-Atauri, I., Kramer, K., & Hänninen, H. (2013). Plant development models. In M. D. Schwartz (Ed.), Phenology: An integrative environmental science (2nd ed., pp. 275–293). Dor- drecht: Springer.

Chuine, I., Mignot, A., & Belmonte, J. (2000). A modelling analysis of the genetic variation of phenology between tree populations. Journal of Ecology, 88, 561–570. https://doi.org/10.1046/j.1365-2745.2000. 00468.x

Clark, J. S., Salk, C., Melillo, J., & Mohan, J. (2014). Tree phenology responses to winter chilling, spring warming, at north and south range limits. Functional Ecology, 28(6), 1344–1355. https://doi.org/10. 1111/1365-2435.12309

Coville, F. V. (1920). The influence of cold in stimulating the growth of plants. Proceedings of the National Academy of Sciences of the United States of America, 6, 434–435. https://doi.org/10.1073/pnas.6.7.434

Cuny, H. E., Rathgeber, C. B. K., Lebourgeois, F., Fortin, M., & Fournier, M. (2012). Life strategies in intra-annual dynamics of wood forma- tion: Example of three conifer species in a temperate forest in north- east France. Tree Physiology, 32(5), 612–625. https://doi.org/10. 1093/treephys/tps039

Delpierre, N., Berveiller, D., Granda, E., & Dufrêne, E. (2016). Wood phe- nology, not carbon input, controls the interannual variability of wood growth in a temperate oak forest. New Phytologist, 210(2), 459–470. https://doi.org/10.1111/nph.13771

Delpierre, N., Guillemot, J., Dufrêne, E., Cecchini, S., & Nicolas, M. (2017). Tree phenological ranks repeat from year to year and corre- late with growth in temperate deciduous forests. Agricultural and For- est Meteorology, 234–235, 1–10. https://doi.org/10.1016/j.agrformet. 2016.12.008

Delpierre, N., Vitasse, Y., Chuine, I., Guillemot, J., Bazot, S., Rutishauser, T., & Rathgeber, C. B. K. (2016). Temperate and boreal forest tree phenology: From organ-scale processes to terrestrial ecosystem mod- els. Annals of Forest Science, 73(1), 5–25. https://doi.org/10.1007/ s13595-015-0477-6

Delpierre, N., Soudani, K., François, C., Le Maire, G., Bernhofer, C., Kutsch, W., ... Dufrêne, E. (2012). Quantifying the influence of climate and biological drivers on the interannual variability of carbon exchanges in European forests through process-based modelling. Agricultural and Forest Meteorology, 154–155, 99–112. https://doi. org/10.1016/j.agrformet.2011.10.010

Deslauriers, A., Huang, J.-G., Balducci, L., Beaulieu, M., & Rossi, S. (2016). The contribution of carbon and water in modulating wood formation in black spruce saplings. Plant Physiology, 170(4), 2072–2084. https://doi.org/10.1104/pp.15.01525

Deslauriers, A., Morin, H., & Begin, Y. (2003). Cellular phenology of annual ring formation of Abies balsamea in the Quebec boreal forest (Canada). Canadian Journal of Forest Research, 33(2), 190–200. https://doi.org/10.1139/x02-178

Deslauriers, A., Rossi, S., Anfodillo, T., & Saracino, A. (2008). Cambial phenology, wood formation and temperature thresholds in two con- trasting years at high altitude in southern Italy. Tree Physiology, 28, 863–871. https://doi.org/10.1093/treephys/28.6.863
Egierszdorff, S. (1981). The role of auxin stored in Scots pine trunk dur- ing spring activation of cambial activity. Biologia Plantarum, 23(2), 110–115. https://doi.org/10.1007/BF02878415
Flynn, D. F. B., & Wolkovich, E. M. (2018). Temperature and photoperiod drive spring phenology across all species in a temperate forest com- munity. New Phytologist, 219, 1353–1362. https://doi.org/10.1111/ nph.15232
Ford, K. R., Harrington, C. A., Bansal, S., Gould, P. J., & St. Clair, J. B. (2016). Will changes in phenology track climate change? A study of growth initiation timing in coast Douglas-fir. Global Change Biology, 22(11), 3712–3723. https://doi.org/10.1111/gcb.13328
Fox, J., & Weisberg, S. (2011). An R companion to applied regression. SAGE, 34, 137–140. https://doi.org/10.1177/0049124105277200
Fu, Y. H., Campioli, M., Van Oijen, M., Deckmyn, G., & Janssens, I. A. (2012). Bayesian comparison of six different temperature-based bud-burst models for four temperate tree species. Ecological Modelling, 230, 92–100. https://doi.org/10.1016/j.ecolmodel.2012.01.010
Fu, Y. H., Zhao, H., Piao, S., Peaucelle, M., Peng, S., Zhou, G., ... Janssens, I. A. (2015). Declining global warming effects on the phenology of spring leaf unfolding. Nature, 526(7571), 104–107. https://doi.org/10. 1038/nature15402
Gelman, A., Carlin, J. B., Stern, H. S., & Rubin, D. B. (2004). Bayesian data analysis. Chapman texts in statistical science series. New York, NY: Chapman and Hall/CRC.
Gelman, A., Meng, X.-L., & Stern, H. (1996). Posterior predictive assess- ment of model fitness via realized discrepancies. Statistica Sinica, 6(4), 733–807. https://doi.org/10.1.1.142.9951
Giagli, K., Gricar, J., Vavrcik, H., & Gryc, V. (2016). Nine-year monitoring of cambial seasonality and cell production in norway spruce. iForest – Biogeosciences and Forestry, 9, 375–382. https://doi.org/10.3832/if or1771-008
Gričar, J., Zupančič, M., Čufar, K., Koch, G., Schmitt, U., & Oven, P. (2006). Effect of local heating and cooling on cambial activity and cell differentiation in the stem of Norway spruce (Picea abies). Annals of Botany, 97(6), 943–951. https://doi.org/10.1093/aob/mcl050
Gričar, J., Zupančič, M., Čufar, K., & Oven, P. (2007). Regular cambial activity and xylem and phloem formation in locally heated and cooled stem portions of Norway spruce. Wood Science and Technology, 41(6), 463–475. https://doi.org/10.1007/s00226-006-0109-2
Gruber, A., Strobl, S., Veit, B., & Oberhuber, W. (2010). Impact of drought on the temporal dynamics of wood formation in Pinus sylvestris. Tree Physiology, 30(4), 490–501. https://doi.org/10.1093/treephys/tpq003
Guillemot, J., Francois, C., Hmimina, G., Dufrêne, E., Martin-StPaul, N. K., Soudani, K., ... Delpierre, N.. (2017). Environmental control of carbon allocation matters for modelling forest growth. New Phytologist, 214 (1), 180–193. https://doi.org/10.1111/nph.14320
Hänninen, H. (2016). Boreal and temperate trees in a changing climate. Dordrecht, The Netherlands: Springer Netherlands. https://doi.org/ 10.1007/978-94-017-7549-6
Hänninen, H., & Kramer, K. (2007). A framework for modelling the annual cycle of trees in boreal and temperate. Silva Fennica, 41(1), 167–205.
Hartig, F., Minnuno, F., & Paul, S. (2017).BayesianTools: General-purpose MCMC and SMC samplers and tools for bayesian statistics. R package version 0.1.5.
Heide, O. (1993). Daylength and thermal time responses of budburst dur- ing dormancy release in some northern deciduous trees. Physiologia Plantarum, 88(4), 531–540. https://doi.org/10.1111/j.1399-3054. 1993.tb01368.x
Huang, J.-G., Deslauriers, A., & Rossi, S. (2014). Xylem formation can be modeled statistically as a function of primary growth and cambium activity. The New Phytologist, 203(3), 831–841. https://doi.org/10. 1111/nph.12859
Immanen, J., Nieminen, K., Smolander, O. P., Kojima, M., Alonso Serra, J., Koskinen, P., ... Helariutta, Y. (2016). Cytokinin and auxin display dis- tinct but interconnected distribution and signaling profiles to stimu- late cambial activity. Current Biology, 26(15), 1990–1997. https://doi. org/10.1016/j.cub.2016.05.053
Jyske, T., Mäkinen, H., Kalliokoski, T., & Nöjd, P. (2014). Intra-annual tra- cheid production of Norway spruce and Scots pine across a latitudi- nal gradient in Finland. Agricultural and Forest Meteorology, 194, 241– 254. https://doi.org/10.1016/j.agrformet.2014.04.015

Kass, R. E., & Raftery, A. E. (1995). Bayes factors. Journal of the American Statistical Association, 90(430), 773–795. https://doi.org/10.1080/ 01621459.1995.10476572
Kramer, K. (1995). Modelling comparison to evaluate the importance of phenology for the effects of climate change on growth of temperate- zone deciduous trees. Climate Research, 5, 119–130. https://doi.org/ 10.3354/cr005119
Larson, P. (1994). The vascular cambium: Development and structure. Ber- lin, Heidelberg: Springer.
Lempereur, M., Martin-stpaul, N. K., Damesin, C., Joffre, R., Ourcival, J., Rocheteau, A., & Rambal, S. (2015). Growth duration is a better pre- dictor of stem increment than carbon supply in a Mediterranean oak forest : Implications for assessing forest productivity under climate change. New Phytologist, 207, 579–590.
Li, X., Liang, E., Gričar, J., Rossi, S., Ćufar, K., & Ellison, A. M. (2017). Crit- ical minimum temperature limits xylogenesis and maintains treelines on the southeastern Tibetan Plateau. Science Bulletin, 62(11), 804– 812. https://doi.org/10.1016/j.scib.2017.04.025
Linkosalo, T., Carter, T. R., Hakkinen, R., & Hari, P. (2000). Predicting spring phenology and frost damage risk of Betula spp. under climatic warming: A comparison of two models. Tree Physiology, 20(17), 1175–1182. https://doi.org/10.1093/treephys/20.17.1175
Little, C. H. A., & Bonga, J. M. (1974). Rest in the cambium of Abies bal- samea. Canadian Journal of Botany, 52, 1723–1730.
Lupi, C., Morin, H., Deslauriers, A., & Rossi, S. (2010). Xylem phenology and wood production: Resolving the chicken-or-egg dilemma. Plant, Cell & Environment, 33(10), 1721–1730. https://doi.org/10.1111/j. 1365-3040.2010.02176.x
Mäkelä, A., Hari, P., Berninger, F., Hänninen, H., & Nikinmaa, E. (2004). Acclimation of photosynthetic capacity in Scots pine to the annual cycle of temperature. Tree Physiology, 24(4), 369–376. https://doi. org/10.1093/treephys/24.4.369
Mäkinen, H., Jyske, T., & Nöjd, P. (2018). Dynamics of diameter and height increment of Norway spruce and Scots pine in southern Fin- land. Annals of Forest Science, 75(1), 28. https://doi.org/10.1007/ s13595-018-0710-1
Mesplé, F., Troussellier, M., Casellas, C., & Legendre, P. (1996). Evaluation of simple statistical criteria to qualify a simulation. Ecological Model- ling, 88, 9–18. https://doi.org/10.1016/0304-3800(95)00033-X
Michelot, A., Simard, S., Rathgeber, C., Dufrêne, E., & Damesin, C. (2012). Comparing the intra-annual wood formation of three European spe- cies (Fagus sylvatica, Quercus petraea and Pinus sylvestris) as related to leaf phenology and non-structural carbohydrate dynamics. Tree Physi-
ology, 32(8), 1033–1045. https://doi.org/10.1093/treephys/tps052
Moser, L., Fonti, P., Büntgen, U., Esper, J., Luterbacher, J., Franzen, J., & Frank, D. (2010). Timing and duration of European larch growing sea- son along altitudinal gradients in the Swiss Alps. Tree Physiology, 30 (2), 225–233. https://doi.org/10.1093/treephys/tpp108
O'Hagan, A. (1995). Fractional bayes factors for model comparison. Jour- nal of the Royal Statistical Society: Series B (Methodological), 57(1), 99–
138. https://doi.org/10.2307/2346088
Olsson, C., & Jönsson, A. M. (2014). Process-based models not always
better than empirical models for simulating budburst of Norway spruce and birch in Europe. Global Change Biology, 20(11), 3492– 3507. https://doi.org/10.1111/gcb.12593
Oribe, Y., Funada, R., & Kubo, T. (2003). Relationships between cambial activity, cell differentiation and the localization of starch in storage tissues around the cambium in locally heated stems of Abies sachali- nensis (Schmidt) Masters. Trees, 17, 185–192. https://doi.org/10. 1007/s00468-002-0231-1
Oribe, Y., & Kubo, T. (1997). Effect of heat on cambial reactivation during winter dormancy in evergreen and deciduous conifers. Tree Physiol- ogy, 17(2), 81–87. https://doi.org/10.1093/treephys/17.2.81
Osada, N., Murase, K., Tsuji, K., Sawada, H., Nunokawa, K., Tsukahara, M., & Hiura, T. (2018). Genetic differentiation in the timing of bud- burst in Fagus crenata in relation to temperature and photoperiod. International Journal of Biometeorology, 62, 1763–1776. https://doi. org/10.1007/s00484-018-1579-2

Pelkonen, P., & Hari, P. (1980). The dependence of the springtime recov- ery of CO2 uptake in scots pine on temperature and internal factors. Flora, 169(5), 398–404. https://doi.org/10.1016/S0367-2530(17) 31974-6

Perrin, M., Rossi, S., & Isabel, N. (2017). Synchronisms between bud and cambium phenology in black spruce: Early-flushing provenances exhi- bit early xylem formation. Tree Physiology, 37(5), 593–603. https://d oi.org/10.1093/treephys/tpx019

Petterle, A., Karlberg, A., & Bhalerao, R. P. (2013). Daylength mediated control of seasonal growth patterns in perennial trees. Current Opin- ion in Plant Biology, 16, 301–306. https://doi.org/10.1016/j.pbi.2013. 02.006

Piñeiro, G., Perelman, S., Guerschman, J. P., & Paruelo, J. M. (2008). How to evaluate models: Observed vs. predicted or predicted vs. observed? Ecological Modelling, 216(3–4), 316–322. https://doi.org/ 10.1016/j.ecolmodel.2008.05.006

Prislan, P., Ćufar, K., Koch, G., Schmitt, U., & Gričar, J. (2013). Review of cellular and subcellular changes in the cambium. IAWA Journal, 34(4), 391–407. https://doi.org/10.1163/22941932-00000032

Prislan, P., Schmitt, U., Koch, G., Gričar, J., & Ćufar, K. (2011). Seasonal ultrastructural changes in the cambial zone of beech (Fagus Sylvatica) grown at two different altitudes. IAWA Journal, 32(4), 443–459. https://doi.org/10.1163/22941932-90000070

Rathgeber, C. B. K., Longuetaud, F., Mothe, F., Cuny, H., & Le Moguédec, G. (2011). Phenology of wood formation: Data processing, analysis and visualisation using R (package CAVIAR). Dendrochronologia, 29(3), 139–149. https://doi.org/10.1016/j.dendro.2011.01.004

Rensing, K. H., & Samuels, A. L. (2004). Cellular changes associated with rest and quiescence in winter-dormant vascular cambium of Pinus contorta. Trees, 18(4), 373–380. https://doi.org/10.1007/s00468- 003-0314-7.

Rinne, P. L. H., Kaikuranta, P. M., & Van Der Schoot, C. (2001). The shoot apical meristem restores its symplasmic organization during chilling- induced release from dormancy. The Plant Journal, 26(3), 249–264. https://doi.org/10.1046/j.1365-313X.2001.01022.x

Rossi, S., Anfodillo, T., & Menardi, R. (2006). TREPHOR: A new tool for sampling microcores from tree stems. IAWA Journal, 27(1), 89–97. https://doi.org/10.1163/22941932-90000139

Rossi, S., Deslauriers, A., & Anfodillo, T. (2006). Assessment of cambial activity and xylogenesis by microsampling tree species: An example at the Alpine timberline. IAWA Journal, 27, 383–394. https://doi.org/ 10.1163/22941932-90000161

Rossi, S., Deslauriers, A., Anfodillo, T., & Carraro, V. (2007). Evidence of threshold temperatures for xylogenesis in conifers at high altitudes. Oecologia, 152(1), 1–12. https://doi.org/10.1007/s00442-006-0625-7

Rossi, S., Morin, H., Deslauriers, A., & Plourde, P.-Y. (2011). Predicting xylem phenology in black spruce under climate warming. Global Change Biology, 17(1), 614–625. https://doi.org/10.1111/j.1365- 2486.2010.02191.x

Rossi, S., Rathgeber, C. B. K., Deslauriers, A., Sergio, R., Cyrille, B. K. R., & Annie, D. (2009). Comparing needle and shoot phenology with xylem development on three conifer species in Italy. Annals of Forest Science, 66(2), 1–8. https://doi.org/10.1051/forest/2008088

Rossi, S., Anfodillo, T., Ćufar, K., Cuny, H. E., Deslauriers, A., Fonti, P., ... Treml, V. (2016). Pattern of xylem phenology in conifers of cold ecosystems at the Northern Hemisphere. Global Change Biology, 22 (11), 3804–3813. https://doi.org/10.1111/gcb.13317

Rossi, S., Deslauriers, A., Griçar, J., Seo, J.-W., Rathgeber, C. B. K., Anfo- dillo, T., ... Jalkanen, R. (2008). Critical temperatures for xylogenesis in conifers of cold climates. Global Ecology and Biogeography, 17(6), 696–707. https://doi.org/10.1111/j.1466-8238.2008.00417.x

Sakai, A. (1966). Studies of frost hardiness in woody plants. II. Effect of temperature on hardening. Plant Physiology, 41(2), 353–359. https://d oi.org/10.1104/pp.41.2.353

Sakai, A., & Larcher, W. (1987). Frost survival of plants: Responses and adaptation to freezing stress. Ecological Studies, 63, 94. https://doi. org/10.1007/BF02854542

Sarvas, R. (1974). Investigations on the annual cycle of development of for- est trees. II. Autumn dormancy and winter dormancy. Helsinki: Commu- nicationes Instituti Forestalis Fenniae.

Schaller, G. E., Street, I. H., & Kieber, J. J. (2014). Cytokinin and the cell cycle. Current Opinion in Plant Biology, 21, 7–15. https://doi.org/10. 1016/j.pbi.2014.05.015

Schiestl-Aalto, P., Kulmala, L., Mäkinen, H., Nikinmaa, E., & Mäkelä, A. (2015). CASSIA – A dynamic model for predicting intra-annual sink demand and interannual growth variation in Scots pine. New Phytolo- gist, 206(2), 647–659. https://doi.org/10.1111/nph.13275

Schmitt, U., Jalkanen, R., & Eckstein, D. (2004). Cambium dynamics of Pinus sylvestris and Betula spp. in the northern boreal forest in Fin- land. Silva Fennica, 38(2), 167–178. https://doi.org/10.14214/sf.426

Seo, J.-W.-J., Eckstein, D., Jalkanen, R., Rickebusch, S., & Schmitt, U. W. E. (2008). Estimating the onset of cambial activity in Scots pine in northern Finland by means of the heat-sum approach. Tree Physiology, 28(1), 105–112. https://doi.org/10.1093/treephys/28.1.105

Singh, R. K., Svystun, T., AlDahmash, B., Jönsson, A. M., & Bhalerao, R. P. (2017). Photoperiod- and temperature-mediated control of phenology in trees – A molecular perspective. New Phytologist, 213, 511–524. https://doi.org/10.1111/nph.14346

Sorce, C., Giovannelli, A., Sebastiani, L., & Anfodillo, T. (2013). Hormonal signals involved in the regulation of cambial activity, xylogenesis and vessel patterning in trees. Plant Cell Reports, 32(6), 885–898. https://doi.org/10.1007/s00299-013-1431-4

Strimbeck, G. R., Schaberg, P. G., Fossdal, C. G., Schröder, W. P., & Kjell- sen, T. D. (2015). Extreme low temperature tolerance in woody plants. Frontiers in Plant Science, 6, 884. https://doi.org/10.3389/fpls. 2015.00884

Swidrak, I., Gruber, A., Kofler, W., & Oberhuber, W. (2011). Effects of environmental conditions on onset of xylem growth in Pinus sylvestris under drought. Tree Physiology, 31(5), 483–493. https://doi.org/10. 1093/treephys/tpr034

Takahashi, S., Okada, N., & Nobuchi, T. (2013). Relationship between the timing of vessel formation and leaf phenology in ten ring-porous and diffuse-porous deciduous tree species. Ecological Research, 28(4), 615–624. https://doi.org/10.1007/s11284-013-1053-x

Vaganov, E. A., Hughes, M. K., & Shashkin, A. V. (2006). Growth dynamics of conifer tree rings: Images of past and future environments (Vol. 83). Berlin: Springer.

van Oijen, M., Reyer, C., Bohn, F. J., Cameron, D. R., Deckmyn, G., Flech- sig, M., ... Rammer, W. (2013). Bayesian calibration, comparison and averaging of six forest models, using data from Scots pine stands across Europe. Forest Ecology and Management, 289, 255–268. https://doi.org/10.1016/j.foreco.2012.09.043

Verdier, B., Jouanneau, I., Simonnet, B., Rabin, C., Van Dooren, T. J. M., Delpierre, N., ... Le Galliard, J. F. (2014). Climate and atmosphere simulator for experiments on ecological systems in changing environ- ments. Environmental Science and Technology, 48(15), 8744–8753. https://doi.org/10.1021/es405467s

Vitasse, Y., Delzon, S., Bresson, C. C., Michalet, R., & Kremer, A. (2009). Altitudinal differentiation in growth and phenology among popula- tions of temperate-zone tree species growing in a common garden. Canadian Journal of Forest Research, 39(7), 1259–1269. https://doi. org/10.1139/X09-054

Vitasse, Y., François, C., Delpierre, N., Dufrêne, E., Kremer, A., Chuine, I., & Delzon, S. (2011). Assessing the effects of climate change on the phenology of European temperate trees. Agricultural and Forest Mete- orology, 151(7), 969–980. https://doi.org/10.1016/j.agrformet.2011. 03.003

von Wuehlisch, G., Krusche, D., & Muhs, H. J. (1995). Variation in temperature sum requirement for flushing of beech provenances. Silvae Genetica, 44(5–6), 343–346.

Weedon, G. P., Balsamo, G., Bellouin, N., Gomes, S., Best, M. J., & Viterbo, P. (2014). The WFDEI meteorological forcing data set: WATCH Forcing Data methodology applied to ERA-Interim reanalysis data. Water Resources Research, 50(9), 7505–7514. https://doi.org/ 10.1002/2014WR015638

CHAPTER 5 | SEASONAL DYNAMICS OF TREE-RING FORMATION

5.0 | Synthesis

5.0.1 | Related papers

Rathgeber CBK, Rossi S, Bontemps J-D. 2011b. Cambial activity related to tree size in a mature silver-fir plantation. *Annals of Botany* 108: 429–438.

Cuny, H., Rathgeber, C.B.K., Lebourgeois, F., Fortin, M., Fournier, M., 2012. Life strategies in intra-annual dynamics of wood formation: example of three conifer species in a temperate forest in north-east France. *Tree Physiology* 32, 612–625. doi:10.1093/treephys/tps039

Cuny H, Rathgeber CBK, Kiessé TS, Hartmann FP, Barbeito I, Fournier M. 2013. Generalized additive models reveal the intrinsic complexity of wood formation dynamics. *Journal of Experimental Botany* 64: 1983–1994.

Rathgeber CBK, Santenoise P, Cuny H. 2018. CAVIAR: an R package for checking, displaying and processing wood-formation-monitoring data. *Tree physiology* 38: 1246–1260.

Rathgeber CBK and GloboXylo Initiative Consortium. Ecological trends in xylem production of coniferous trees. In preparation for *Ecology Letters*.

Saderi S, Rathgeber CBK, Rozenberg P, Fournier M. Seasonal dynamics of wood formation in larch trees growing along a 1,000 m elevation gradient in the French Southern Alps. In preparation for *Forest Ecology and Management*.

5.0.2 | Summary

In the first section of this chapter, we presented our approach to describe and quantify accurately tree-ring formation dynamics using Gompertz functions thanks to the CAVIAR R package.

In the second section, we explored the variability of tree-ring formation dynamics according to tree age, size and social status, tree species, and site conditions. We discovered two distinct strategies concerning the dynamics of wood formation: the "intensive" strategy, in which trees grow quickly but not long; and the "extensive" strategy, in which trees grow long but slowly. We have also shown that trees adapt to high elevation by increasing their number of cambial cells to compensate for the increase in cell cycle length caused by lower temperatures.

In the third section, we explored the large GloboXylo data base to show that the rate of xylem cell production by the cambium is the main driver of tree-ring width and wood production, while the duration of the growing season only played a secondary role. We also confirmed that temperatures have a major influences on the duration of wood production, while the rate is more influenced by microsite, stand and tree conditions.

Our results confirm that recent global warming could have resulted in extended period of growth explaining enhanced forest growth. However, we showed that the rate of growth, along with species behaviour and site conditions must be considered

into vegetation models to assess the impact of future climatic changes on forest productivity.

5.0.3 | Key words

Wood production, Tree radial growth, Cambial activity, Speed and duration of xylem formation, Forest productivity, Climatic changes

5.1 | Introduction

5.1.1 | General scientific framework

As a consequence of recent climatic changes, many studies have reported an increase in tree growth {Pretzsch et al. 2014}, forest ecosystem net primary productivity (NPP) {Nemani et al 2003}, and terrestrial biosphere carbon up-take {Ballantyne et al. 2012}, forests being one of the largest carbon sink at the moment {Pan et al. 2011}.

Earth-system models, however, do not agree on the future behavior of that sink. Some models predicts that forests will continue to soak up massive amounts of carbon in the coming decades, whereas others suggest that forests could become stressed by droughts and high temperatures and die back, releasing carbon into the atmosphere {Keenan 2014}.

So, identifying the mechanisms and locations responsible for increasing global carbon uptake remains a critical challenge in constraining the modern global carbon budget and predicting future carbon–climate interactions {Ballantyne et al. 2012}.

Direct {Pretzsch et al. 2014} and remote observations {Nemani et al. 2003}, as well as bioclimatic {Barichivich et al. 2012}, eco-physiological {Delpierre et al. 2012}, and earth-system models {Keenan 2014}, have suggested that it is mainly the rise in temperatures and the resulting extended vegetation period that are responsible for forest enhanced productivity.

However, several other environmental factors (e.g., nitrogen deposition, CO2 fertilization, global dimming) may have promoted increases or decreases in NPP {Nemani et al. 2003}.

5.1.2 | Nature of the problem and scope of the chapter

Climatic factors like temperature, radiation, and water interact to impose complex and varying limitations on vegetation activity in different parts of the world {Churkina & Running 1998}.

While tree-rings are the most important source of information to study climate-growth relationships, tree-ring studies have mainly documented thermal contraints on tree-ring width and density {Briffa et al. 1998}, exploring more occasionally water constraints {Shi et al. 2014}, and very rarely the effect of other climatic factors like light availability {Stine and Huybers 2014}.

Also, despite the crucial importance of tree growth for reconstructing past climate conditions and assessing impacts of current climatic changes on forest ecosystems, the processes that drive wood formation and shape tree-ring structure are still poorly understood {but see for example Vaganov et al. 2010; Rathgeber et al. 2016; Cuny et al. 2018}.

5.1.3 | Research questions

Plants are well known to follow the cycle of seasons, being active only during a certain period of time that is favorable for them, and proceeding at their own pace during this period.

Plant phenology (which is mainly concerned with the dates of occurrence of biological events in their annual cycle) and growth dynamics (which is principally concerned with the speed of the biological process in their annual cycle) may be related to different plant functions and influenced by different environmental factors.

Plant phenology, on the one hand, determines individual survival {Chuine and Beaubien 2001}, driving plant species distribution {Chuine 2010}, and being principally controlled by temperature and photoperiod {Menzel 1999, Körner and Basler 2010}.

Plant growth, on the other hand, determine individual competitiveness, driving stand dynamics in forest ecosystems {Landsberg and Sands 2011}. Growth is controlled by the developmental stage of the plant {Lachenbruch et al. 2011}, but is strongly influenced by climatic factors and site conditions {Fritts 1976, Schweingruber 1996}.

Only few wood-formation-monitoring studies investigated the question of the contribution to the annual tree-ring width of the rate of xylem cell production vs. its duration, presenting contradictory results. Rathgeber et al. {2011} comparing suppressed, intermediate, and dominant 40-years old firs, grown in a temperate forest stand in north-east of France, showed that the rate of cell production accounted for 75 % of the annual radial growth (results presented hereafter). However, Rossi et al. {2014} comparing different black-spruce stands, spread along a latitudinal gradient in Quebec boreal forest (Canada), showed that xylem cell production duration accounted for 86 % of the annual radial growth. Finally, in a recent paper, Ren et al. {2019} found that growth rate rather than growing season length determines wood biomass production in dry environments.

5.1.4 | Aim of the chapter (and hypotheses to challenge)

In this chapter we aim to: (1) unravel the contribution of the different wood formation traits (i.e. rates vs. dates) to the final annual growth (i.e. tree-ring width or number of cells), and (2) disentangle the influence of the environmental factors on these traits.

H1 — To reconcile the former case studies on the subject, we hypothesized that, at the global scale, rate and duration of cambial activity, both play an important role in determining annual tree growth. However, when looking in more details, we expect their role to vary: durations being more important for temperature limited biomes (Boreal and Alpine forests for example; H1a), and rates more important for water limited biomes (Arid and Mediterranean forests for example; H1b), while rate and duration may be of similar importance for biomes where no particular environmental constraints dominate (temperate forests, for example; H1c).

H2 — Based on these former works, we also hypothesized that, while xylem phenology is mainly driven by broad scale climatic factors (e.g. temperature; H2a); cambial activity is more related to local conditions (e.g., soil characteristics, stand

structure; H2b), and individual tree characteristics (e.g., species, size, age, vitality; H2c).
H3 — Finally concerning the influence of the climatic factors, because duration of the growing season must be related to climatic conditions occurring at the boundaries of the period, we expect temperature to be the main driver (H3a). On the contrary, because the maximal growth rate occurs in the central part of the growing season, we expect water or light availability to be the main drivers (H3b).

5.1.5 | Presentation of the materials and methods

The analyses were based on a large wood-formation-monitoring dataset, encompassing 52 sites, distributed in 12 countries from North-America, Europe, and Asia, and representing conifer forests distribution in mediterranean, temperate, and boreal regions.
The dataset (GloboXylo Initiative) was collected from temperate to northern boreal sites of three continents, ranging from the Southern Italy to the Northern Finland and Canada, from the low altitudes in France and Slovenia to the highlands of Alps, Bohemian Massif in Czech Republic and Tibetan Plateau in China. The sites represented temperate to boreal forests, timberline and treeline areas, including the highest treeline of the North hemisphere in the Tibetan Plateau.
Sensitivity analyses of the basic physical model were used to reveal global ecological patterns in tree-ring formation, while mixed effect models were used to quantify the influences of the environmental factors on the variables of the model.

5.2 | Describing and quantifying the intra-annual dynamics of tree-ring formation

5.2.1 | Application of the Gompertz model to describe tree-ring formation dynamics

For temperate and cold forest trees, the accumulation all along the growing season of xylem cells in the forming ring is classically described using a Gompertz function {e.g., Camarero et al., 1998; Deslauriers et al., 2003; Rossi et al., 2003, 2006b, 2006c; Cufar et al., 2008; Rathgeber et al. 2011b}. In the Gompertz function {Gompertz, 1825} the exponential increase is controlled by a limiting term as in the equation:
$dG(t) = a - b \ln(G(t))$ [Equation 5.1]
Where a is the exponential term, and $b.\ln(G(t))$ the limiting term.
Theoretical ecologists classically interpret the limiting term as the effect of the increase of competition for limited resources (e.g., space, light, substrate, nutrients) accompanying any growing population {e.g., Zeide 1980, 1993, 2003}.

5.2.2 | Fitting Gompertz models

The evolution of the three cumulated cell counts (nM, nWM and nEWM) over a growing season can be fitted using the Gompertz function.
For fitting purpose, the Gompertz function is classically defined as:
$n(t) = \alpha \cdot \exp(-\exp(\beta - \kappa t))$ [Equation 5.2]

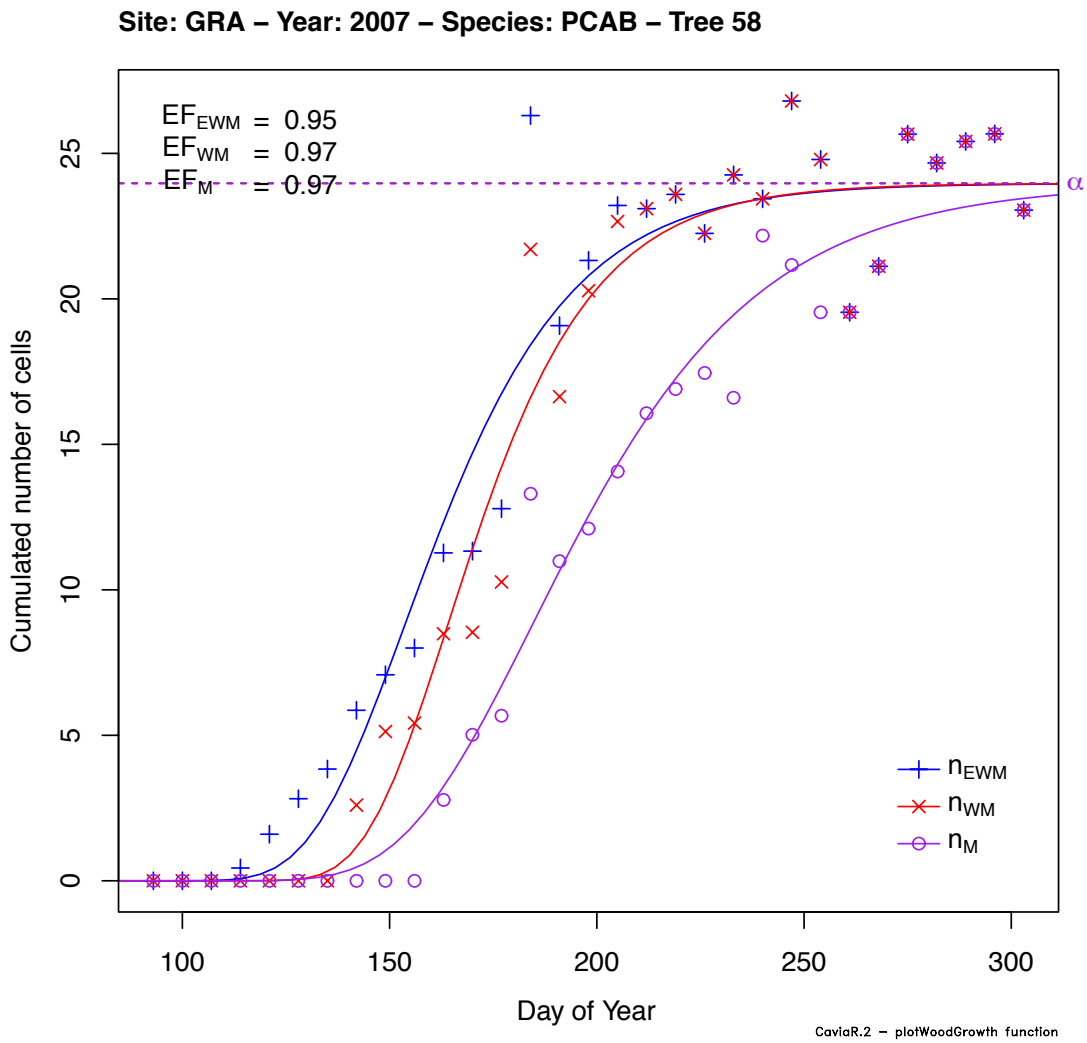
Where $n(t)$ is the total number of cells at time t; α is the upper horizontal asymptote parameter representing the final number of cells reached at the end of the growing season; β is the displacement along the time axis parameter, which reflects the choice of the origin of time; and κ is the growth rate parameter, which determines the spread of the curve along the time axis.

In CAVIAR, the *fitGompertzModels* function automatically fits a Gompertz function to each of the three cumulated cell counts, for each year, and each tree available in the dataset, using the *nls* function of the R stats package {R Development Core Team, 2015} [Fig. 5.1]. The *nls* function determines the weighted least-squares estimates of the parameters of a nonlinear model {Bates and Chambers, 1992}.

The goodness-of-fit of the model is evaluated using the *fitGompertzModels* function, which plots time-series of predicted values along with the observations {Piñeiro et al., 2008}. This function also plots a linear regression analysis of the observed vs. predicted data {Mayer and Butler, 1993}, which is used to check for any biases in the models.

The overall measure of fit is assessed using the modelling efficiency (EF), a statistic close to the coefficient of determination R^2 that can be used for nonlinear models {Mayer and Butler, 1993}. The EF is close to 1 for good fittings, and is ≤ 0 when the model is equal to or less good than the mean of the observations. In addition to EF, the root mean square deviation (RMSD) is also computed. The RMSD represents the standard deviation of the differences between predicted and observed values.

The RMSD can be used to aggregate the magnitudes of the errors in predictions over the whole dataset, providing a single measure of the predictive power of all the models together {Hyndman and Koehler 2006}. It can then be used to evaluate the effects of different data treatments (e.g., fitting Gompertz functions on raw data vs. standardized data), or to compare different type of models (e.g., Gompertz vs. general additive models using standardized data).



30 — Figure 5.1 | Example of a verification plot produced by the plotWoodGrowth function.
Observations (points) and fitted Gompertz functions (lines) for the three cumulated cell counts (nM: mature zone; nWM: wall thickening plus mature zone; nEWM: enlarging plus wall thickening and mature zone). Modelling efficiency coefficients (EF) are given in the top left corner for the three Gompertz models. This figure was produced using standardized data from one silver fir monitored in 2007 in Grandfontaine {Cuny et al. 2012}. Figure from Rathgeber et al. {2018}.

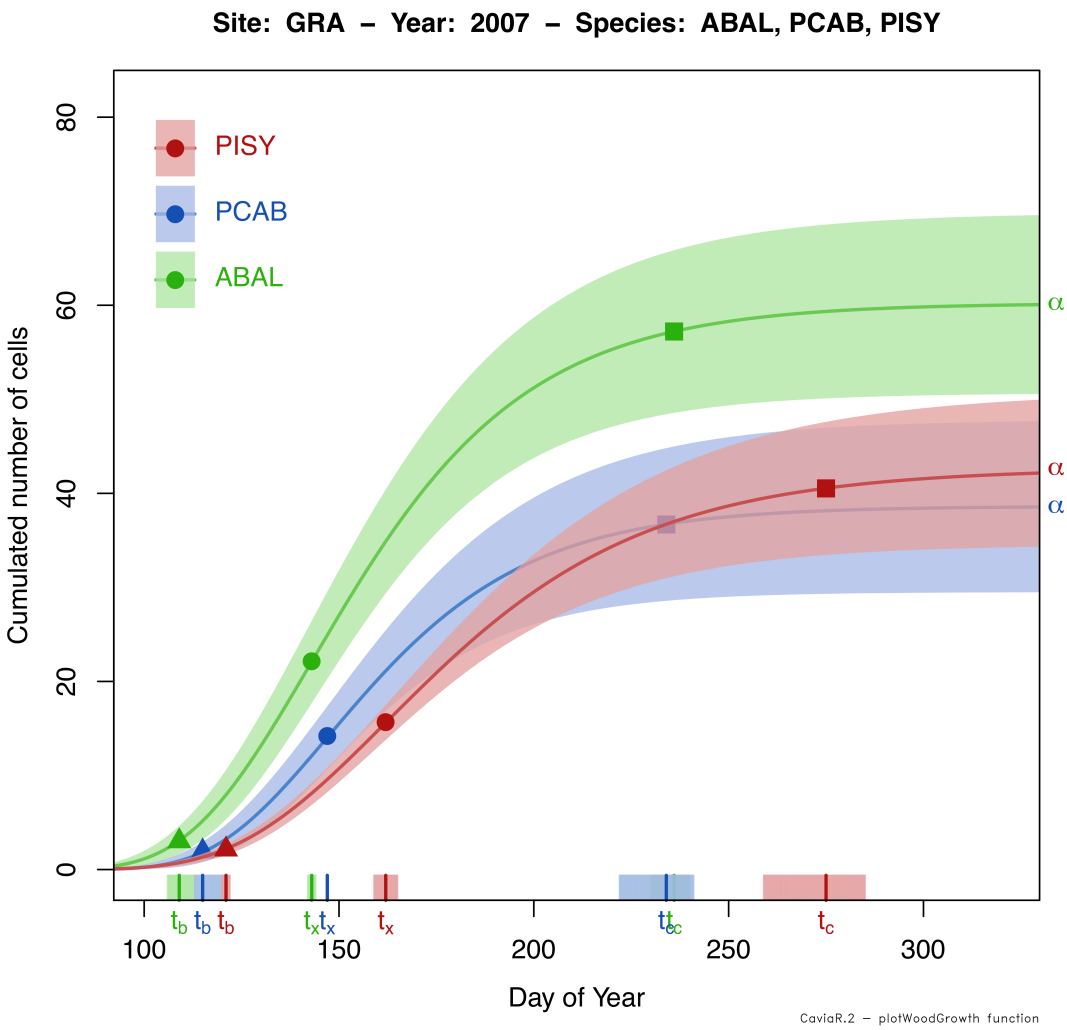
5.2.3 | Computing biological meaningful parameters from the Gompertz models

From the fitted parameters of the Gompertz function (α, β and κ), six additional biologically meaningful parameters can be computed {Rathgeber et al. 2011b}. These parameters are: (i) tb and (ii) tc, the dates at which 5 and 95% of the growth is completed, respectively; along with (iii) dg, the time elapsed between tb and tc; and (iv) gm and (v) gx, the average and maximum growth rates, respectively; and also (vi) tx, the date of occurrence of gx.
tb, tc and dg are computed as follows:

$tb = \beta - \log(-\log(0.05)) / \kappa$ [Equation 5.3]
$tc = \beta - \log(-\log(0.95)) / \kappa$ [Equation 5.4]
$dg = tc - tb$ [Equation 5.5]
gm, gx and tx are computed as follows:
$gm = 0.9\ \kappa\ \alpha / \log(\log(0.05)/\log(0.95))$ [Equation 5.6]
$gx = \kappa\alpha / \exp(1)$ [Equation 5.7]
$tx = \beta/\kappa$ [Equation 5.8]
The CAVIAR fitGompertzModels function automatically outputs a data frame containing, for each year, and each tree available in the dataset, the three fitted parameters of the Gompertz model (α, β and κ), along with the six computed biological meaningful parameters (tb, tc, dg, gm, gx and tx), and the three estimators of the goodness-of-fit (R^2, EF and RMSD).

5.2.4 | Visualization of the fitted Gompertz models

In order to check the behaviour of all the trees individually, the results of the Gompertz function fittings have to be displayed and verified for all the trees of a dataset. This can be done automatically in CAVIAR using the plotWoodGrowth function (see an example in Figure III.9).
Once individual fittings have been approved, Gompertz curves can then be plotted for a group of trees — using plotWoodGrowth function again.
If the behaviours of the different trees of the group are coherent, the group can then be represented by its mean and standard error — using plotWoodGrowth function again.
Finally, averaged wood growth dynamics can be compared between groups of trees representing different, e.g., years, species or sites [see an example in Fig. 5.2], using plotWoodGrowth function again.



31 — Figure 5.2 | Example of a presentation plot produced by the plotWoodGrowth function.
The average wood growth dynamics of five silver fir (ABAL), five Norway spruce (PCAB), and five Scots pine (PISY), during the growing season 2007 at Grandfontaine, in the Vosges Mountains (France), using data from Cuny et al. {2012}. The triangles and the squares represent the dates (tb and tc, respectively) when the Gompertz curves reach 5 or 95% of the total final ring cell number. The circles represent the Gompertz curve inflexion points (tx), i.e., the dates when cell production is maximal. The α signs mark the asymptotes of the Gompertz models. The envelopes represent the confidence interval. Figure from Rathgeber et al. {2018}.

5.2.5 | Statistical analysis of the fitted parameters

As for the critical dates, the fitted parameters of the Gompertz functions can be compared between groups of trees using bootstrap permutation tests. These tests can be performed in CAVIAR using the computeBoostrapTest function (see the section on the statistical analysis of wood formation critical dates for more details).

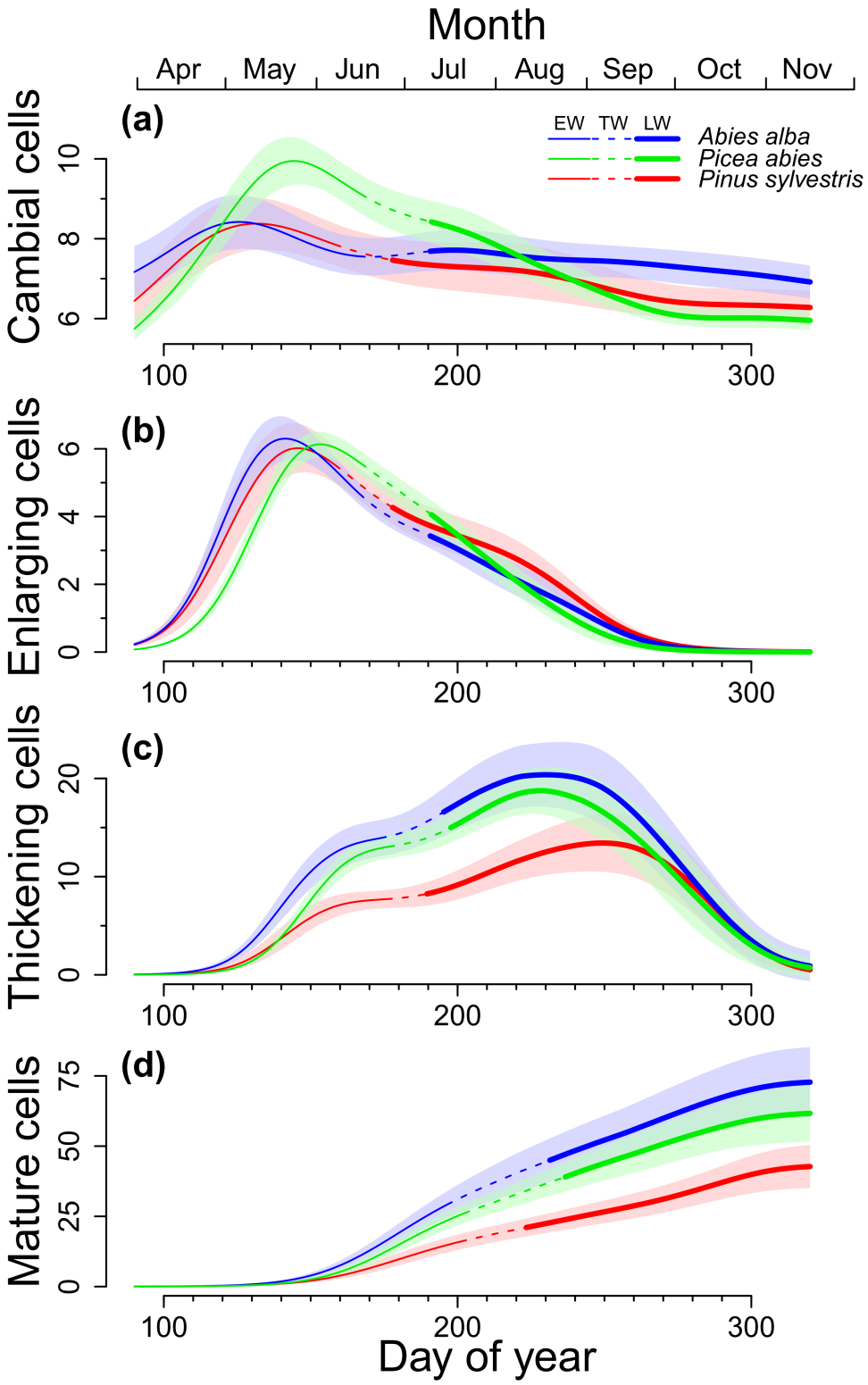
5.2.6 | A simple physical model of xylem cell production

The total number of cells in the ring at the end of the growing period (RCN) is computed as the median of the total number of cells of all the samples taken after the cessation of cell production. We propose expressing RCN as a function of the

average cell production rate computed from the Gompertz function and the cell production duration computed from the observations:
RCN = f (rm × DtE) [Equation 5.9]
A sensitivity analysis of the model was performed to determine the specific contribution of the production rate and duration to the total number of cells produced. Sensitivity analysis of a model involves assessing the impact of relative changes in its input variables (which are generally varied over a range of two standard deviations around the mean) on the model output variables {Cariboni et al., 2007}.

5.2.7 | Application of the General Additive Models to describe tree-ring formation dynamics

The intra-annual dynamics of wood formation, which involves the passage of newly produced cells through three successive differentiation phases (division, enlargement, and wall thickening) to reach the final functional mature state, has traditionally been described in conifers as three delayed bell-shaped curves followed by an S-shaped curve. However, this classical view represented by the 'Gompertz function approach' (GFA) has been challenged using a novel approach based on 'data-driven' generalized additive models (GAMs). We demonstrated that GAMs outperformed GFA in describing intra-annual wood formation dynamics {Cuny et al. 2013}.
Wood formation dynamics captured using GAMs for our Grandfontaine stand in the Vosges Mountains revealed distinctive patterns for the seasonal changes in cell number in the different developmental zones. The number of cells counted weekly in the cambial and enlargement zones both followed bell-shaped curves skewed to the left [Fig. 5.3a, b]. The number of cambial and enlarging cells rapidly increased from April onwards to culminate in May, after which it decreased slowly until mid-September. By contrast, the number of cells in the thickening phase followed a bimodal, right-skewed curve [Fig. 5.3c]. A rapid increase in spring led to a first peak at the beginning of June. A second increase in summer resulted in a maximum in August. From then on, the number of thickening cells decreased rapidly until November. The number of mature cells followed two periods of rapid accumulation separated by a period of slower accumulation [Fig. 5.3d]. The first mature cells were completed during the second half of May. Earlywood cells reached maturity during the initial period of fast accumulation, from spring until mid-July; transition wood cells matured during the intermediate period of slower increase (mid-July to mid-August); finally, latewood cells were completed during the last period of steady accumulation between late August and late October.



32 — Figure 5.3 | Generalized additive models (GAMs) fitted to the number of cells counted weekly in the cambial (a), enlargement (b), wall-thickening (c) and mature (d) zones for *Abies alba*, *Picea abies* and *Pinus sylvestris*.
For each species, the line represents the mean values for 15 trees over 3 year (2007–2009), and the shadowed area delimits the 90 % confidence interval. The different line types represent the separation between earlywood (EW), transition wood (TW) and latewood (LW) cells. Figure from Cuny et al. {2014}.

5.3 | Exploring the variability of tree-ring formation dynamics

5.3.1 | Variability of tree-ring formation dynamics within tree

Circumferential variability and cambial stimulation by punching

Tree-ring characteristics variability around the stem (i.e. circumferential variability) is an overlooked problem in forest and tree-ring sciences in general. Wodzicki and Zajaczkowski {1970} already showed in a pioneer study that this problem may completely confuse the results from wood-formation-monitoring studies.

Standardisation (see chapter 3 for more details) of wood-formation-monitoring data was proposed by Rossi et al. {2003} as a mean to solve this problem. However our experience has shown that despite standardisation looks like a good practical solution, it cannot be applied blindly and most of the time do not improve the data significantly.

Another related problem we discovered working on the MLZ dataset is what we call the 'cambial stimulation'. Indeed, we observed a general decreasing trend in the number of xylem cell at the end of the growing season, when cambial activity is over. Some colleagues (Campelo com. pers.) also reported the same observations. This 'artificial' decreasing trend can only be explained by the fact that the cambium is stimulated by our punching. Despite the time we have devoted to this problem in the framework of Masoumeh Saderi's PhD thesis, we have not been able to demonstrate it in a statistically unambiguous way or to systematically correct for it. An interesting way to revisit this problem could be to explore the GloboXylo database for species that are particularly sensitive to punching and species that are not affected at all.

Variability along the stem

The variability of the timing of cambial activity and cell differentiation with stem height has been investigated by Anfodillo at al. {2012} using microcores taken every four days at nine different heights (from 1 to 9 m) along the stem of a Norway spruce (*Picea abies*).

The first enlarging cells appeared almost simultaneously all along the tree axis indicating that cambium activation is not height-dependent in this case. The rate of cell production must then increase with height in the same proportion of tree-ring width, but this is not documented in the literature.

Variability between branches and stem

The variability between the branches and the stem is not documented at all for now in the literature despite the fact that this is a very important matter concerning carbon allocation dynamics.

Several studies, however, use wood formation monitoring data from branches, handling them as if they were from the stem. This approach poses serious problems because while we do not know how the branches behave in relation to the stem, we do know that the variability is much greater in the branches than in the stem.

Variability between the roots and the stem

The secondary growth of higher-order roots has seldom been studied {Chaffey 2013}, but is subjected to the same cascade of phenological events as stems or branch wood. The timing, however, differs and generally follows an extended calendar, with an earlier timing of onset and later cessation of the differentiation period compared to stem wood {Thibeault-Martel et al. 2008}.

5.3.2 | Variability of tree-ring formation dynamics with tree social status

Introduction

As we already mentioned it in Chapter 4, section 4.3.3, our knowledge about the influences of environmental factors on tree growth is principally based on the study of dominant trees, despite the fact that intermediate and suppressed trees represent a large proportion of the stems in a stand and have an important contribution in wood production.

In this section we come back to our CPX dataset, where cambial activity was monitored weekly in 2006 for three crown classes in a 40-year-old silver-fir (*Abies alba*) plantation near Nancy (France) and timings, duration and rate of tracheid production were assessed from anatomical observations of the developing xylem.

The main objective of this study was to understand how the timings, duration and rate of xylem cell production – the most important features of tree-ring formation – change according to tree size, while the other main influencing factors (site conditions, climate, species and age) are fixed.

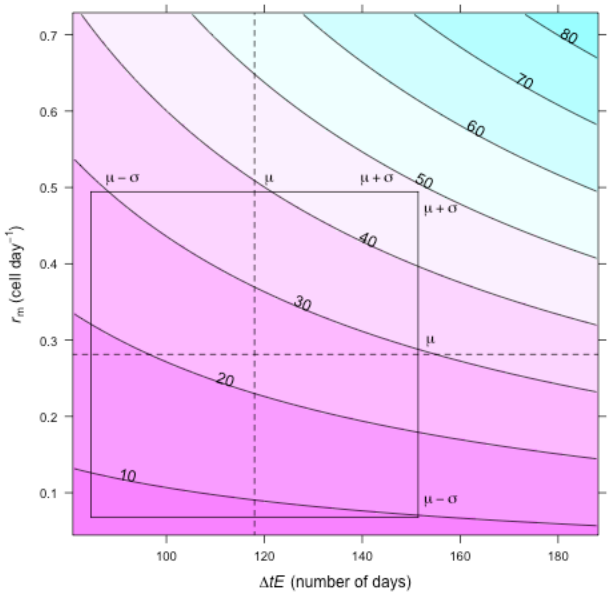
Our general hypothesis is that the timings and duration of cambial activity are principally under the control of environmental factors external to the stand, while its rate is principally under the control of factors related to tree social status within the stand. Following this idea, the hypothesis was tested that all the trees have similar timings (initiation, culmination and cessation) of cambial phenology because they all follow the same external environmental forcings (this was developed in chapter 4). Moreover, the hypothesis was tested that the rate of xylem cell production is related to tree size, because in a closed stand, the bigger a tree is, the greater its access to resources.

Summary of the results

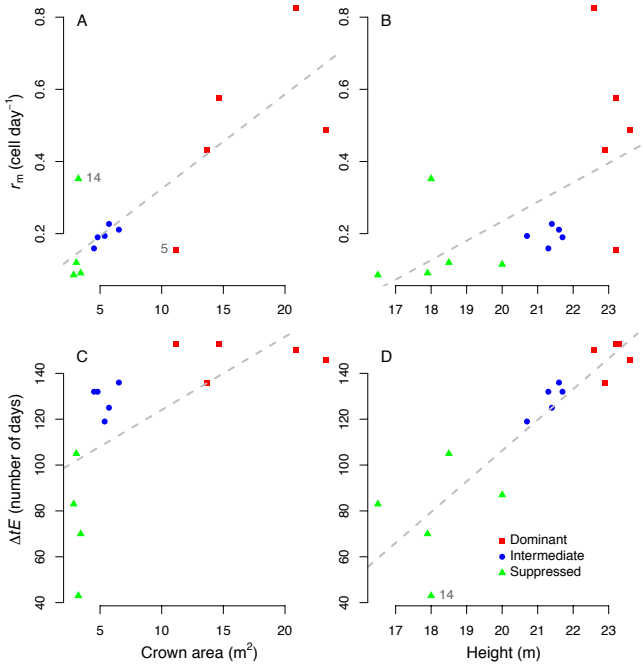
This study on the intra-annual dynamics of wood formation in a silver-fir plantation has shown that the timings, duration and rate of xylem production change according to the social status of trees. Cambial activity started earlier, stopped later and thus lasted longer in dominant trees than in intermediate and suppressed ones. Within the stand, the duration of the growing season varied from 3 to 5 months, a difference as wide as what can be observed when comparing temperate and boreal forests.

Moreover, during the growing season, cambial activity was more intense for dominant trees than for intermediate and suppressed ones. Although the duration and rate of cambial activity acted together so that dominant trees produced wider tree rings than intermediate and suppressed ones, it was estimated that about 75% of the variation was attributable to the rate of growth, while only 25% was

attributable to growth duration [Fig. 5.4]. These results show that the rate of cell production is an essential component of the growth process that should be considered in studies of intra-annual dynamics of wood formation in addition to cambium phenology.
Moreover, growth duration was correlated with tree height, while growth rate was better correlated with crown area [Fig. 5.5]. These results show that, in a closed conifer forest, stem diameter variations resulted principally from differences in the rate of xylem cell production.



33 — Figure 5.4 | Simulated total cell number (RCN) when the average rate (rm) and the duration (ΔtE) of cell production varied around their mean within a range of twice their standard deviation.
Dashed lines represent the means, while the frame delimits the area inside the mean ± s.d. Figure from Rathgeber et al. {2011}.



34 — Figure 5.5 | Relationships between the rate (rm) and duration (DtE) of xylem cell production and tree height (H) and crown area (CA).

Dashed lines illustrate the linear regressions. Tree numbers indicate outliers. Figure from Rathgeber et al. {2011}.

Influence of tree social status on the rate of cambial activity

The present results confirmed the hypothesis that cambial activity culminates at the same period for most of the trees of the stand. The fact that this date occurred before or close to the summer solstice confirms the proposition from Rossi et al. {2006c} that the maximal day length is interpreted by trees from boreal and temperate regions as a safety limit, after which the rate of xylem cell production must decrease, allowing cell-wall lignification to be completed before winter.

This study also demonstrated that the rate of cell production is greater in dominant trees than in intermediate and suppressed trees, casting new light on the previous knowledge on the intra-annual dynamics of wood formation. Numerous studies have recorded higher rates of cell production for trees exhibiting wider tree rings (i.e. containing a greater number of cells), regardless of the cause of the differences, i.e. either climate {Horacek et al., 1999; Rossi et al., 2007; Deslauriers et al., 2008}, vitality {Gricar et al., 2005, 2009}, age {Rossi et al., 2008a} or competition {Grotta et al., 2005; Linares et al., 2009}. However, these studies did not conclude – probably because they focused on cambium phenology – that cell production rate is an important, if not the main, driver determining wood production.

This work has also shown that the timing and rate of xylem cell production are not independent. In the dominant trees, for example, cambial activity started earlier and finished later (and thus lasted longer), and was also more intense with higher rates of cell production. This agrees with the results from De Luis et al. {2007}, showing that, in the Mediterranean region, only Aleppo pine trees with higher rates of cell production in spring were able to resume cambial activity in autumn. Thus, it is difficult to differentiate the effect of timing and rate on wood production. In this study, it was estimated that the final number of produced cells was mainly determined by the level of the cell production rate and only partially by the duration of the growing season. These results complement those from Lupi et al. {2010}, which demonstrated that the end of xylogenesis (i.e. the completion of cell wall lignification) is determined by the number of produced cells, which is in turn influenced by the onset of cambial activity. Indeed, the end of xylogenesis directly depends on the number of produced cells because more cells are produced and more time is needed to complete differentiation. However, the number of produced cells itself depends on the duration of cambial activity (i.e. not only on the beginning but also, to a lesser extent, on the end) and primarily on its rate.

Influence of tree dimensions on cambial activity

The height of a tree correlated very significantly with the duration of cambial activity, while the crown area correlated very significantly with the rate of cambial activity. This suggests that the advantages for a tree of bearing a larger crown and being taller than its neighbours were converted differently in terms of benefits for the dynamics of wood formation. The advantages for a tree of bearing a larger crown than its neighbours are quite obvious. Trees with larger crowns benefit from larger access to resources and larger photosynthetic capabilities, loading their phloem with a higher concentration of assimilates and delivering more carbohydrates to the cambium, allowing them to reach a higher level of radial

growth. The relationship between the rate of cambial activity and the availability of carbohydrates has been demonstrated by Deslauriers et al. {2009}, who showed that the highest rates of cell production were associated with the highest sugar contents in the outer wood of the most productive poplar clones.

The advantages for a tree of being taller than its neighbours are not so obvious. The forest overstorey environment is characterized by higher solar radiation, atmospheric vapour pressure deficit and wind velocity than in the understory {Aussenac, 2000}. An explanation for the earlier start of cambial activity in taller trees than in smaller ones could be that, in spring, the top of taller trees are warmed earlier in the morning and later in the afternoon by direct sunlight, allowing the level of critical temperatures triggering the onset of growth to be reached earlier in the season. This 'height-mediated thermal explanation' has the advantage of reconciling the well-established knowledge that spring temperatures trigger the onset of cambial activity {e.g., Rossi et al., 2008b; Seo et al., 2008; Begum et al., 2010} and the present observations that in the same stand, under the same climatic conditions, the onset of cambial activity was spread over several weeks according to tree height.

The relationship observed in this study between cambial activity duration and tree height could suggest a control of the apical meristem on cambial activity that translates the advantages for a tree of being taller than its neighbours into a signal triggering cambial activity onset and/or cessation in the stem. It has been suggested since the 1960s that dominant trees, showing larger and more vigorous crowns, have higher auxin availability, promoting early onset of cambial activity and a high rate of cell production {Kozlowski and Peterson, 1962; Larson, 1962}. More recent studies showing (a) that cambial activity can resume without any help from buds or growing needles {Little and Wareing, 1981; Oribe and Kubo, 1997; Rossi et al., 2009c}, (b) that auxin can be transferred to the cambium from both mature needles and dormant buds {Savidge and Wareing, 1982} and (c) that the amount of auxin required for cambial resumption can be found in dormant conifer tissues even during autumn and winter {Little and Wareing, 1981; Sunberg et al., 1991} demonstrate that the hormonal control of cambial activity is a very complex process that leaves the way open for the above-discussed 'differentiated thermal explanation' of cambial activity resumption. Thus, the relationships observed between tree dendrometric characteristics and cell production rate and duration are based on functional processes involving source–sink relationships principally, but also, presumably, hormonal control of cambial activity. However, more work is needed to understand the relative importance of these biological processes.

Conclusion

In this work the hypothesis emerged that the duration of cambial activity was influenced by tree height, which provides indications on the sensitivity of a tree to the increases in spring temperatures, while the intensity of cambial activity was rather influenced by crown area, which provides indications on the photosynthetic capability of a tree under normal conditions during the growing season. These results suggest that the intra-annual dynamics of wood formation is strongly related to tree size (social status and dimensions) through functional processes involving source – sink relationships principally, but also hormonal control.

5.3.3 | Variability of tree-ring formation dynamics between different species — life strategy in wood formation dynamics

Introduction

Living organisms develop different life strategies to access vital resources and cope with intra- and inter-species competition {Grime 1977}. These strategies can be seen in functional traits, which are measurable features that determine individual and species performance (e.g., survival, growth and reproduction) and, ultimately, their fitness in a given environment {Ackerly 2003}. For tree species, growth timing and intensity may provide critical functional traits portraying their life strategies, and the result of these strategies may be evaluated using the annual growth increment recorded in tree rings, which is known to be closely related to individual tree fitness {Baraloto et al. 2005, Poorter et al. 2008, Martinez-Vilalta et al. 2010}. Indeed, during an annual cycle, trees must adjust their period of activity to maximize resource exploitation and minimize exposure to harmful events. According to their life strategies, tree species may respond differently to this unavoidable trade-off between growth and survival. Early onset and late termination of growth may involve high risks because they increase exposure to disturbance, such as herbivore damage, and harmful climatic events such as spring or autumn frosts {Lockhart 1983, Hanninen 1991}. However, a tree that begins growth early in spring can benefit from favorable conditions and avoid competition with its later neighbors {Augspurger et al. 2005}. In contrast, a tree that begins late and ends early but demonstrates a high rate of growth may surpass its neighbors in longer-period growth.

It has been theorised that early-successional species are more prone to take risks, while late-successional species are associated with safer life strategies {Korner 2006, Korner and Basler 2010}. Pioneer species, for example, are often photoperiod insensitive in spring and become temperature sensitive once their chilling demand has been fulfilled, allowing them to grow as soon as the climate is favorable. Late-successional species, however, can be controlled by photoperiod in spring, with temperature only exerting a limited modulating effect once the critical day length has come, preventing them from beginning too early. Such intertwined influences of environmental factors and life strategies are not documented in boreal and temperate conifer species, for which temperature is known to control spring phenological events {Wielgolaski 1999, Deslauriers et al. 2008, Rossi et al. 2008a, Lebourgeois et al. 2010a}. However, the differences observed between early- and late-successional species in cambial activity resumption {Rossi et al. 2006a, 2008a} and budburst {Lebourgeois et al. 2010a} seem consistent with the theory of riskier life strategies in pioneer species.

The contrasting strategies adopted by early- and late-successional species are also visible in their long-term growth dynamics. At the seedling stage, in an open field, early-successional species generally reach higher growth rates than late-successional species because they have higher capacities to capture resources {Reich et al. 1998, Lusk 2004, Niinemets 2006}. Late-successional species, however, are more efficient in their resource utilization, which ultimately allows them to grow at higher rates and surpass early-successional species during forest closure {Lusk 2004, Niinemets 2006, Boyden et al. 2009}.

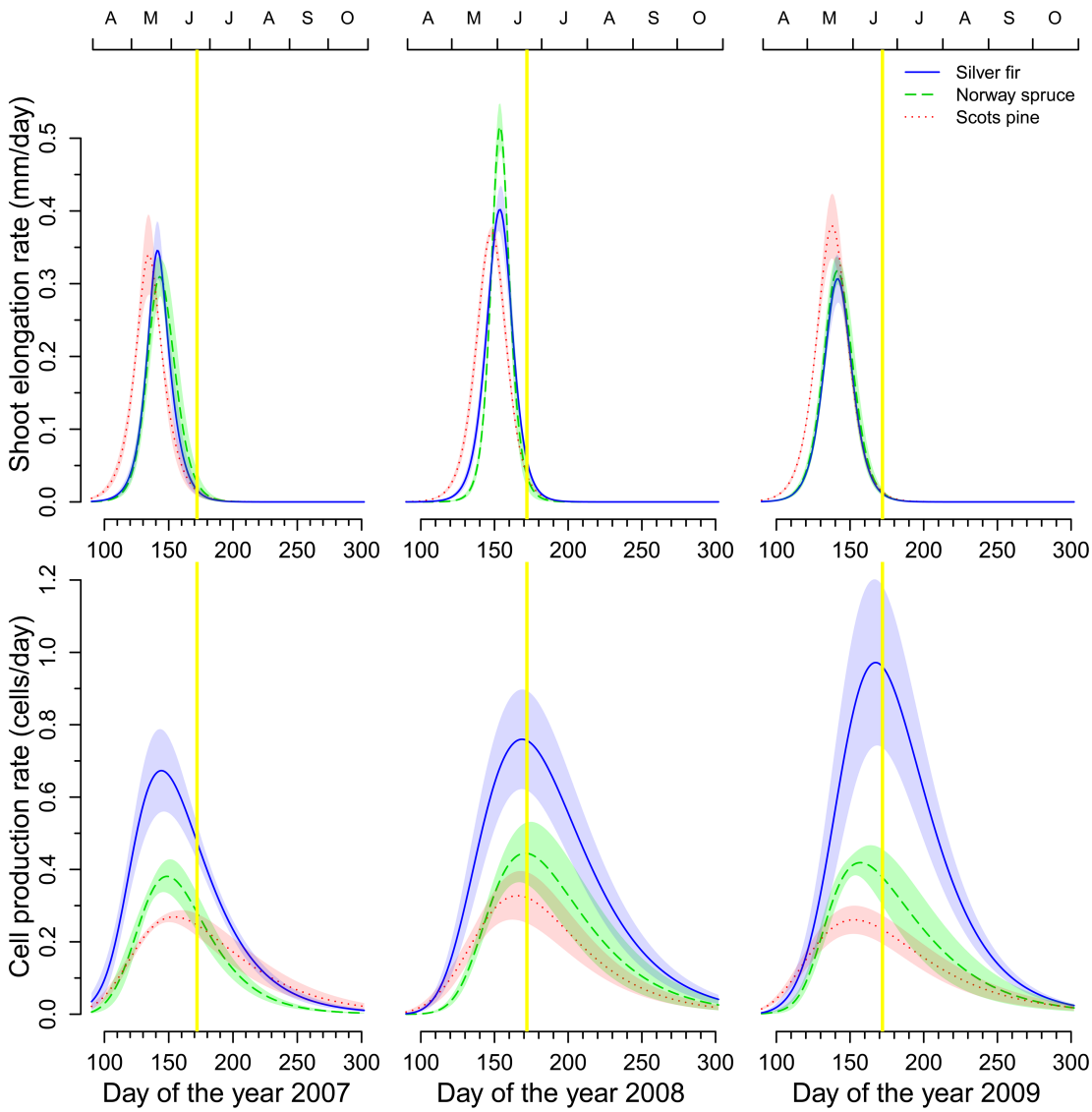
Over 3 years, from 2007 to 2009, we monitored (on the Grandfontaine site located in the Vosges Mountains) needle phenology and intra-annual dynamics of shoot elongation, cambial activity and wood formation in five silver firs (*Abies alba*), five Norway spruces (*Picea abies*) and five Scots pines (*Pinus sylvestris*). We chose to base our study on silver fir, Norway spruce and Scots pine because they are the three main conifer species in Europe and can be found growing in the same site despite contrasting autecologies.
Selecting species of the same plant functional type (i.e., ever- green conifer) allowed us to compare their life strategies according to pioneer or late-successional status, without confusion with the deciduous or evergreen character. Finally, conifers produce wood comprising 90% of tracheids, which facilitates monitoring and comparing their intra-annual wood formation dynamics. The very shade-tolerant silver fir is more frost- and drought- sensitive than the intermediate shade-tolerant Norway spruce, while the light-demanding Scots pine is the most drought- and cold-resistant of the three species {Lebourgeois et al. 2010b}. Silver fir is also more thermophilic and has higher nutrient requirements than Norway spruce or Scots pine {Pinto and Gegout 2005}.
The three species also differ in their long-term growth dynamics {Bouriaud and Popa 2009}. Scots pine has optimal growth at younger ages, and its productivity then rapidly decreases to become the lowest of the three species. In contrast, silver fir presents a slow growth rate at young age, but its productivity gradually increases and remains high even for old trees. Norway spruce demonstrated an intermediate behavior: its optimal growth is observed later than for Scots pine, then its productivity decreases but remains the highest of the three species until intermediate age. All these characteristics make Scots pine a pioneer species, while Norway spruce is an intermediate- and silver fir is a late-successional species.
The main objective of this study was to understand how the intra-annual dynamics of wood formation is related to species life strategies. As a pioneer species, we expected Scots pine to present longer growth durations due to earlier onsets and later cessations. As intermediate- and late-successional species, we expected Norway spruce and silver fir to produce greater growth increments due to higher cell production rates. Considering the contrasting life strategies of the investigated species, we expected that the functional diversity of the stand, with respect to the intra-annual dynamics of wood formation, was sufficient to lead to a mutual beneficial sharing of the resources in time, resulting in a 'soft' competition between trees.

Results and discussion

Intra-annual dynamics of wood formation in temperate and cold forest ecosystems

In our study site cambial activity began between late April and early May, a few days before the onset of needle unfolding and shoot elongation [Fig. 4.6]. While needle unfolding and shoot elongation lasted only 1–1.5 months, tracheids accumulated over 3–4.5 months and matured over 5–6 months. During the growth period, the cambium produced cells at an average rate of 0.31 ± 0.03 cells day−1, for a final number of 44 ± 4 cells in the annual tree ring [Fig. 5.6].
Conifers grown in subalpine and boreal forests exhibit similar rates of cambial activity {Deslauriers and Morin 2005, Rossi et al. 2008b}, but with very different timings. In such cold environments, cambial activity begins 2–4 weeks later,

finishes 2–4 weeks earlier, and thus lasts 1–2 months less than those in temperate forests, which results in lower annual production, generally <30 tracheids {Rossi et al. 2006c, 2007, 2011, Deslauriers et al. 2008, Lupi et al. 2010}.



35 — Figure 5.6 | Rates of shoot elongation and tracheid production in Grandfontaine (Vosges Mountains).
For each species, the line represents the mean of the five monitored trees, and the shadowed area delimits the 90% confidence interval. The vertical yellow line represents summer solstice. Figure from Cuny et al. {2012}.

Influence of tree age and size on wood formation dynamics

In chapter 4, we saw that our observations support the hypothesis that early-successional species tend to adopt riskier life strategies, starting wood formation earlier and finishing it later compare to late-successional species.

On our site, pines exhibited a lower cell production rate than firs and spruces. However, Rossi et al. {2008b} and Rathgeber et al. {2011b} found that cambial activity was weaker for older and smaller trees. Therefore, we also calculated the rates of cell production in the CPX dataset in order to compare trees of the same age and of comparable size, and we confirmed that the maximum and mean rates

of cambial activity were higher for silver firs (0.82±0.18 and 0.49±0.11 cells/day, respectively) than for Scots pines (0.50±0.12 and 0.30±0.09 cells/day, respectively). The use of cell cycle length was also a method to minimize the effects of tree age and size by 'standardizing' the rate of cell production by the number of dividing cells, amplifying the intrinsic species behavior. The computation of this crucial functional trait slightly reduced the differences in cell production rate observed between species. However, the contrasting behaviors remained strong, with the minimal cell cycle duration being twice as long for Scots pine as for silver fir, Norway spruce being in between. Once again, this body of evidence supports the idea that early-successional species present a lower rate of cell production than late-successional species because they are less efficient at converting environmental resources into growth.

Life strategies in intra-annual wood formation dynamics

Our results demonstrated that the contrasting life strategies adopted by the three species were clearly apparent in the functional traits depicting the key features of their intra-annual wood formation dynamics. For example, to produce similar radial increment, cambial activity lasted 40% longer in pine than in spruce because its cell production rate was 30% slower. Firs were more difficult to compare with the two other species because they produced radial annual increments twice larger. However, the fir-specific relationships between radial increment and duration of cambial activity and the non-specific relationship between radial increment and cell production rate indicated that firs used less time than pines to produce similar radial increments because they exhibited higher rates. In summary, we described the Scots pine growth strategy as 'extensive' because it was characterized by long durations and slow rates, illustrating the characteristic behavior of early-successional species, which are prone to explore space and time to capture resources but are not very efficient in their utilization. In contrast, we described the growth strategy adopted by fir and spruce as 'intensive' because it was characterized by higher rates and shorter durations, illustrating the behavior of late-successional species, which are more cautious in their exploration of space and time but are more efficient in the utilization of the limited resources they have access to. The high rates of growth associated with the 'intensive' strategy suggest that at the dominant stage, silver fir, and to a lesser extent Norway spruce, are better competitors than Scots pine, while the long growing periods associated with the 'extensive' strategy suggest that Scots pine is more stress tolerant — see the Grime's C-S-R triangle for a nice representation of these different strategies {Grime 1977}.

Firs reached higher rates of cambial activity than spruces, which themselves reached higher rates than pines because their cambiums count more dividing cells during the growing season and, above all, because they have shorter cell cycles. The number of cambial cells is known to depend on tree species {Larson 1994} and vitality {Gricar et al. 2009}, and its influence on the rate of cell production is already well documented {Vaganov et al. 2006}. The strong influence of the cell cycle length is consistent with the results obtained by Deslauriers et al. {2009}. As cell cycle length is closely linked to the quantity of sucrose available around dividing cells {Riou-Khamlichi et al. 2000}, the shorter cell cycles observed in firs and spruces suggest that they can produce more assimilates than pines. The

results of Deslauriers et al. {2009}, however, suppose differences in cell cycle length between two poplar genotypes that have similar photosynthetic capacities, indicating that genetic control can also be envisaged. The suggested ability of fir and spruce to produce more assimilates may be related to their management of foliage turnover. Indeed, late-successional evergreen conifer species have slower foliage turnover than pioneer species. For example, leaf life span reaches 6–7 years in firs and spruces, compared with only 2–3 years in pines {Becker et al. 1995}. A slow foliage turnover allows the accumulation of several leaf cohorts that finally develop larger leaf areas than those of shorter turnover species without spending more energy {Lusk 2004}.

Life strategies, fitness and functional convergence of conifer species

When regrouping the data from the three species, we estimated that about 75% of the annual radial increment variability was attributable to the rate of cell production and only 25% to its duration. We found the same values to explain the tree-ring width variability between trees belonging to different social status in a mature silver fir plantation (previous section). These results suggest that the rate of cell production is the main component of the growth process that influences the fitness of a particular tree compared with its neighbors, whether of the same or different species.

Mixed stands have been theorized to achieve greater overall productivity than monocultures because of the 'competitive production principle', assuming that the competition acting between trees of different species is inferior to that between trees of the same species {Vandermeer 1989}. This reduction of competition is a consequence of the functional diversity between species, which leads to a complementary resource use in two dimensions: first in space, e.g., by the complementarity of crown and root architectures; and second in time, by the occupation of different time windows to carry out different vital processes {Richardson and O'Keefe 2009, Ishii and Asano 2010}. Indeed, we observed that needle phenology and primary growth critical dates (onset, maximal rate occurrence and cessation) were spread over a few weeks depending on the species. In contrast, we found that the maximal rate of tracheid production culminated at the same time for the three species, indicating that most wood production occurs at the same time for all trees. For conifers, shoot production and wood formation mainly depend on current-year assimilates {Hansen and Beck 1990, Oribe et al. 2003}. Shoots, however, represent less than 10% of the annual carbon allocation, whereas wood is the largest carbon sink, representing about 45% of the annual allocation {Grote 1998}. Therefore, even if the spreading observed in shoot production suggests complementary resource use in time, the low quantity of energy needed to produce new shoots indicates that its benefit is limited.

On the contrary, the synchronicity observed in the occurrence of the maximal rate of wood production shows that species allocate great quantities of carbon to their stem at the same time. We found similar results for silver firs of different social status within a close monospecific stand (previous section). Thus, within a close conifer stand, the occurrence of the maximal rate of cell production is controlled by the same environmental factors regardless of tree species or size. Its convergence toward the summer solstice may suggest, as proposed by Rossi et al. {2006a}, that the maximum day length is this common environmental factor triggering growth

decrease, allowing trees to safely finish secondary wall lignification before winter. However, the inter-annual variations we observed concerning this trait are not fully consistent with this hypothesis and suggest that other climatic factors (soil humidity for example) may play a role. Whatever the environmental control behind this synchronization, it demonstrates a strong functional convergence between conifer species, leading to a 'hard' head-on competition for resources, with all the trees fully expressing their needs at the same time. This functional convergence may be heightened by the evergreen character of the studied species, which makes a real 'phenological escape' impossible and thus limits the benefit of a competition-avoidance strategy {Richardson and O'Keefe 2009}. The fact that early-successional species are not able to avoid the head-on competition imposed by this synchronization with late-successional species may be a clue to explain their elimination during conifer forest maturation.

Conclusion and perspectives

For the three studied conifer species, the growing season (as delimited by cambial activity onset and cessation) lasted about 4 months, while the whole process of wood formation lasted about 5–6 months. Needle unfolding and shoot elongation followed the onset of cambial activity and lasted about one-third of the season. Species-specific life strategies were recognizable through critical functional traits of intra-annual wood formation dynamics. Pines exhibited an 'extensive strategy' characterized by long duration and low rate of growth, while firs and spruces adopted an 'intensive strategy' with shorter durations and higher rates. The opposition between Scots pine extensive strategy and silver fir and Norway spruce intensive strategy is consistent with the theory that pioneer species are more disposed to take risks for exploring space and time in order to capture new resources, while shade-tolerant species safely favor maximal efficiency in the use of available resources.

Despite the fact that both the timing and rates of wood formation provided pertinent functional traits indicative of contrasting species life strategies, we estimated that about 75% of the annual radial increment variability could be attributed to the rate of cambial activity, and only 25% to its duration. Considering the strong link between secondary growth and fitness of forest trees, this result suggests that, whatever the life strategy, fitness relies mainly on one single crucial functional trait: the rate of cambial activity.

Moreover, this rate of cambial activity culminated at the same time for all the trees of the stand, whereas shoot elongation, for example, reached its maximal rate earlier in pines than in firs and spruces. These results suggest a strong functional convergence between co-existing conifer species concerning key traits that influence fitness. Furthermore, the synchronization of the rates of cambial activity indicates that all the trees express their maximal resource need at the same time, which must result in a hard head-on competition. The fact that early-successional species are not able to avoid this competition while being less efficient in using resources may be a clue to explain their elimination during conifer forest maturation.

5.3.4 | Variability of tree-ring formation dynamics along elevation gradients

Introduction

Xylogenesis is a very dynamic process, which creates the wood cells that accumulate, year after year, in tree-rings. The variations in the timings and rates of cell production along the growing season determine the total annual number of xylem cell and tree-ring width.

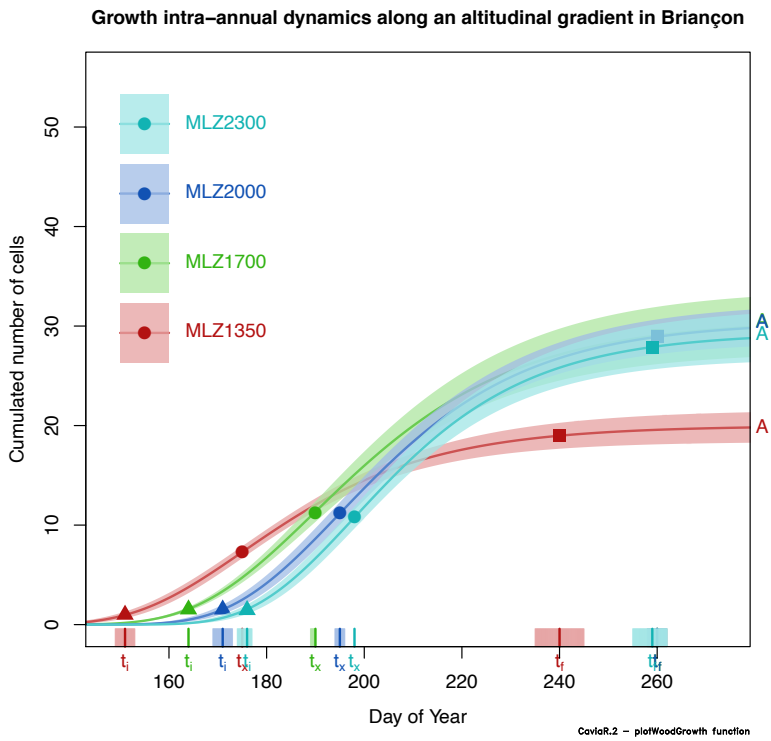
Analyzing wood formation dynamics along elevation gradients can provide crucial information about the adjustment of growth parameters (timings and rates) with elevation and related environmental factors such as temperature. The main objective of this study is to understand how trees are able to grow in contrasting climatic conditions by adjusting their seasonal dynamics of wood formation.

Following Rathgeber et al. {2011}, we hypothesize that: (1) the rate of xylem cell production (i.e. the intensity of cambial activity) is mainly driven by internal factors that can be related to e.g., size, age, vitality; while (2) the time length of xylem cell production (i.e. the duration of cambial activity) is mainly driven by environmental factors such as e.g., temperature, photoperiod, water availability.

To test this hypothesis, the xylogenesis of 60 larch trees, distributed in four stands, and spread along an elevation gradient of 1,000 m in the French Southern Alps was monitored all along the 2013 growing season (MLZ dataset, see chapter 3 for a detailed description of material). Wood growth dynamics was described by fitting Gompertz models on cell count data and the obtained growth parameters were compared along the elevation gradient using bootstrap tests. The relative contribution of rates vs. durations to the total annual production was assessed using a sensitivity analysis of a basic physical model (see section 5.2 for a detailed description of methods). Additionally, data from DNN + LST elevation gradient were used to contribute to the question.

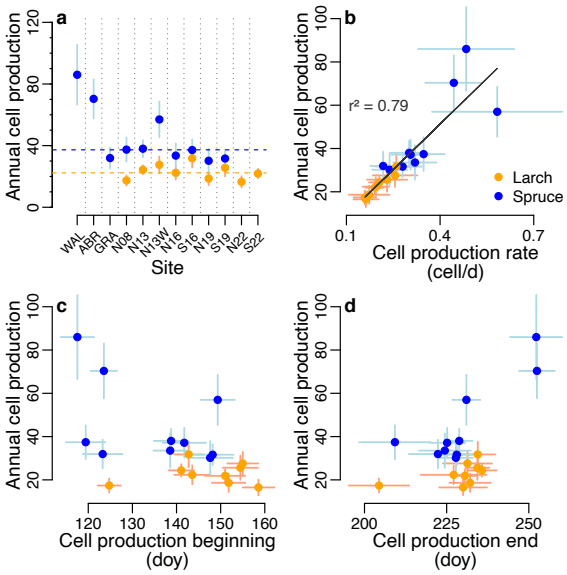
Result and discussion

As we saw in chapter 4, cambial activity duration (CAD) in MLZ exhibited parabolic a trend along the elevation gradient, with the shortest duration occurring at 2300 m (CAD of about 3 months), and the longest one at the 1700 m site (CAD of about 4 months). However, the shortening of the growing season with increasing elevation was compensated by an increase in the cell production rate leading to comparable tree-ring widths in the three upper stands [Fig. 5.7]. The lowest stand, on the contrary, exhibited narrow tree-rings due to both low production rates and short durations related to water stress. The sensitivity analysis of the basic physical model parametrized for MLZ, DNN and LST data showed that the final tree-ring widths were mainly related to the rate of cell production (contribution of about 60 %) and only secondly to the duration of cell production (contribution of about 40 %) [Fig. 5.8].



36 — Figure 5.7 | Average wood growth dynamics for the four sites of the MLZ elevation gradient, where 15 trees per site were sampled weekly during the 2013 growing season.

The triangles and the squares represent the dates (t_b and t_c, respectively) when the Gompertz curves reach 5 or 95% of the total final ring cell number. The circles represent the Gompertz curve inflexion points (t_x), i.e., the dates when cell production is maximal. The α signs mark the asymptotes of the Gompertz models. The envelopes represent the confidence interval. Figure from Saderi et al. {in preparation}.

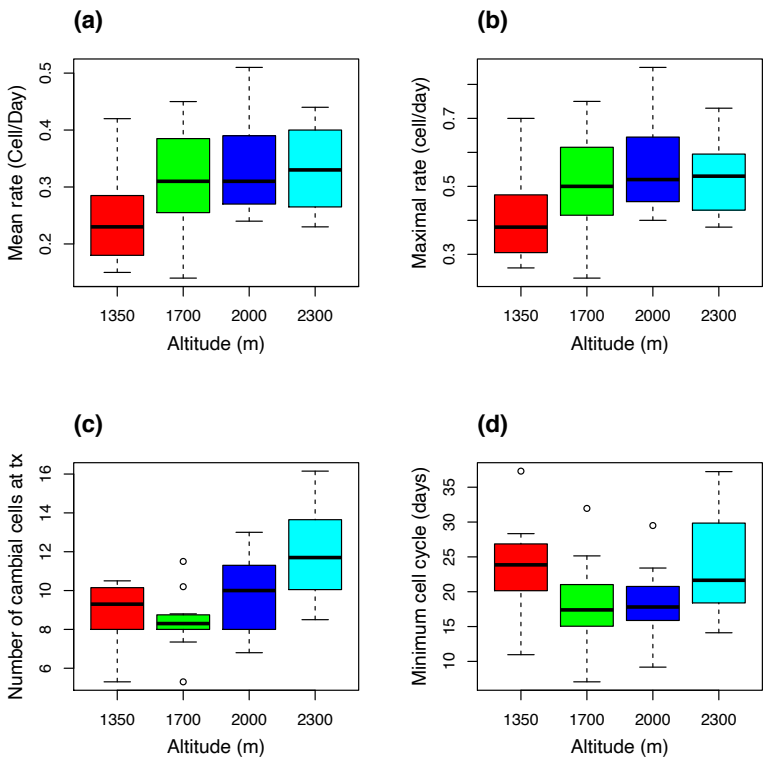


37 — Figure 5.8 | Annual cell production in Donon (DNN) and Lötschental (LST).

a, Number of xylem cells produced each year at the sites along the gradient (DNN + LST sites ordered by increasing altitude). **b**, Relationship between the annual cell production

and the daily rate of cell production. **c**, Relationship between the annual cell production and the onset of wood formation. **d**, Relationship between the annual cell production and the cessation of cell production. For each panel, every point represents the site and species average, while the bars symbolize the associated standard deviations. Figure from Cuny et al. {2018}.

We already discuss in chapter 4 how the duration of the growing season, which is determined by the onset and cessation of cambial activity, is changing in relation to elevation and environmental factors. The rate of cell production, on the other hand, is determined by the number of cell that are able to divide in the cambial zone, and by the time these cells need to complete their division process (i.e. the length of the cell cycle) {Vaganov 2006}. As we saw in chapter 2, cell division is known to be a very slow process in the cambium, which is sensitive to temperature {Larson 1994}. Our investigations showed that while moving towards higher elevation, the length of the cell cycle increases (from about 25 days at 1700 m to 30 days at 2300 m) as expected with decreasing temperatures [Fig. 5.9]. However, the number of dividing cells in the active cambium —and also in the dormant cambium— increases in the same proportion (from about 11 to 15 dividing cells in the cambium, when the production is maximal). This compensation of the increase of the cell cycle length by the concomitant increase of the number of dividing cells allows larch trees to maintain high production rates, even at high elevation. We believe that this mechanism, which was reported for the first time in this study, can be considered as an adaptation of larch trees to cold environments.



38 — Figure 5.9 | Change of cambium characteristics with elevation.
Comparison of (**a**) mean rate of cell production (cell/day); (**b**) maximal rate of cell production (cell/day); (**c**) number of cambial cells at the day of occurence of the maximal

rate of tracheid production (tx); and (**d**) minimum cell cycle length (days). Figure from Saderi et al. {in preparation}.

Our results highlighted two different types of adaptation mechanisms, allowing larch trees to maintain viable secondary growth at high elevation. We named the first type of mechanism: 'gradient resistance' since its action aim to reduce the rate of change along the gradient. In our works, this mechanism was observed for example for the durations of: (1) the cambial activity {Cuny et al. 2018, Saderi et al. in prep}, (2) the cell cycle {Saderi et al. in prep}, the different cell differentiation processes {Cuny et al. 2018}. We have shown, for example, that the onset of cambial activity is delayed at the top of the gradient, but less than would be expected from the sensitivity of the trees at the bottom of the gradient {Cuny et al. 2018}. Thus, in this type of adaptation mechanism, trees respond well to changes in environmental factors along the gradient, but their response is not proportional and does not reflect the full range of changes. We can therefore say that trees "resist" to environmental changes. We named the second type of mechanism: 'counter-gradient reaction' since its action aim to counter-balance the effects of environmental changes along the gradient. In our works, we observed this mechanism in the increase in the number of dividing cells of the active cambium, which counter-balance the increase of cell cycle duration due to the decrease of temperatures along the elevation gradient {Saderi et al. in prep}.

Conclusion and perspectives

In our MLZ elevation gradient, we observed more dividing cells in the active cambium, and also more resting cells in the dormant cambium, when moving upwards to higher elevation. This means that larch trees at higher elevation keeps a higher number of cambial cell all along the year, including during winter time when temperature are extremely cold. Further research is needed to understand what mechanisms are used by these trees to protect their fragile, living cambial cells from extreme frost occurring during winter at high elevations. What are the costs of maintaining such a high number of cambial cells is also an important question.

We believe we are the first to report such a mechanism of adaptation of tree growth to low temperature. The question of the importance of such a mechanism naturally arises: is this mechanism stable over time, is it common in different tree species, is it occurring in both altitudinal and latitudinal gradients? Exploring GloboXylo database will allow us to address these questions at the global scale for conifers.

5.4 | Ecological trends and life strategies in tree-ring formation dynamics

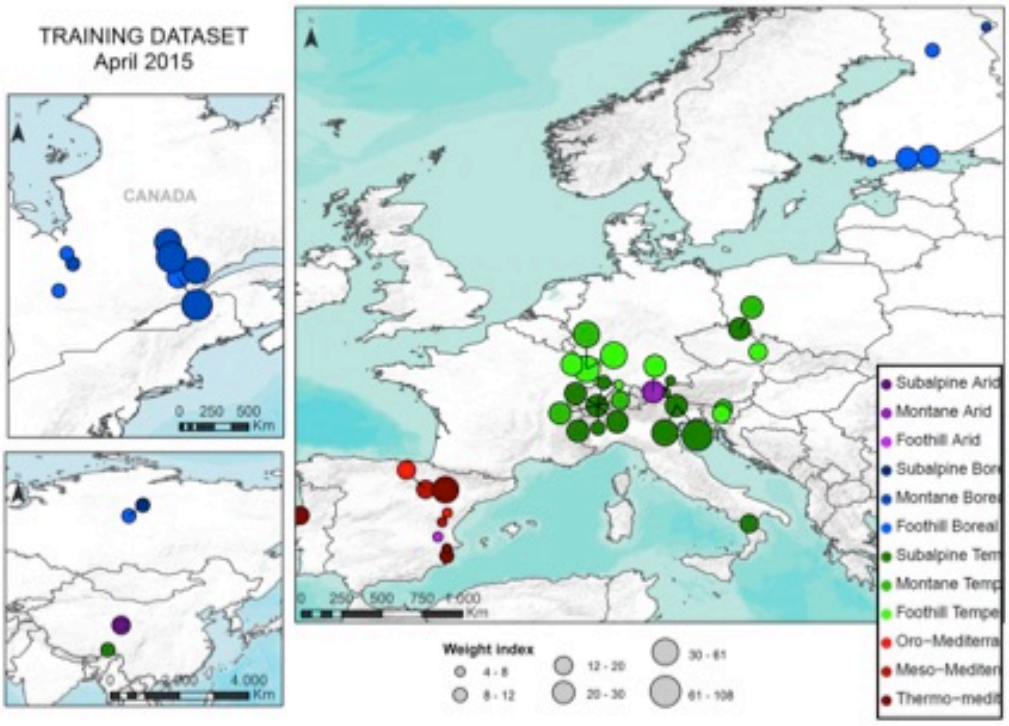
5.4.1 | Introduction

As a consequence of recent climatic changes, many studies have reported an increase in tree growth, forest ecosystem net primary productivity, and terrestrial biosphere carbon up-take, making forest one of the largest carbon sink on earth. Direct and remote observations, as well as eco-physiological models, have suggested that it is mainly the rise in temperature and the resulting extended

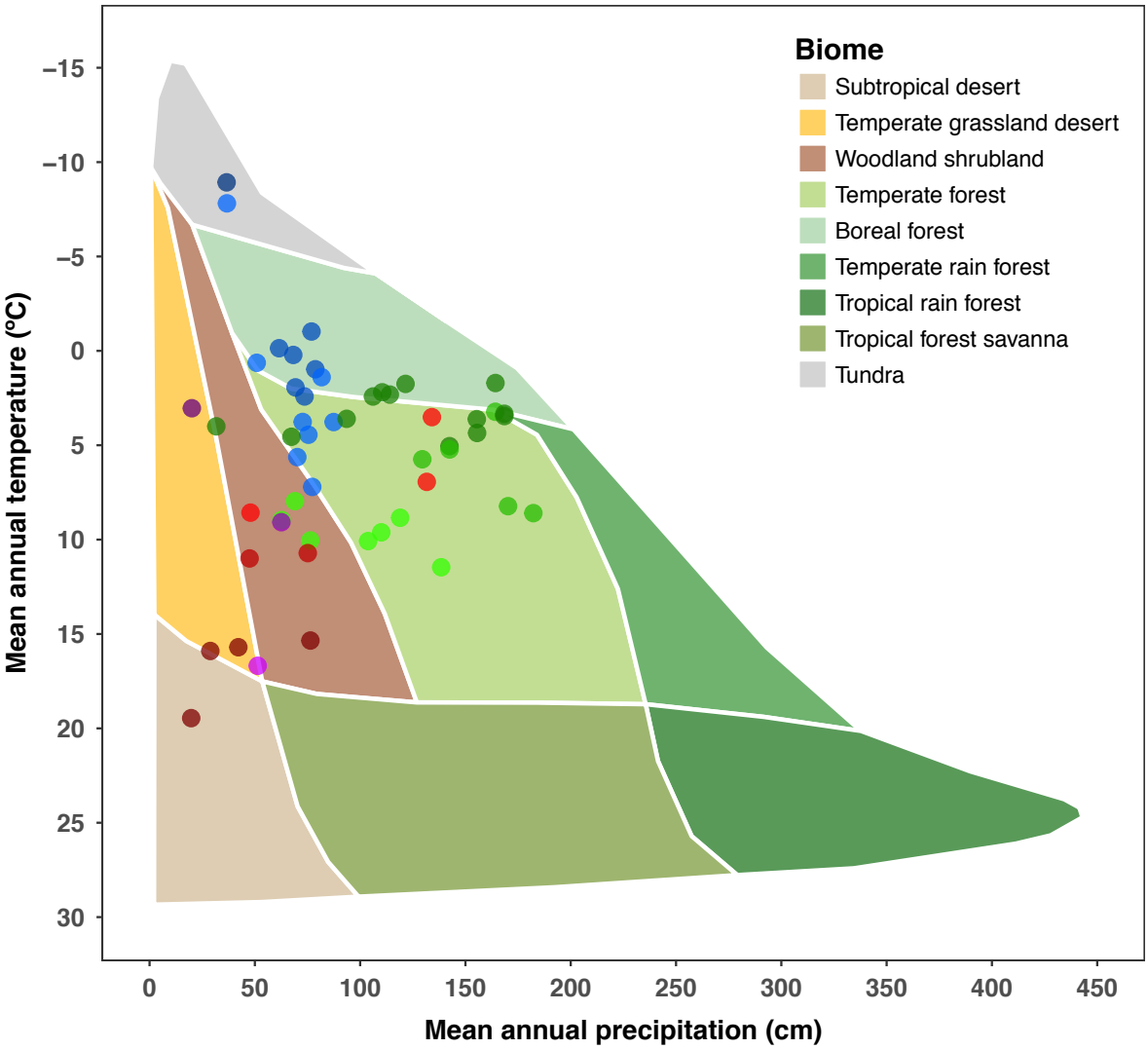
vegetation period that are responsible for forest enhanced productivity. However, a comprehensive study of the influences of the environment on forest productivity has still not been expressed in terms of the influence of limiting factors to the duration vs. the rate of growth.
Based on a large wood formation monitoring dataset, encompassing numerous sites from Mediterranean to Boreal conifer forests, we tested the hypothesis that the rate of xylem growth is even more important than its duration to explain forest productivity. Moreover, we explored the influence of the environmental conditions on the variation in both timings and rates of xylem cell production.

5.4.2 | Material and Methods

We collected data from 51 study sites spread at various altitudes and latitudes, on three continents (America, Europe, Asia), in extra tropical regions of the Northern Hemisphere (Boreal, Temperate and Mediterranean bioclimatic zones) [Fig. 5.10 and 5.11]. Wood formation was monitored at a weekly time-scale using histological sections of forming wood collected from the stems of 15 different conifer species. The critical dates and rates of xylem cell production were computed at tree level using logistic regressions and Gompertz fittings. A basic physical model was developed relating the total number of xylem cells with the rate and duration of their production. Sensitivity analyses of the basic physical model were used to reveal global ecological patterns in tree-ring formation, while regression analyses and mixed effect models were used to quantify the influences of the environmental factors.



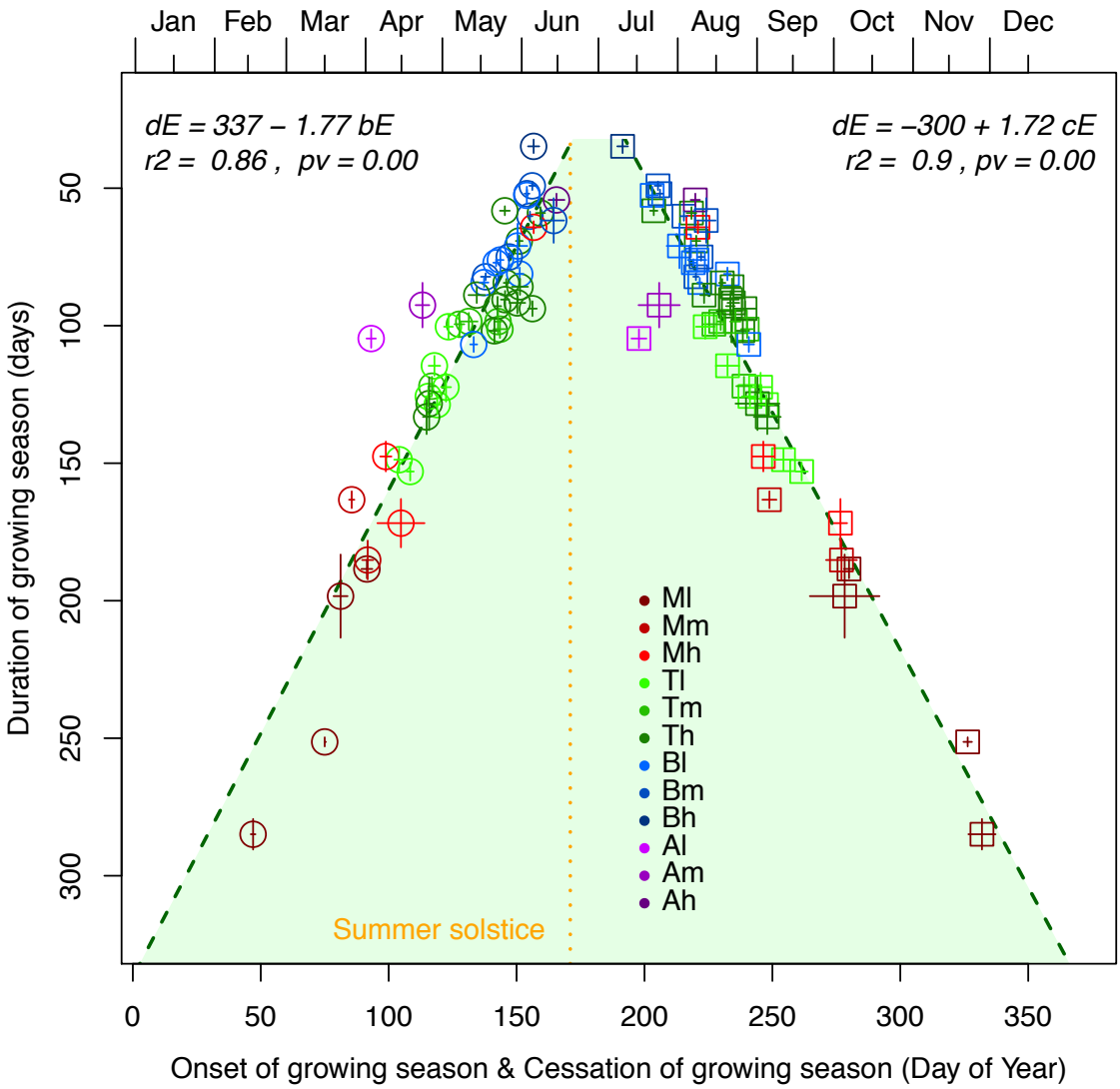
39 — Figure 5.10 | Map of GloboXylo sites.
Figure from Rathgeber et al. {in preparation}, prepared by Edurne Martínez del Castillo.



40 — Figure 5.11 | Distribution of GloboXylo data in a Whittaker biome figure. Figure from Rathgeber et al. {in preparation}, prepared by G. Perez-de-Lis.

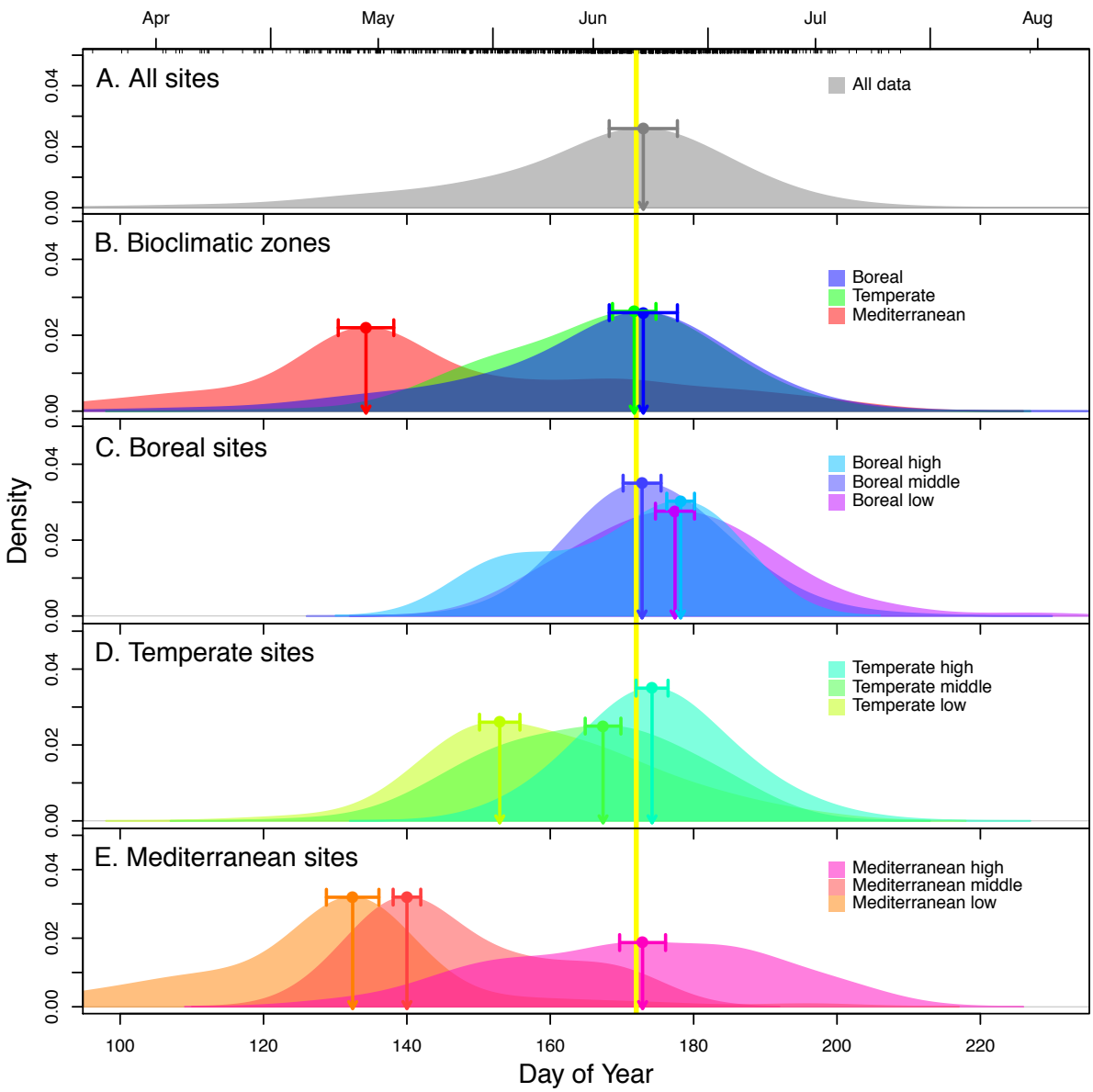
5.4.3 | Results

The duration of xylem cell production (DCP) exhibited a huge range of variation with a value going from about 20 days to more than 300 days. The DCP exhibited a very clear biogeographic pattern with values increasing when moving southward from the boreal forests (60 ± 2 days), to the temperate (107 ± 2 days), and the mediterranean regions (177 ± 5 days) [Fig. 5.12]. DCP data also exhibited a clear altitudinal trend with durations decreasing when going up in the mountains from 133 ± 3 days in average in the low lands, to 108 ± 4 for the montane zone, and 80 ± 3 for the sub-alpine zone.



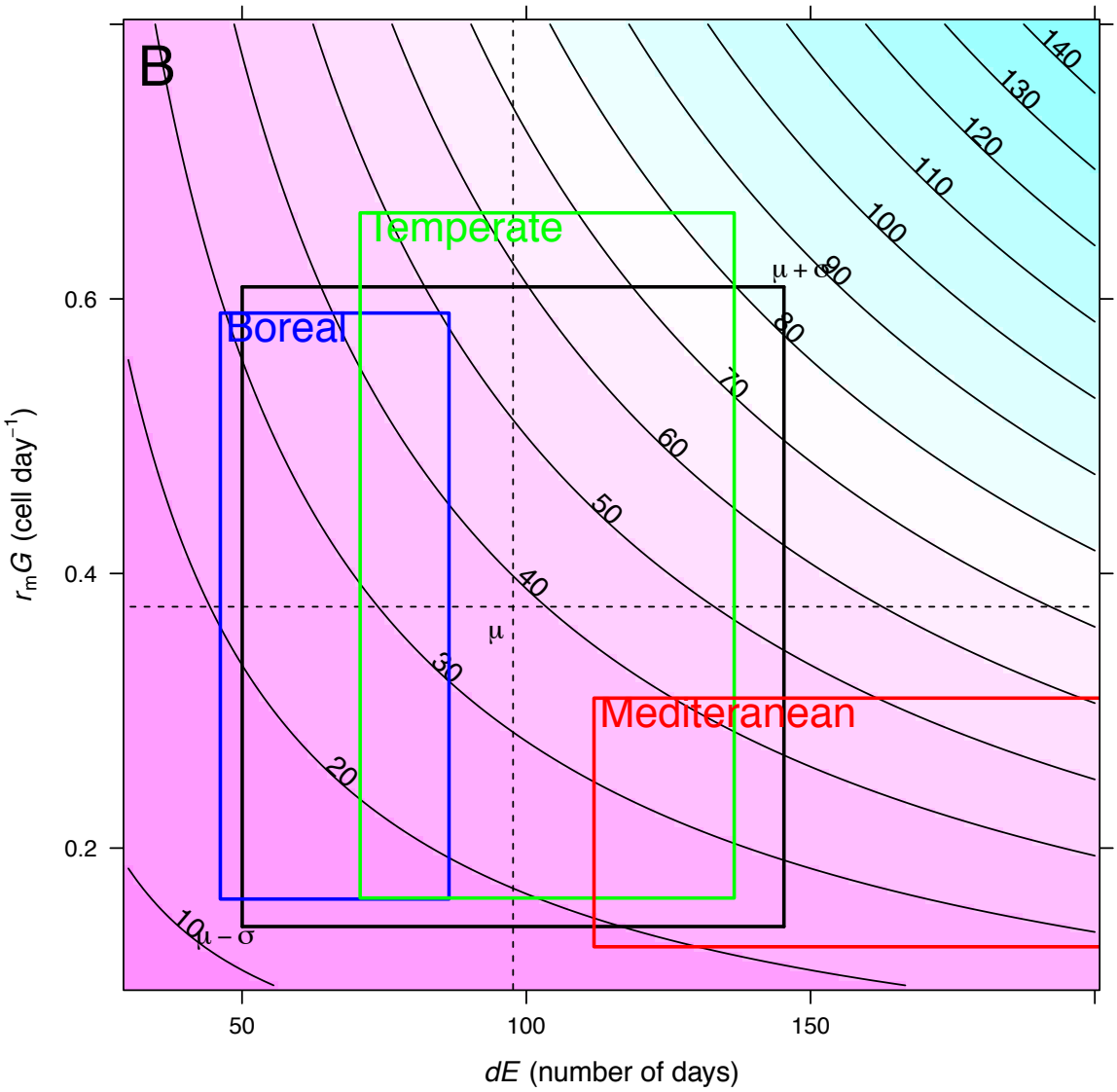
41 — Figure 5.12 | Onset and cessation (x-axis) of wood formation in function of duration (y-axis) exhibit significant (see bootstrap test histograms) linear relationships at global scale.
Figure from Rathgeber et al. {in preparation}, prepared by G. Perez-de-Lis.

Although the maximum rate of cell production is centred on the summer solstice as expected {Rossi et al. 2016}, we also observed large variations by biome and altitude: trees in the driest conditions being earlier, and those in the coldest conditions being later [Fig. 5.13].



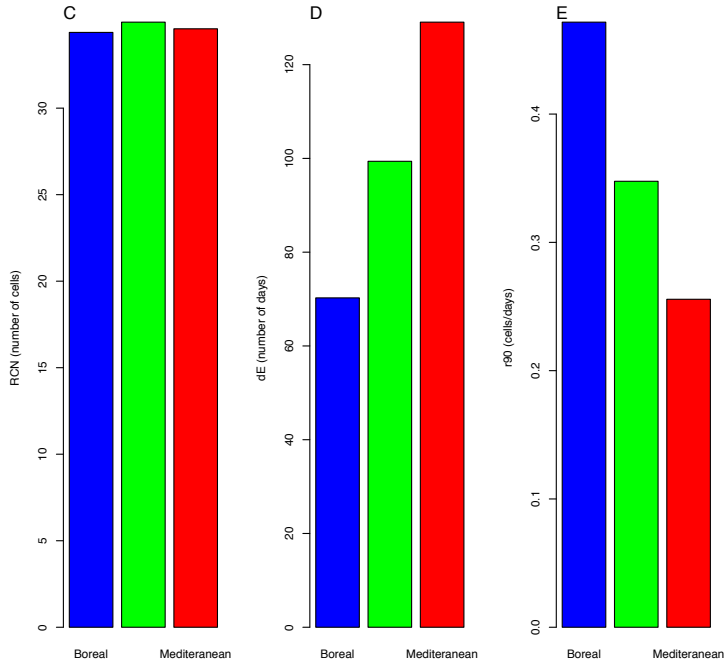
42 — Figure 5.13 | Global patterns the date of occurrence of the maximal rate of wood cell production.
Figure from Rathgeber et al. {in preparation}.

The basic physical model of xylem cell production was applied successfully to the whole dataset (including Mediterranean sites) explaining more than 80 % of the observed variability. The sensitivity analysis showed that the speed and duration contributed approximately equally to the variation in final number of cells (55% and 45% respectively) [Fig. 5.14].

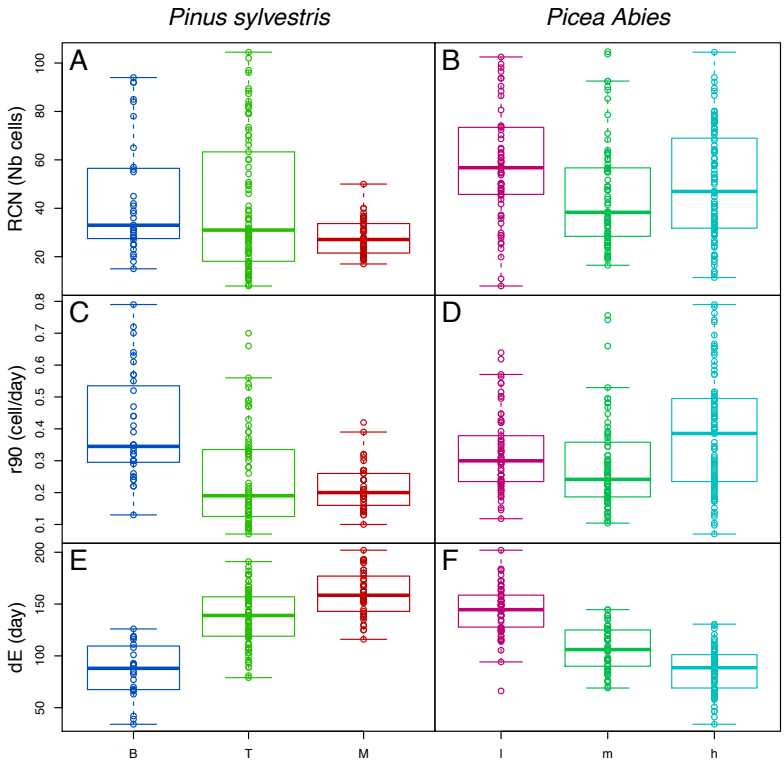


43 — Figure 5.14 | Sensitivity analysis of the basic physical model RCN = dE x r90.
The black square represents the variation between mean ± sd, while the coloured rectangle represent the variations for a given Biome. Figure from Rathgeber et al. {in preparation}.

Trees presented contrasted strategies according to the bioclimatic zone they belong to: while Boreal trees grew at a high speed during a short time; Mediterranean trees proceeded slowly, but for an extended period of time; temperate trees adopting an intermediate behaviour. Nevertheless, even for Mediterranean trees, the rate of growth remained the main driver of the final number of cell of a tree-ring [Fig. 5.15].

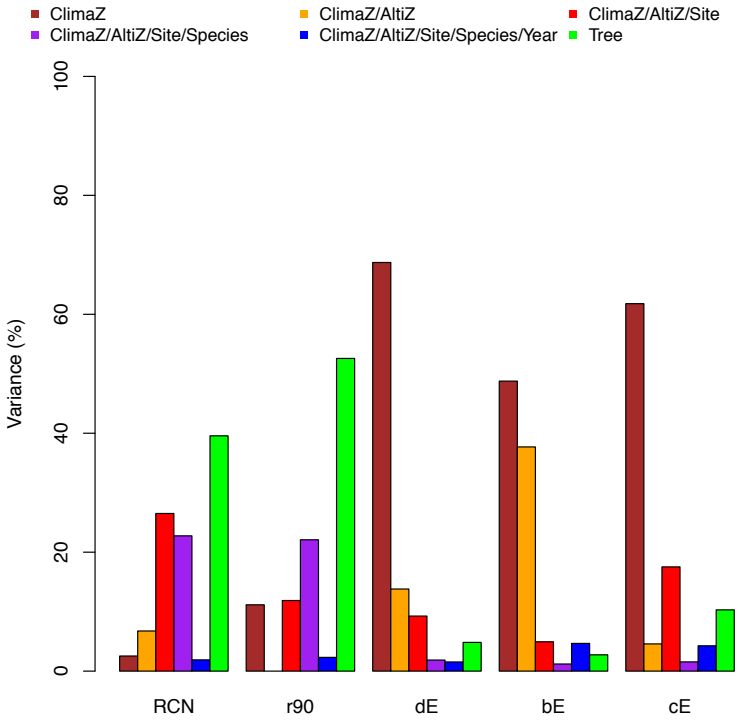


44 — Figure 5.15 | Growth strategy in terms of duration and rate of growth in the different biomes (Boreal, Temperate, Mediterranean).
Figure from Rathgeber et al. {in preparation}, prepared by G. Perez-de-Lis.



45 — Figure 5.16 | Growth strategy of Scots pine (Pinus sylvestris) in the different biomes (Boreal, Temperate, Mediterranean), and of Norway spruce (Picea abies) at different elevations (low, medium, high).
Figure from Rathgeber et al. {in preparation}, prepared by G. Perez-de-Lis.

Moreover, we showed that xylem growth phenology was consistently explained by the variation of thermal conditions occurring with altitude or latitude, while growth intensity was more related to species effect and site conditions [Fig. 5.17].



46 — Figure 5.17 | Contribution of the different factors that structure the GloboXylo dataset to the variability of each wood formation dynamics variables.

5.4.4 | Conclusion

Our results confirm that recent global warming could have resulted in extended period of growth explaining enhanced forest growth. However, we showed that the rate of growth, along with species behaviour and site conditions must be considered into vegetation models to assess the impact of future climatic changes on forest productivity.

5.5 | Conclusion and perspectives

5.5.1 | Synthesis

In the first section of this chapter, we presented our approach to describe and quantify accurately tree-ring formation dynamics using Gompertz functions thanks to the CAVIAR R package.

In the second section, we explored the variability of tree-ring formation dynamics according to tree age, size and social status, tree species, and site conditions. We discovered two distinct strategies concerning the dynamics of wood formation: the "intensive" strategy, in which trees grow quickly but not long; and the "extensive" strategy, in which trees grow long but slowly. We have also shown that trees adapt

to high elevation by increasing their number of cambial cells to compensate for the increase in cell cycle length caused by lower temperatures.
In the third section, we explored the large GloboXylo data base to show that the rate of xylem cell production by the cambium is the main driver of tree-ring width and wood production, while the duration of the growing season only played a secondary role. We also confirmed that temperatures have a major influence on the duration of wood production, while the rate is more influenced by microsite, stand and tree conditions.
Our results confirm that recent global warming could have resulted in extended period of growth explaining enhanced forest growth. However, we showed that the rate of growth, along with species behaviour and site conditions must be considered into vegetation models to assess the impact of future climatic changes on forest productivity.

5.5.2 | Perspectives

In the near future we would like to include more arid sites {Ren et al. 2018} in the GloboXylo data base and complete our work on the 'Ecological trends and life strategies in tree-ring formation dynamics'.
In a second time we would like to integrate the generalized additive model approach in CAVIAR to describe wood formation dynamics. This will allow to work on more extreme sites or years and provide more accurate estimates of timings and rate of wood formation dynamics.
Working with more accurate rates will allow to launch a new analysis of the GloboXylo data base to check the synchronisation between the maximal rate of xylem cell production and the summer solstice {Rossi et al. 2006}, and look for the influence of other potentially important climatic factors (e.g., soil water content, temperature).

5.6 | References

This reference list is incomplete and not properly formatted

Ackerly, D.D. 2003. Community assembly, niche conservatism, and adaptive evolution in changing environments. Int. J. Plant Sci. 164:165–184.

Augspurger, C.K., J.M. Cheeseman and C.F. Salk. 2005. Light gains and physiological capacity of understorey woody plants during phe- nological avoidance of canopy shade. Funct. Ecol. 19:537–546.

Baraloto, C., D.E. Goldberg and D. Bonal. 2005. Performance trade- offs among tropical tree seedlings in contrasting microhabitats. Ecology 86:2461–2472.

Bates, D., M. Maechler and B. Bolker. 2011. lme4: linear mixed-effects models using S4 classes. R package version 0.999375–39. http:// CRAN.R-project.org/package=lme4

Becker, M., D. Bert, G. Landmann, G. Lévy, J.C. Rameau and E. Ulrich. 1995. Growth and decline symptoms of silver fir and Norway spruce in northeastern France: relation to climate, nutrition and sil- viculture. In Forest Decline and Air Pollution Effects in the French Mountains. Eds. G. Landmann and M. Bonneau. Springer, Berlin, pp 120 –142.

Bouriaud, O. and I. Popa. 2009. Comparative dendroclimatic study of Scots pine, Norway spruce, and silver fir in the Vrancea Range, Eastern Carpathian Mountains. Trees Struct. Funct. 23:95–106.

Boyden, S.B., P.B. Reich, K.J. Puettmann and T.R. Baker. 2009. Effects of density and ontogeny on size and growth ranks of three compet- ing tree species. J. Ecol. 97:277–288.

Cariboni, J., D. Gatelli, R. Liska and A. Saltelli. 2007. The role of sensitivity analysis in ecological modelling. Ecol. Model. 203:167–182. Deslauriers, A. and H. Morin. 2005. Intra-annual

tracheid production in balsam fir stems and the effect of meteorological variables. Trees Struct. Funct. 19:402–408.
Deslauriers, A., H. Morin and Y. Begin. 2003. Cellular phenology of annual ring formation of Abies balsamea in the Quebec boreal forest (Canada). Can.J. For. Res. 33:190–200.
Deslauriers, A., S. Rossi, T. Anfodillo and A. Saracino. 2008. Cambial phenology, wood formation and temperature thresholds in two con- trasting years at high altitude in southern Italy. Tree Physiol. 28:863–871.
Deslauriers, A., A. Giovannelli, S. Rossi, G. Castro, G. Fragnelli and L. Traversi. 2009. Intra-annual cambial activity and carbon availability in stem of poplar. Tree Physiol. 29:1223–1235.
Gricar, J. 2007. Xylo- and phloemogenesis in silver fir (Abies alba Mill.) and Norway spruce (Picea abies (L.) Karst.). PhD Thesis, Slovenian Forestry Institute, University of Ljubljana, Ljubljana, 106 p.
Gricar, J., L. Krze and K. Cufar. 2009. Number of cells in xylem, phloem and dormant cambium in silver fir (Abies alba), in trees of different vitality. Iawa J. 30:121–133.
Grime, J.P. 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. Am. Nat. 111:1169–1194.
Grote, R. 1998. Integrating dynamic morphological properties into for- est growth modelling—II Allocation and mortality. For. Ecol. Manage. 111:193–210.
Hanninen, H. 1991. Does climatic warming increase the risk of frost damage in northern trees. Plant Cell Environ. 14:449–454.
Hansen, J. and E. Beck. 1990. The fate and path of assimilation prod- ucts in the stem of 8-year-old Scots pine (Pinus sylvestris L.) trees. Trees Struct. Funct. 4:16–21.
Ishii, H. and S. Asano. 2010. The role of crown architecture, leaf phe- nology and photosynthetic activity in promoting complementary use of light among coexisting species in temperate forests. Ecol. Res. 25:715–722.
Karkach, A.S. 2006. Trajectories and models of individual growth. Demogr. Res. 15:347–400.
Korner, C. 2006. Significance of temperature in plant life. In Plant Growth and Climate Change. Eds. J.I.L. Morison and M.D. Morecroft. Blackwell Publishing Ltd, Oxford, pp 48–69.
Korner, C. and D. Basler. 2010. Phenology under global warming. Science 327:1461–1462.
Larson, P.R. 1994. The vascular cambium: development and structure. Springer, Berlin, 736 p.
Lebourgeois, F., J.-C. Pierrat, V. Perez, C. Piedallu, S. Cecchini and E. Ulrich. 2010a. Simulating phenological shifts in French temperate forests under two climatic change scenarios and four driving global circulation models. Int. J. Biometeorol. 54:563–581.
Lebourgeois, F., C.B.K. Rathgeber and E. Ulrich. 2010b. Sensitivity of French temperate coniferous forests to climate variability and extreme events (Abies alba, Picea abies and Pinus sylvestris). J. Veg. Sci. 21:364–376.
Lockhart, J.A. 1983. Optimum growth initiation time for shoot buds of deciduous plants in a temperate climate. Oecologia 60:34–37.
Lupi, C., H. Morin, A. Deslauriers and S. Rossi. 2010. Xylem phenology and wood production: resolving the chicken-or-egg dilemma. Plant Cell Environ. 33:1721–1730.
Lusk, C.H. 2004. Leaf area and growth of juvenile temperate ever- greens in low light: species of contrasting shade tolerance change rank during ontogeny. Funct. Ecol. 18:820–828.
Martinez-Vilalta, J., M. Mencuccini, J. Vayreda and J. Retana. 2010. Interspecific variation in functional traits, not climatic differences among species ranges, determines demographic rates across 44 temperate and Mediterranean tree species. J. Ecol. 98:1462–1475.
Mayer, D.G. and D.G. Butler. 1993. Statistical validation. Ecol. Model. 68:21–32.
Niinemets, U. 2006. The controversy over traits conferring shade- tolerance in trees: ontogenetic changes revisited. J. Ecol. 94: 464–470.
Oribe, Y., R. Funada and T. Kubo. 2003. Relationships between cambial activity, cell differentiation and the localization of starch in storage tissues around the cambium in locally heated stems of Abies sachali- nensis (Schmidt) Masters. Trees Struct. Funct. 17:185–192.
Pinto, P.E. and J.C. Gegout. 2005. Assessing the nutritional and cli- matic response of temperate tree species in the Vosges Mountains. Ann. For. Sci. 62:761–770.
Poorter, L., S.J. Wright, H. Paz, et al. 2008. Are functional traits good predictors of demographic rates? Evidence from five neotropical for- ests. Ecology 89:1908–1920.
Rathgeber, C.B.K., S. Rossi and J.-D. Bontemps. 2011b. Cambial activity related to tree size in a mature silver-fir plantation. Ann. Bot. 108:429–438.

Reich, P.B., M.G. Tjoelker, M.B. Walters, D.W. Vanderklein and C. Bushena. 1998. Close association of RGR, leaf and root morphol- ogy, seed mass and shade tolerance in seedlings of nine boreal tree species grown in high and low light. Funct. Ecol. 12:327–338.

Richardson, A.D. and J.F. O'Keefe. 2009. Phenological differences between understory and overstory: a case study using the long- term Harvard Forest records. In Phenology of Ecosystem Processes. Ed. A. Noormets. Springer, New York, pp 87–117.

Riou-Khamlichi, C., M. Menges, J.M.S. Healy and J.A.H. Murray. 2000. Sugar control of the plant cell cycle: differential regulation of Arabidopsis D-type cyclin gene expression. Mol. Cell. Biol. 20: 4513–4521.

Rossi, S., A. Deslauriers and H. Morin. 2003. Application of the Gompertz equation for the study of xylem cell development. Dendrochronologia 21:33–39.

Rossi, S., A. Deslauriers, T. Anfodillo, H. Morin, A. Saracino, R. Motta and M. Borghetti. 2006a. Conifers in cold environments synchronize maximum growth rate of tree-ring formation with day length. New Phytol. 170:301–310.

Rossi, S., T. Anfodillo and R. Menardi. 2006b. Trephor: a new tool for sampling microcores from tree stems. Iawa J. 27:89–97.

Rossi, S., A. Deslauriers and T. Anfodillo. 2006c. Assessment of cam- bial activity and xylogenesis by microsampling tree species: an example at the alpine timberline. Iawa J. 27:383–394.

Rossi, S., A. Deslauriers, T. Anfodillo and V. Carraro. 2007. Evidence of threshold temperatures for xylogenesis in conifers at high altitudes. Oecologia 152:1–12.

Rossi, S., A. Deslauriers, J. Gricar, et al. 2008a. Critical temperatures for xylogenesis in conifers of cold climates. Glob. Ecol. Biogeogr. 17:696–707.

Rossi, S., A. Deslauriers, T. Anfodillo and M. Carrer. 2008b. Age- dependent xylogenesis in timberline conifers. New Phytol. 177: 199–208.

Rossi, S., C.B.K. Rathgeber and A. Deslauriers. 2009. Comparing nee- dle and shoot phenology with xylem development on three conifer species in Italy. Ann. For. Sci. 66:1–8.

Rossi, S., H. Morin, A. Deslauriers and P.Y. Plourde. 2011. Predicting xylem phenology in black spruce under climate warming. Glob. Change Biol. 17:614–625.

Ulrich, E. and S. Cecchini. 2009. Manuel de référence n°12 pour les observations phénologiques—Placettes des niveaux A et B (Level II plots within the European Union)—Deuxième version. Office National des Forêts (ONF), Fontainebleau, 27 p.

Vaganov, E.A., M.K. Hughes and A.V. Shashkin. 2006. Growth dynamics of conifer tree rings. Ecological studies. Springer, Berlin, 354 p.

Vandermeer, J. 1989. The ecology of intercropping. Cambridge University Press, Cambridge, 237 p.

Wielgolaski, F.E. 1999. Starting dates and basic temperatures in phe- nological observations of plants. Int. J. Biometeorol. 42:158–168. Wodzicki, T.J. 1971. Mechanism of xylem differentiation in Pinus sylvestris L. J. Exp. Bot. 22:670–687.

Wodzicki, T.J. 2001. Natural factors affecting wood structure. Wood Sci. Technol. 35:5–26.

Wodzicki, T.J. and S. Zajaczkowski. 1970. Methodical problems in studies on seasonal production of cambial xylem derivatives. Acta Soc. Bot. Pol. 39:509–520.

Zuur, A.F., E.N. Ieno, N.J. Walker, A.A. Saveliev and G.M. Smith. 2009. Mixed effects models and extensions in ecology with R. statistics for biology and health. Springer, New York.

CHAPTER 6 | KINETICS OF TRACHEID DIFFERENTIATION IN CONIFERS

6.0 | Synthesis

6.0.1 | Related papers

Balducci L, Cuny H, Rathgeber CBK, Deslauriers A, Giovannelli A, Rossi S. 2016. Compensatory mechanisms mitigate the effect of warming and drought on wood formation. *Plant, Cell & Environment* 39: 1338–1352.

Cuny H, Fonti P, Rathgeber CBK, Arx von G, Peters RL, Frank DC. 2018. Couplings in cell differentiation kinetics mitigate air temperature influence on conifer wood anatomy. *Plant, Cell & Environment* 11: 328–11.

Cuny H, Rathgeber CBK, Frank D, Fonti P, Fournier M. 2014. Kinetics of tracheid development explain conifer tree-ring structure. *New Phytologist* 203: 1231–1241.

Cuny H, Rathgeber CBK, Kiessé TS, Hartmann FP, Barbeito I, Fournier M. 2013. Generalized additive models reveal the intrinsic complexity of wood formation dynamics. *Journal of Experimental Botany* 64: 1983–1994.

Cuny H, Rathgeber CBK. 2016. Xylogenesis: Coniferous Trees of Temperate Forests Are Listening to the Climate Tale during the Growing Season But Only Remember the Last Words! *Plant physiology* 171: 306–317.

Hartmann FP, Rathgeber CBK, Fournier M, Moulia B. 2017. Modelling wood formation and structure: power and limits of a morphogenetic gradient in controlling xylem cell proliferation and growth. *Annals of Forest Science* 74: 14.

Hartmann FP, Rathgeber CBK, Fournier M, Moulia B. Wood formation dynamics and tree-ring structure in conifers can be explained by the interaction between two biochemical signals. in preparation for *Annals of Forest Science*.

6.0.2 | Summary

In the second section of this chapter, we presented our approach to describe and quantify accurately the kinetics of xylem cell differentiation using a conceptual model of wood formation dynamics and generalized additive models.

In the third section, we provided novel quantifications of wood formation kinetics that explain the development of the typical, widely observed, conifer tree-ring structure. Importantly, we refute the long-lasting assumption that the increase in wall thickness and wood density along the ring is driven by the fixation of more biomass during the wall-thickening process. Instead, we demonstrated that the amount of wall material per cell is almost constant along a ring. We also shown that the duration of cell expansion decreases along the ring (from about 3 to 1 week), while the duration of cell-wall deposition increase (from 1 to 2 month), and the rate of cell-wall deposition decrease. Consequently, changes in cell-wall thickness and wood density are principally driven by changes in cell size.

Moreover, we estimated that the duration of cell enlargement contributes to 75 % of the changes in cell size, whereas the rate contributes to the other 25 %.
In the fourth section, we explored the relationship between tracheid differentiation kinetics and environmental factors to show that temperature and water stress have significant influences on the rate of cell expansion and cell wall deposition. However, these influences only remain visible in the very last latewood cells of a tree-ring.
In the fifth and sixth section of this chapter we show how couplings between the rate and the duration of cell differentiation kinetics allow trees to adjust the xylogenesis process to the changing environment conditions to produce typical conifer tree-ring structure. We also show how compensatory mechanisms allow trees to adapt to cold temperature at high elevation or to water stress in arid conditions.
Finally, in the last section of this chapter, we present XyDyS the modelling framework we developed to simulate tracheid differentiation kinetics and resulting tree-ring structure. This approach allows us to demonstrate that the classical morphogenetic gradient theory was not working and that at least two distinct biochemical signals (e.g., auxin and cytokine or TDIF) must form gradients of concentration over the developing xylem to organise cell division in the cambium and cell differentiation in the developing xylem, and creates realistic conifer tree-ring structure.
The kinetic model presented here has been established on three major European conifer species, whose typical tree-ring structure is representative of most conifers {Schweingruber, 1990; Schoch et al., 2004}. We believe that the conceptual framework provided by this model can be applied to many species and other environments. However, further investigations are needed to understand how the relationships between cell development kinetics and wood anatomy are modified for conifer species with narrow latewood (e.g., *Pinus cembra*, *Juniperus* species) or rings formed under stressful conditions (e.g. xeric site, drought year, insect attacks, late frosts) and typically associated with wood anatomical anomalies (e.g. light latewood, false rings).

6.0.3 | Key words

Xylem cell differentiation, Tracheid morphology, Tree-ring structure, Wood anatomy, Wood formation modelling, Climate changes

6.1 | Introduction

6.1.1 | The beauty of conifer tree-ring structure

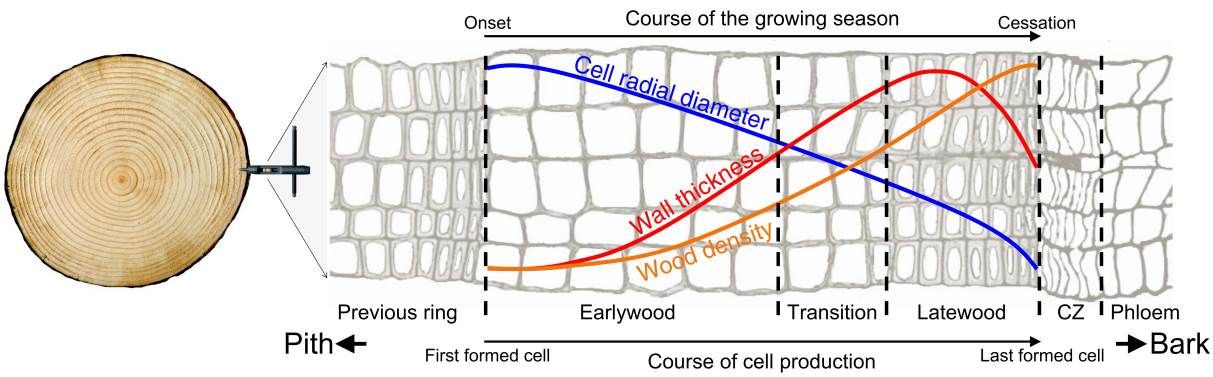
A remarkable aspect of the wood formation process (i.e. xylogenesis) is its capacity to generate various wood forms in response to developmental or environmental constraints {Rowe and Speck, 2005}. Contrasting wood anatomy can thus be observed between different phylogenetic groups {Carlquist, 1975}, but also between different individuals of the same group, between different organs inside the same individual {Lachenbruch et al., 2011}, or even during the ontogenic trajectory of the plant for the same organ {Bao et al., 2001}. On top of this, wood anatomy is also known to change with environmental conditions either in space or in time {Jansen et al., 2004; Fonti et al., 2010}.

Conifers, for example, display an extraordinary biogeography with more than 600 species widely distributed across the globe: from the latitudinal limits of tree growth in the Northern and Southern Hemispheres to the Equator; from lowland plains to near the perpetual snow line of the highest mountains; and from the wettest forests to the driest savannas {Farjon & Filer, 2013}. However, despite growing in such contrasting environments, conifers generally develop very similar tree-ring structures {Schoch et al. 2004}.

In fact, some of the most spectacular variations in conifer's wood anatomy occur within a single tree ring. Indeed, tracheids, which provide both water transport and mechanical support and represent about 90% of the xylem, show very large variations in size along a ring. The conifer tree-ring structure is characterized by a transition from wide, thin-walled earlywood cells produced at the beginning of the growing season, to narrow, thick-walled latewood cells formed at the end of the growing season [Figure 6.1]. Tracheid diameter, for example, is commonly divided by a factor five, when going from the beginning to the end of a tree ring {Vaganov et al., 2006; Cuny et al., 2014, Schoch et al., 2004}.

This typical coniferous tree-ring structure reflects structural and physiological trade-offs that are important for tree functioning and performance. Wider tracheids are more efficient in transporting water but disputably more prone to cavitation {Pratt et al., 2007}; whereas narrower thick-walled tracheids provide most of the mechanical support yet are less efficient in transporting water {Chave et al., 2009; Cochard et al., 2004; Sperry et al., 2006}. These changes in tracheid morphology also drive many fundamental wood properties, including the ratio between early- and late-wood and the tree-ring density profile {Rathgeber et al., 2006}.

Yet, to what extent these anatomical changes are related to climatic constraints, tree physiological state or developmental control is still debated. Xylogenesis is the key process during which trees balance the aforesaid functional and structural trade-offs, and fix them permanently in the wood tissue. However, which and how climatic factors influence xylem cell differentiation and the resulting tree-ring structure have still not been fully unravelled yet {Vaganov et al., 2011}.



47 — Figure 6.1 | Typical tree-ring structure generally observed in conifers. The cambial zone (CZ) lies between the latewood and the phloem. Figure from Cuny et al. {2014}.

6.1.2 | From wood formation dynamics to the kinetics of tracheid cell differentiation

In recent years, much effort has been made in molecular biology, biochemistry and biophysics of xylogenesis. Such advances include: (i) the description of the biosynthetic pathways of cellulose {Somerville, 2006; Mutwil et al., 2008} and lignin {Boerjan et al., 2003; Vanholme et al., 2010}; (ii) identification of the molecular regulatory mechanisms at the genetic {Du & Groover, 2010; Zhong et al., 2011} or hormonal {Uggla et al., 1996; Nilsson et al., 2008} levels; and (iii) the study of cambial cell ultrastructure {Chaffey et al., 2002; Oda & Hasezawa, 2006}. It is striking how the details of xylogenesis are being intensively studied and increasingly understood, whereas general aspects of wood formation dynamics still remain insufficiently known to yield an integrated understanding of how wood is formed {Vaganov et al., 2011}.

During xylogenesis, cell enlargement and secondary cell-wall deposition and lignification (hereafter named wall thickening) are the two fundamental subprocesses that shape xylem cell dimensions and the resulting tree-ring structure {Skene, 1969; Wodzicki, 1971; Cuny et al., 2014}. In his pioneering work, Skene {1969} set the framework for studying the kinetics of tracheid differentiation: the tracheid's final radial diameter is the product of the duration and the rate of cell enlargement; whereas the final amount of secondary cell-wall is the product of the duration and the rate of wall material deposition; cell wall thickness is the result of the total amount of wall material deposited for one cell, relative to its final size.

The complex inter-plays between the durations and the rates of xylogenesis subprocesses determine the changes in cell features (e.g., cell and lumen diameter, lumen area, and wall thickness) that, in turn, create the anatomical structure driving the wood density profile {Cuny et al., 2014}. Subsequent studies, however, mainly focused on the duration of the processes (cell enlargement and wall deposition) to explain the observed changes in cell features (cell diameter and wall thickness) along the ring, discounting the rates without assessing their importance {Wodzicki, 1971; Denne, 1972; Skene, 1972; Dodd & Fox, 1990; Horacek etal., 1999}. Consequently, these pioneering studies failed to unravel how the durations and the rates of xylogenesis subprocesses interact to create the observed tree-ring structures.

6.1.3 | The drivers behind the kinetics of tracheid cell differentiation

Two schools of thoughts have developed to explain the intriguing changes in cell dimensions along conifer tree rings. The developmental theory supposes that tree-ring formation is primarily and directly regulated by internal signals {Uggla et al., 1996, 1998; Sundberg et al., 2000}. According to this view, a morphogenetic gradient of phytohormone (e.g. auxin) concentration shapes the zonation of the developing xylem by giving a positional information to the forming cells {Uggla et al., 1996}. The development of the plant generates changes in signal concentration or in tissue sensitivity through the growing season, creating the typical tree-ring structure {Uggla et al., 2001; Bhalerao and Fischer, 2014}. Such internal control may be tightly coupled with daylength, which has long been regarded as a stable indicator of the time of year that helps to coordinate plant development by influencing the balance of growth regulators {Larson, 1962; Wodzicki, 1964; Jackson, 2009}.

In contrast, the environmental theory proposes that the within-ring anatomical changes are mainly driven by environmental constraints {Larson, 1963; Deleuze and Houllier, 1998; Fritts et al., 1999; Vaganov et al., 2011}. However, a precise assessment of the influence of the climatic factors on tree-ring formation is complex, because the processes involved are tightly coupled and depend on the physiological state of the tree (carbon and hormonal balances), which itself is under the influence of the environment {Denne and Dodd, 1981}.

Consequently, the influence of climatic factors can be direct through physical inhibition or stimulation of the xylogenesis processes; or indirect, i.e. mediated by carbohydrate availability or growth regulators {Denne and Dodd, 1981}. For example, unusual climatic events may occasionally indirectly influence tissue development by altering the phytohormonal gradient {Schrader et al., 2003}, showing that the developmental and environmental theories are not mutually exclusive.

Moreover, the direct or indirect nature of the climatic influence depends on the factor considered {Faitichi et al. NP 2016}. For example, in addition to possible indirect effects, temperature and water availability can directly influence wood formation processes by modulating cellular metabolic activity and/or cell turgor pressure. In contrast, the effects of light conditions are undoubtedly indirect, being mediated by photosynthate availability (which can be related to the amount of light radiation) and/or phytohormone concentration (which can be related to the daylength).

Furthermore, cell enlargement and wall thickening are expected to be influenced by different factors, as they are very different processes in nature {Rathgeber et al. 2016}. Indeed, cell enlargement requires water to exert pressure on the cell wall and increase its surface area {Nonami and Boyer, 1990b; Cosgrove, 1997}. When a drought occurs, cell enlargement is physically and physiologically inhibited {Nonami and Boyer, 1990a, 1990b}, resulting in a rapid reduction of the diameter of the cells produced {Abe et al., 2003; Rossi et al., 2009b; Balducci et al. 2016}. Cell enlargement is thus often depicted as the plant process the most sensitive to water stress {Hsiao, 1973}. These experimental observations have fueled the long-lasting belief that, in nature, decreasing water availability triggers the transition from the

wide earlywood cells to the narrow latewood cells {Kramer, 1964; Deleuze and Houllier, 1998}.
In contrast to cell enlargement, wall thickening involves high energetic costs and represents the bulk of biomass allocation in trees {Demura and Ye, 2010; Cuny et al., 2015}. Thus, this process relies mainly on the carbohydrates produced by photosynthesis, for which light is the indispensable energy source {Kozlowski and Pallardy, 1997}. However, cell wall lignification involves numerous and complex metabolic processes, which are considered the most sensitive to temperature among all the physiological processes related to tree carbon balance {Donaldson, 2001; Körner, 2003; Simard et al., 2013; Cuny et al., 2015}.

6.1.4 | Objective of the chapter

Because of the lack of integrated understanding exposed here above, even basic xylogenesis mechanisms have not been consistently considered in environmental {Briffa et al., 1998; Bouriaud et al., 2005; Gagen et al., 2006; Castaneri et al, 2017}, ecophysiological {Helle & Schleser, 2004; Offermann et al., 2011} or modeling {Deleuze & Houllier, 1998; Fritts et al., 1999; Ogée et al., 2009; Drew et al., 2010; Eglin et al., 2010} tree-ring-based studies. In contradiction with observations, most of these studies assume that the rates of the xylogenesis processes are the drivers of the system, whereas the durations are fixed or derived directly from the rates. A comprehensive investigation of the relative importance of the rates and the durations of xylogenesis subprocesses, in relation to environmental conditions, tree developmental status and xylem tissue physiological state appears thus as a timely and necessary endeavor.
In this chapter, we will first quantify the contribution of each kinetic parameter in shaping the typical conifer tree-ring structure and related wood properties {Cuny et al., 2014}. Secondly, we will unravel the influence of the climatic factors on the kinetics of tree-ring formation {Balducci et al. 2016; Cuny et al. 2016}, assuming that the environmental factors influence the rates of the processes, which directly reflects the physiological state of the plant as well as the influence of the environment; rather than their durations, which are assumed to be tightly associated with plant growth genetic controls and developmental constraints {Uggla et al., 1996, 2001; Tuominen et al., 1997}. Finally, we will investigate how tree-ring formation kinetic parameters vary across changing environments to maintain efficient wood tissues and adapt trees to environmental constraints {Cuny et al. 2018}.
In this chapter, we aim to draw a detailed picture of how the intra-annual dynamics of xylogenesis generates the typical tree-ring structure generally observed in conifers. To reach this goal, we assess the kinetics (duration and rate) of tracheid differentiation processes (enlargement and secondary wall formation) over several growing seasons, and explore the relationships between the kinetics and the resulting tracheid features (cell diameter and cell wall thickness) and wood structure (wood density profile). To assess the kinetics of tracheid differentiation processes, we have developped a novel statistical approach {Cuny et al., 2013} based on General Additive Models (GAMs). Here we will use this approach to analyze an extensive dataset collected in the Donon area (Vosges Mountains, France).

In this chapter, we also aim to understand how the seasonal variations in environmental factors influence the dramatic changes in cell dimensions observed along conifer tree-rings. For that, we will relate the kinetics of each differentiation process (cell enlargement and cell wall thickening) with the resulting tracheid dimensions (cell diameter and cell-wall area) and the environmental factors occurring during the corresponding time window. These relationships will be explored using data from the Donon elevation gradient and the Chicoutimi experiment.
Finally, to explore the variations of tree-ring formation kinetics across changing environments we pulled together the Donon mountains and the Lötschental valley datasets.

6.2 | Material and Methods

6.2.1 | Study sites

The Donon elevation gradient dataset (DNN)

The Donon dataset (called hereafter DNN) comprise 45 trees grown in three mixed stands (Walscheid, Abreschviler, Grandfontaine) spread along an elevation gradient (350, 450, 650 m a.s.l. respectively) in the Donon area (Vosges Mountains, northeast France) [Cuny et al. 2014]. The selected trees belong to 3 conifer species: Norway spruce (*Picea abies* (L.) Karst.), Scots pine (*Pinus sylvestris* L.) and silver fir (*Abies alba* Mill.); and were monitored from April to November over 3 years (2007–2009). Weekly stem microcores were collected from each tree using a Trephor [Rossi et al., 2006] and prepared in the laboratory for histological observations.

The Lötschental valley network dataset (LST)

The nine sites of the Lötschental valley, in the Swiss inner Alps (46°23′N, 7°45′E), were selected along a 1,400-m elevational transect (from 800 to 2,200 m a.s.l.) including both north and south aspects. At each site Norway spruce and European Larch (*Larix decidua* Mill.) grow intermixed, with the exception of the two highest sites (north and south aspects) where only Larch is present. At each site, 3 to 4 mature and dominant trees per species were monitored weekly, throughout the whole growing season, and since 2007 for the very first sites installed. Because of the long-term monitoring performed, the monitored trees were changed after the 2007, 2009, and 2011 vegetation period in order to reduce impact of sampling-related wound reaction.

The Chicoutimi experiment dataset (C2TM)

The Chicoutimi dataset (called hereafter C2TM) was obtained thanks to an experiment conducted during the 2011 growing season in Chicoutimi (QC, Canada) on 4-year-old black spruce (*Picea mariana* Mill.) saplings {Balducci, 2016}. In April 2011, about 1,200 saplings of uniform size were transferred into three greenhouses where two thermal treatments were applied in addition to the control (in which greenhouse temperature was similar to external air temperature): day warming treatment kept a temperature 6 °C warmer than the control during the day (7 h – 19 h), while night warming treatment kept a temperature 6 °C warmer than the

control during the night (19 h – 7 h). In addition, two water treatments were applied for each thermal condition: the wet treatment, in which soil water content was maintained above 80 % of the field capacity; and the dry treatment, in which irrigation was withheld for 25 days in June. So in total there were six combinations of treatments: three thermal conditions × two irrigation regimes.

6.2.2 | Sampling, preparation, and microscopic observations of xylem development

The assessment of the timing of tracheid formation was based on repeated cellular observations performed on microcores taken over the full growing season at different position around the stem circumference.

Microcores were collected weekly at breast height (1.3 m) on the trunk of the selected trees from April to November, using a Trephor tool {Rossi, Anfodillo, & Menardi, 2006}. Successive microcores were then taken at least 1 cm apart from each other and following a slightly ascending spiral pattern to avoid wound reaction. Microcores were then prepared in the laboratory, and 5- to 15-µm thick transverse sections were cut with a microtome. Sections were stained and permanently mounted on glass slides. Xylogenesis observations were performed on the sections using an opti- cal microscope under visible and polarized light at ×100–400 magnification to distinguish the different phases of differentiation among the cells. Thin-walled enlarging cells were distinguished from cambial cells by their larger size. Cells in the thickening zone developed secondary walls that could be detected under polarized light because of the orientation of cellulose microfibrils. Staining was used to follow the advancement of lignification. Thickening cells exhibited two-coloured walls indicating that lignification was in progress, whereas mature tracheids presented entirely lignified and thus monochromatic walls.

Count data of cells in different differentiation phases were standardized by the total number of cells of the previous ring {Rossi, Deslauriers, & Morin, 2003} using the R package CAVIAR {Rathgeber 2012; Rathgeber et al. 2011; and Rathgeber et al. 2018}. This standardization process reduces the noise in the data, thus increasing the signal-to-noise ratio by about 50%.

6.2.3 | Measuring xylem anatomy and computing morphometric density

For each tree, anatomical sections from microcores (DNN), standard 5-mm cores (LST), or stem sections (C2TM) taken after the end of the growing season were used to characterize the structure of the tree rings produced during the monitoring period.

Digital images of the tree rings were analysed using image analysis software specifically developed for wood cell analysis in order to measure the dimensions of tracheids along radial files.

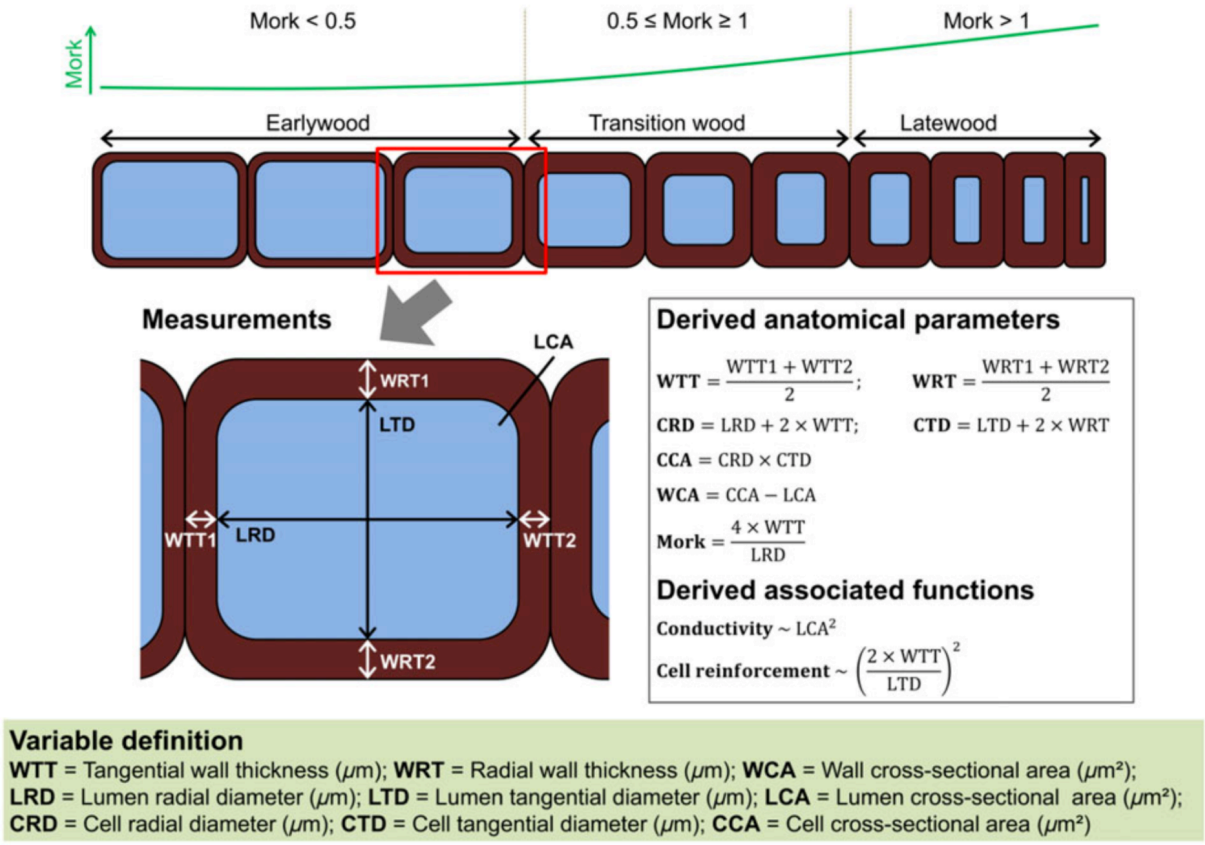
The WinCell software (Regent instruments, Canada) was used for trees at DNN and C2TM to measure cell dimensions along an average of five radial files per tree. ROXAS {Prendin et al. 2017; von Arx & Carrer 2014} and RAPTOR {Peters et al. 2018} were used for trees at LST to measure cell dimensions along an average of 30 radial files. Both programmes measure the radial and tangential lumen

diameter, the lumen area, and the wall thickness of each tracheid from transversal cuts. However, WinCell measures only the tangential wall thickness, while ROXAS measures both the tangential and the radial wall thicknesses. ROXAS measurements revealed that for both species, the radial and tangential wall thicknesses were similar in earlywood, whereas in latewood the radial wall thickness is about 1.3 times larger. We therefore multiplied the tangential wall thickness values by this factor to estimate the radial wall thickness at DNN. From these anatomical variables, the cell and wall cross-sectional areas (CCA and WCA) were calculated and considered as the final results of the differentiation phases of cell enlargement and wall deposition, respectively.

To show variations in tracheid dimensions along a ring, individual cell morphological measurements were grouped by radial file in profiles called tracheidograms {Vaganov, 1990}. Because the number of cells varied between radial files within and between trees, tracheidograms were standardized according to Vaganov's method {Vaganov, 1990} using a dedicated function of the R package CAVIAR {Rathgeber 2012}. This standardization allows adjusting the length of the profiles (cell numbers) without changing their shape (cell dimensions). We checked visually that this standardization did not alter the shape of the anatomical profiles. The standardized tracheidograms were then averaged by site, year, and species.

Mature tracheids were classified into three different types of wood: earlywood, transition wood, and latewood; according to Mork's criterion (MC), which is computed as the ratio between four times the tangential wall thickness divided by the radial lumen diameter {Denne, 1988}. According to Park & Spiecker {2005}, tracheids were classified as follows: $MC \leq 0.5$, earlywood; $0.5 < MC < 1$, transition wood; $MC \geq 1$, latewood [Figure 6.2].

To explore the relationships between cell wall thickness, cell size and the amount of wall material deposited within the cell, we developed a geometric model of tracheid dimensions expressing wall radial thickness as a function of wall cross-sectional area, cell radial diameter and cell tangential diameter (for more details see mathematical development in Methods S1 in Cuny et al. {2014}). The influence of tracheid dimension variability on wood density variation along the ring was assessed using the geometric model of Rathgeber et al. {2006}, which allowed the calculation of the morphometric density from WCA, CRD and CTD (for more details see mathematical development in Methods S2 in Cuny et al. {2014}).



48 — Figure 6.2 | Illustration of the cell anatomical measurements performed. For every cell along a radial file, we measured the lumen radial and tangential diameter and the cross-sectional area of the lumen, as well as the radial and tangential wall thicknesses. From these measurements, we computed the diameters and area of the cells, and the area of the wall. These dimensions were used to derive some indexes related to cell functional performances, specifically the conductivity and the cell wall reinforcement. Figure from Cuny et al. {2018}.

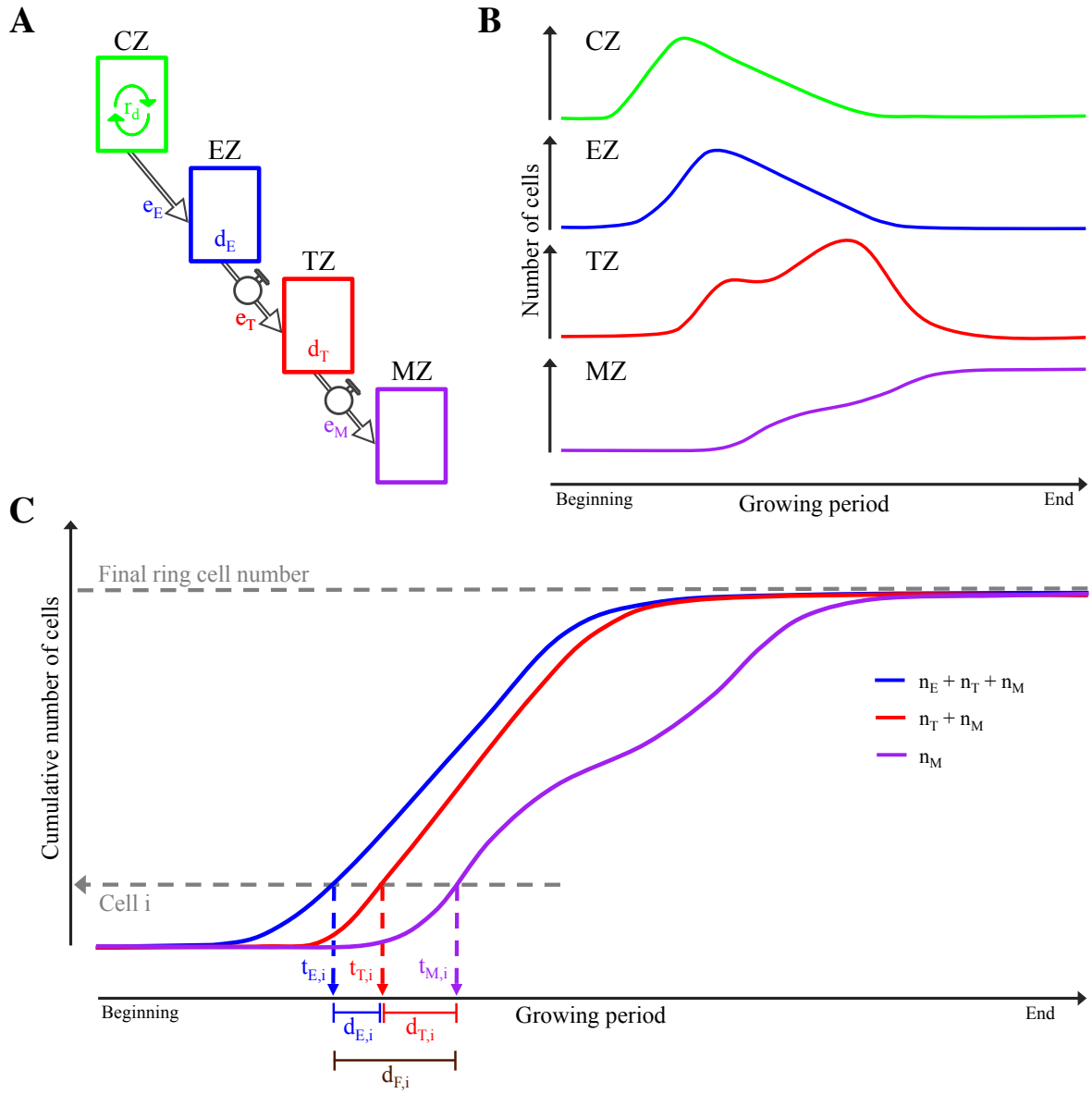
6.2.4 | Wood density measurements

At the end of 2009 for DNN, two standard cores were taken from each tree, one from each side of the stem (at breast height and perpendicular to the slope), using a Pressler increment borer. A radial strip was cut from each core and exposed to X-ray radiation {Polge & Nicholls, 1972} to obtain a digital map of wood density and tree-ring boundaries using CERD software {Mothe et al., 1998}. Each annual ring was divided radially into 20 zones and the mean density of each zone was calculated. Thus, from each ring of each core, we obtained 20 values that described density variation along the ring (i.e. a 'microdensity' profile). The microdensity data of the two cores for each tree were averaged to obtain individual series. The individual microdensity profiles were then averaged for the three stands and years to obtain species-specific microdensity profiles.

6.2.5 | Quantifying tracheid differentiation kinetics

A very recent methodological breakthrough — based on generalized additive models (GAMs) — allowed us to model the intrinsic complexity and variability of wood formation dynamics (see chapter 5 for more details). Here, we took advantage

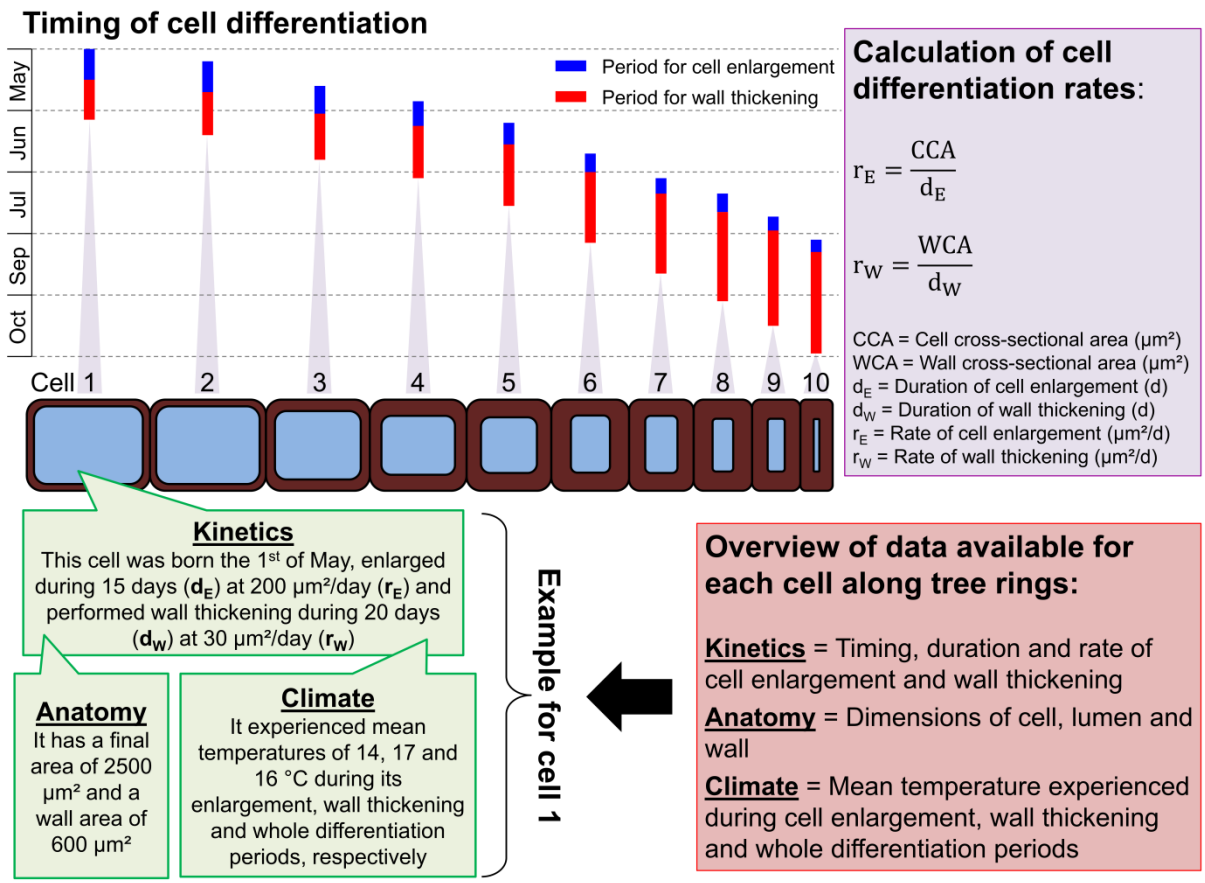
of this innovative method in order to assess properly the durations and rates of tracheid differentiation [Figure 6.3].



49 — Figure 6.3 | Revisited conceptual view of the wood formation dynamics along with the computation of xylem cell timings.
(A) The zones of differentiation in the developing xylem are considered as interconnected reservoirs: cells are generated in the cambial zone (CZ) according to a rate of cell production (rC), and then successively enter the enlargement (EZ), thickening (TZ), and mature (MZ) zones at rates eE, eT, and eM — Valves below EZ and TZ indicate the possibility for the cell residence durations in EZ and TZ (dE and dT) to change independently during the season. Consequently, EZ and TZ can have their own dynamics, which in turn influence the dynamics of eT, eM, and MZ. **(B)** The complex intra-annual dynamics resulting from the interplay of rC with decreasing dE and increasing dT during the season: two left-skewed bell-shaped curves for CZ and EZ, a right-skewed bimodal curve for TZ, and a double sigmoid curve for MZ. **(C)** shows the sums of the cell numbers simulated with generalised additive models used to compute the timing of cell development. The dates of entrance and the duration spent in the enlargement (tE,i and dE,i) and thickening (tT,i and dT,i) zones, as well as the date of

entrance into the mature zone (tM,i) and the total duration of tracheid formation (dF,i) are computed for cell i. Figure modified from Cuny et al. {2013}.

In order to accurately characterize wood formation dynamics, generalized additive models (GAMs) were fitted on the standardized numbers of cells for each zone of xylem development, each year, and each individual tree {Cuny et al., 2013}, using the R package mgcv {R Core Team, 2015; Wood, 2006}. The predictions of the fitted models were then averaged in order to characterize the mean behaviour of each species, during each year, and at each site.
Thus, for each species, year and site combination, we used the average cell numbers predicted by the GAMs to calculate the date of entrance of each *in silico* cell into each differentiation zone (enlargement, wall thickening, and mature). From these dates, the residence time of each cell i in the enlargement (dE,i) and wall-thickening (dW,i) zones was computed {for more details on the computation method, see Cuny et al., 2013}. For each cell i, we then estimated the rate of enlargement (rE,i) and wall thickening (rW,i) by dividing its final dimensions (CCAi and WCAi) by the duration (dE,i and dW,i) it spent in the corresponding phases [see Figure 6.4].
To explore the relationship between tracheid differentiation kinetics and the resulting cell features, we expressed the tracheid dimensions (CRD and WCA) as:
CRD = dE x rE (Eqn X) [Equation 6.1]
WCA = dW x rW (Eqn X) [Equation 6.2]
where dE and dW are the durations and rE and rW the rates of cell enlargement and wall deposition, respectively.



50 — Figure 6.4 | Overview of the information inferred by the « kinetic approach » applied here for every site, year and species.
For each xylem cell along a tree ring, the timing, duration, and rate of its enlargement and wall thickening are calculated. This approach allows linking cell anatomy with the co-occurring environmental conditions [see Figure 6.8 for illustration]. Figure from Cuny et al. {2018}.

6.2.6 | Meteorological data

For the LST, climatic conditions (air temperature, air relative humidity, soil moisture) were measured in situ at each site beneath the canopy at 15-min intervals during the monitoring period. The same kind of data was also collected at DNN sites, but these data were not exploited yet.
At the DNN, daily meteorological data (air temperature, precipitation, cumulative global radiation, wind speed, and air relative humidity) during the monitoring period were gathered from three meteorological stations distributed over the studied area, following the location of the selected sites.
For the LST and DNN sites, the soil relative extractable water (REW) was calculated. For the LST, the REW was directly calculated using the measurements of soil moisture and the soil depth. For the DNN, the REW was assessed with the model Biljou©, which takes as input the measured meteorological parameters along with several soil (e.g., number and depth of layers as well as proportion of fine roots per layer) and stand (forest type and maximum leaf area index) parameters, and gives as output the REW at a daily scale {Granier, Breda, Biron, & Villette, 1999}.

Concerning the C2TM experiments, greenhouse climatic conditions as well numerous ecophysiological parameters were recorded {Balducci et al. 2016}.
Air temperature can be considered as a good indicator of cambium temperature {see Cuny & Rathgeber, 2016}.

6.2.7 | Unravelling the influence of the climatic factors on cell differentiation kinetics and tracheid final dimensions

In order to unravel the mechanisms by which xylogenesis respond to environmental conditions, we related, for each tracheid, the differentiation kinetics and the resulting dimensions to the exact conditions prevailing during the process of its differentiation. For example, the kinetics of enlargement and the final cross-sectional area of a cell were compared with the mean soil relative extractable water experienced during the enlargement of this cell. Similarly, the kinetics of wall thickening and the final wall cross-sectional area of a given cell were compared with the mean air temperature it experienced during its wall thickening. Finally, the lumen area and wall thickness, which integrate both cell size and wall amount, were compared with the mean air temperature experienced during the whole period of cell differentiation (i. e., enlargement plus wall thickening).
Furthermore, in order to test the existence of a transition zone sensing environmental stimuli and transmitting them to further stages of cell differentiation {Vaganov et al., 2011}, we performed comparable analyses but using only the climatic factors that were occurring when the cell was in the cambial zone (i.e. during the time between its appearance in the cambial zone and its entrance into the enlargement zone).
As we wanted to focus on the variations in kinetics and resulting cell anatomy according to the different environmental conditions (represented by sites or treatments), data (cell differentiation kinetics, cell dimensions, and associated environmental conditions) were averaged per site, species, and also by separating earlywood and latewood cells in order to test the possibility of different environmental sensitivities between these two tree-ring zones.

6.2.8 | Data analysis

Sensitivity analysis of the mechanistic models

Sensitivity analyses were conducted in R {R Development Core Team, 2014} to estimate the relative contributions of each input variable to the results of the different mechanistic models (models of cell enlargement and wall deposition, and geometric models of wall thickness and wood density). These analyses measured differences in model output when values of input variables were changed one at a time within a range of twice their standard deviations whilst holding the other variables constant at their mean values {Cariboni et al., 2007}. Advantages of sensitivity analyses are the possibility to deal with the non-linearity and non-independence of the variables of the model {Saltelli et al., 2004}. Contrary to the correlation analyses typically used in previous studies, the results of sensitivity analyses thus allowed us to accurately quantify the individual contributions of the duration and rate of cell enlargement and wall deposition to cell feature and tree-ring structure (CRD, WCA, WRT and MD). To demonstrate the influence of each

kinetic parameter on the whole tree-ring structure, sensitivity analyses were illustrated by drawing simulated tree rings. For this, each kinetic parameter was made to vary within its natural range of variations whilst holding the other parameters constant at their mean values. The simulated cell features were used to build virtual radial files assuming rectangular-shaped tracheids.

Statistical analysis of the influences of the environmental factors on the cell differentiation kinetics

Relationships between environmental conditions, kinetics parameters (rate and duration of cell enlargement and wall thickening), and anatomical variables (cell area, lumen area, wall area, and wall thickness) were assessed using linear mixed effects models.

Additionally, to account for possible biases for the assessment of the influence of air temperature on xylem cell dimension and kinetics due to missing independence of data caused by pseudo-replication (the same trees have been monitored for two to three consecutive years), the relationships were assessed by bootstrapping 1,000 times the same linear model where only one tree and year combination is randomly selected from each tree. In particular, covariance analyses were performed to evaluate the effects of species (European larch and Norway spruce) and wood types (earlywood and latewood) on the relationships. Significant variables and interactions were identified by backward elimination using the R function drop1 {R Core Team, 2015}, which allows selecting the best model based on the Akaike information criterion. The best model was chosen based on the lowest Akaike information criterion.

Finally, as the assessed kinetics and anatomical parameters are on different scales and have different absolute values between them and between early- and late-wood, relationships were also assessed on normalized data in order to compare the slopes between the different variables and wood types.

In addition, covariance analyses were performed to test the effect of species on the relationships between variables.

Model simulations of xylogenesis response to developmental and environmental changes

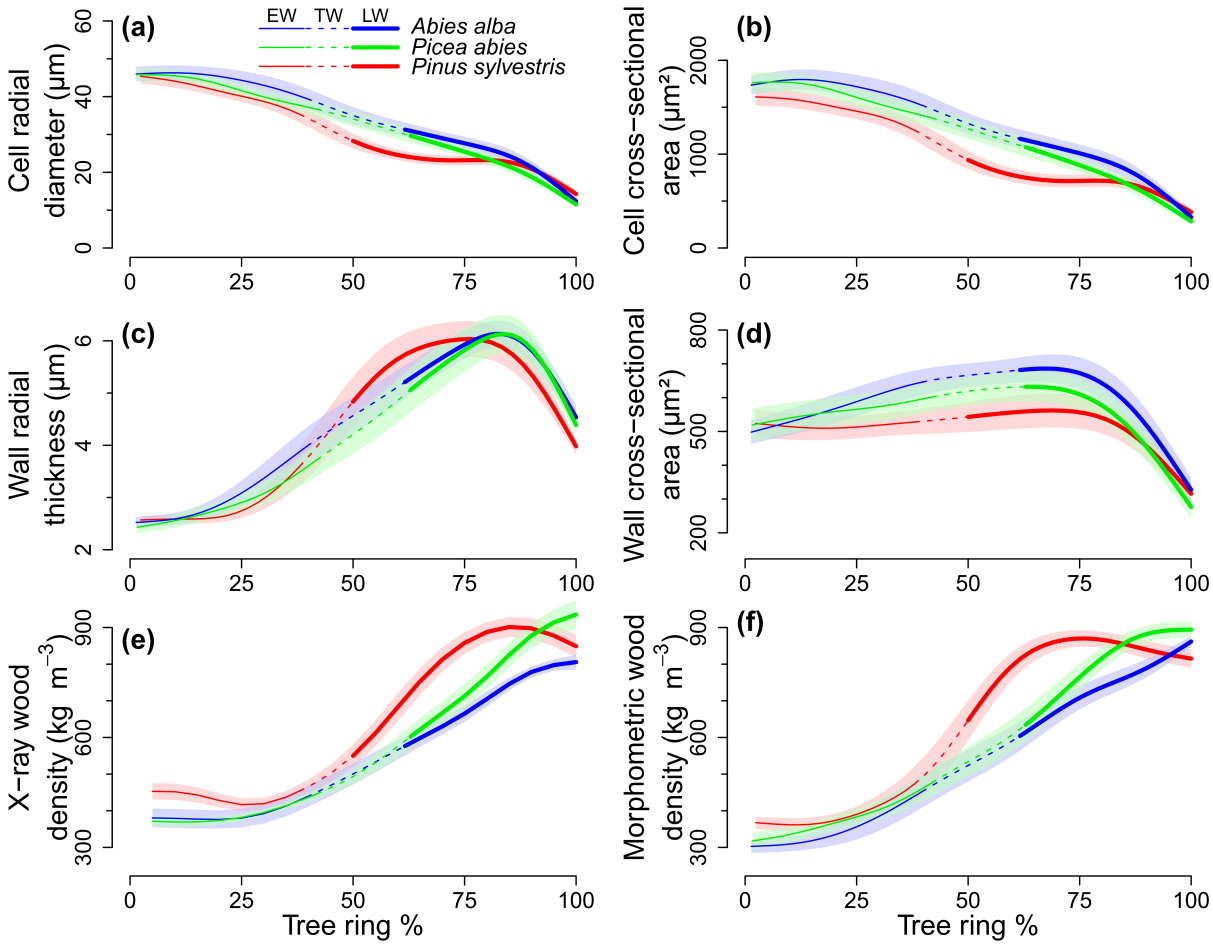
To assess the implications of the xylogenesis response to thermal conditions in terms of tree-ring structure and associated functions, we used the relationships established to simulate the average kinetics and resulting dimensions of the cells produced at two theoretical sites representing a 5°C gradient (~3.5°C and 8.5°C in average air temperature, respectively). For that, the relationships established between thermal conditions and kinetics parameters were used to simulate the rate and duration of differentiation processes for the two thermal modalities. The simulated kinetics were then used to calculate cell dimensions. For example, the cell cross-sectional area was calculated by multiplying the simulated rate and duration of cell enlargement, whereas the wall cross-sectional area was calculated by multiplying the simulated rate and duration of wall thickening. Other dimensions (lumen area, cell and lumen diameters, and wall thickness) were then calculated from these two anatomical dimensions and used to build virtual cells assuming rectangular-shaped tracheids. Finally, the calculated cell dimensions

were used to infer some indices about the functions (conductivity and cell wall reinforcement) associated to the simulated tracheids. Conductivity was thus calculated as the square of the lumen cross-sectional area according to Hagen–Poiseuille law {Sutera & Skalak, 1993}, whereas the cell wall reinforcement was calculated according to Hacke et al. {2001}:
Cell wall reinforcement = ((2 x WTT) / LTD)2; [Equation 6.2]
where WTT is the tangential wall thickness and LTD the lumen tangential diameter.

6.3 | The kinetics of the tracheid differentiation explains the typical conifer tree-ring structure

6.3.1 | Tree-ring structure of silver fir, Norway spruce and Scots pine

The three studied species in the Vosges mountains (silver fir, Norway spruce and Scots pine) displayed the same characteristic tree-ring structure, with a progressive transition from (i) large, thin-walled cells of light earlywood; to (ii) narrow, thick-walled cells of dense latewood. Earlywood represented ca. 40% of the tree rings [Fig. 6.5], but the transition to latewood was more abrupt in pine, where transition wood represented only 8% of pine tree rings, compared with 20% in fir and spruce.
A regular decrease in cell radial diameter (CRD) was observed from the beginning to the end of the rings [Fig. 6.5a]; the first earlywood cells were three times larger (ca. 45 µm) than the last latewood cells (ca. 15 µm). Because the tangential diameter was almost constant (35 ± 2 µm, mean ± SD), the changes in cell cross-sectional area followed those of CRD [Fig. 6.5b].
The tracheid wall thickness was lowest (2.7 ± 0.2 µm) in the first 25 % of the rings, increased to a maximum of 6 µm around 75 % of the ring, before to decreased abruptly to a minimum of ca. 4 µm in the very last cells of the rings [Fig. 6.5c]. By contrast, wall cross area (WCA) increased slightly in the first 75 % of the rings, before decreasing abruptly to reach its minimum in the very last latewood cells [Fig. 6.5d].
Sensitivity analysis of the geometric model of cell wall thickness (not presented here) indicated that 67 % of the changes in wall thickness along a ring could be attributed to changes in CRD, and only 33 % to changes in WCA {Cuny et al. 2014}.
These anatomical properties were broadly reflected in the integrative X-ray density. Density profiles [Fig. 6.5e] follow S-shaped curves, with minimal values in earlywood (ca. 375 kg m-3 in fir and spruce and 450 kg m-3 in pine) and maximum values in latewood (800, 900 and 930 kg m-3 in fir, pine and spruce, respectively). Only in pine, X-ray density decreased slightly in the last 15 % of the rings.
The morphometric density [Fig. 6.5f] computed from the tracheid dimensions agreed with the X-ray density. Sensitivity analysis of the geometric model of wood morphometric density (not presented here) suggested that changes in CRD accounted for 75 % of the changes in wood density along the rings, whereas variation in WCA accounted for the remaining 25 %.



51 — Figure 6.5 | Tree-ring structure of three conifer species (*Abies alba*, *Picea abies* and *Pinus sylvestris*).
Variations in **(a)** cell radial diameter, **(b)** cell cross-sectional area, **(c)** wall radial thickness, **(d)**, wall cross-sectional area, **(e)** wood density measured by X-ray, and **(f)** morphometric wood density, along tree rings divided into earlywood (EW), transition wood (TW), and latewood (LW). For each species, the line represents the mean values for 15 trees over 3 year (2007–2009), while the shadowed area delimits the 90 % confidence interval. Figure from Cuny et al. {2014}.

6.3.2 | Kinetics of tracheid differentiation along a tree ring

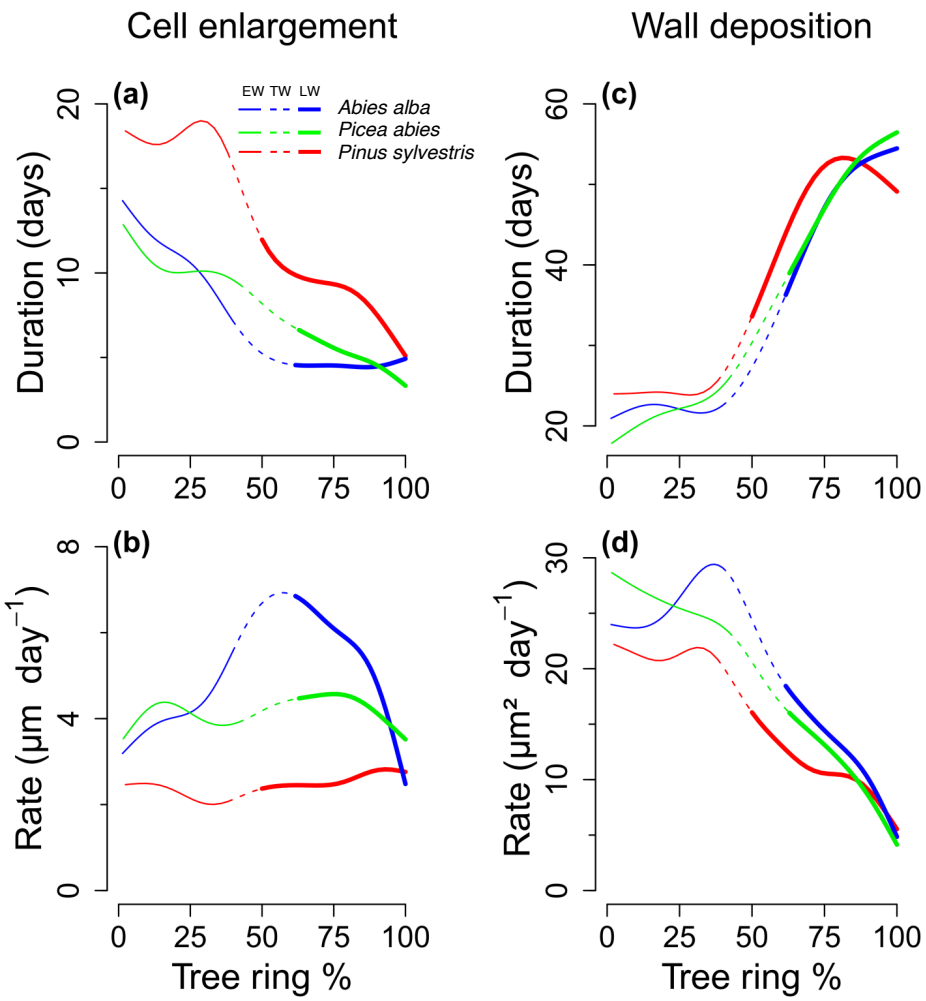
Thanks to detailed weekly observations of the numbers of cells in each zone of xylem development (see chapter 3), a new method of quantification of wood formation dynamics (see chapter 5), and a revisited approach to accurately compute the timings of xylem cell differentiation (this chapter), we can describe the evolution of the kinetics of tracheid differentiation all over the growing season [Fig. 6.6].

Enlarging tracheids needed, on average, slightly more than one week (dE = 9 ± 1 d) at a rate of ca. 4 µm a day to reach their final size [Fig. 6.6a, b]. Yet, the duration of cell enlargement in pine was 40 % longer than in fir and spruce, and was associated with a 40 % lower enlargement rate (rE). Despite these differences in levels, dE followed similar variations along the ring for the three studied species: it decreased progressively by ca. two-thirds from the first to the last cells of the rings (from 18 to 6 days in pine, 14 to 5 in fir, and 12 to 4 in spruce). The decrease was particularly abrupt for transition wood cells in pine. By contrast, rE showed

little variations for spruce and pine, but a strong increase in fir transition wood [Fig. 6.6b].

Thickening tracheids required, on average, slightly more than 1 month to mature their cell walls, with very small variations between species (dW = 35 ± 13 d). The average rate of wall material deposition (rW) was ca. 19 ± 7 µm2 d-1. The duration of cell wall thickening increased from 20 to 55 days along the successively cells in the ring, exhibiting the same stretched S-shaped curve for the three species [Fig. 6.6c], whereas rW exhibited the opposite trend [Fig. 6.6d]. Secondary wall formation of early-, transition- and late-wood tracheids required three weeks (22 ± 3), one month (29 ± 4) and two months (50 ± 6 d), respectively. In earlywood cells, rW remained stable at 25 ± 2 µm2 d-1, and then decreased continuously during the production of transition- and late-wood cells to reach a minimum of ca. 5 µm2 d-1 for the very last cells.

Our data revealed remarkable patterns in the relationships between the duration and the rate of cell enlargement and wall deposition. Although dE and rE were not correlated, a very significant and non-species-specific relationship linked dW and rW (P < 0.001, r2 = 0.92, n = 177). This negative relationship held for the majority of the cells of a ring, with the exception of the very last latewood cells.



52 — Figure 6.6 | Kinetics of cell differentiation for the successive « in silico » tracheids along tree rings of Abies alba, Picea abies and Pinus sylvestris.

With **(a)** the duration of cell enlargement, **(b)** the rate of cell enlargement, **(c)** the duration of wall deposition and **(d)** the rate of wall deposition. The different line types represent the separation between earlywood (EW), transition wood (TW) and latewood (LW) cells. Figure from Cuny et al. {2014}.

6.3.3 | Relationships between the tree-ring structure and the kinetics of tracheid differentiation

We found highly significant, linear and species-specific relationships between the cell radial diameter and the duration of the enlargement phase ($P < 0.001$, $r2 = 0.85$, $n = 177$): the longer dE, the larger CRD {Cuny et al. 2014}. By contrast, the rate of cell enlargement did not present any significant linear relationship with the final size of the tracheids. We did not find any linear relationships between the wall cross area and the rate or duration of wall deposition because of the more complex nature of their interactions.

The sensitivity analysis of the basic model of cell enlargement (CRD = dE x rE) allowed us to estimate that the variation in dE along the rings contributed to 75 %, on average, of the changes in CRD, whereas rE contributed to only 25% {Cuny et al. 2014}. The studied species, however, presented species-specific modulations of this general trend. Thus, in agreement with the slightly different behaviors observed for fir, we found a lower contribution of dE and a higher contribution of rE in fir than in pine and spruce.

The sensitivity analysis of the basic model of cell wall deposition (WCA= dW x rW) suggested that the variation in dW and rW along the rings contributed equally to the changes in WCA, independent of the species {Cuny et al. 2014}.

The combined results of the sensitivity analyses allowed us to estimate the contribution of the different kinetic parameters to the changes in tracheid dimensions and wood density along the rings. We found that dE contributed to 75 % of the changes in CRD, which, in turn, contributed to 67 % of the changes in wall thickness. Therefore, dE contributed to 50 % (75 % of 67 %) of the changes in wall thickness along the rings. Similarly, dE contributed to 56 % (75 % of 75 %) of the changes in wood density. Proceeding in the same way, we found that rE, dW and rW contributed to 19%, 12.5% and 12.5%, respectively, of the changes in wood density along the rings {Cuny et al. 2014}.

6.3.4 | Re-thinking tree-ring formation in conifers

In Cuny et al. {2014} we combined an original and extensive dataset (45 trees from three conifer species monitored over 3 years) with recent methodological advances in statistical analysis to accurately describe the change in tracheid differentiation kinetics all along the growing season. This work allowed us to quantify concomitantly the contribution of both the durations and rates of xylogenesis subprocesses to the anatomical changes observed along the rings, and thus to draw the first detailed picture of how the kinetics of tracheid differentiation creates the typical tree-ring structure that characterizes conifers.

The results we obtained in Cuny et al. {2014} question the relationship between cell wall thickness and wood density. These two parameters are generally considered to reflect the amount of biomass allocated to xylem. For example, the dense latewood made of thick-walled cells is thought to hold most of tree biomass

{e.g. Begum et al. 2012}, and several authors have attempted to associate latewood formation with carbohydrate availability {e.g., Larson 1964; Uggla et al. 2001; Rossi et al. 2009; Carteni et al. 2018}. Therefore, patterns of wall thickness and wood density along conifer rings are regularly assumed to be primarily driven by the process responsible for filling the cells with biomass, namely wall deposition. Based on this concept, wall thickness changes have been generally related to the kinetics of wall deposition {Wodzicki, 1971; Denne, 1972; Dodd & Fox, 1990; Horacek et al., 1999}, and the idea that the wall thickness is mainly driven by the wall-thickening duration is deeply anchored in the scientific literature {e.g., Uggla et al. 2001; Bouriaud et al. 2005; Vaganov et al. 2011}.

More recently, Carteni et al. {2018} proposed a very original model of tracheid differentiation relating cell enlargement, cell-wall deposition, cell-wall lignification and programmed cell death to a hypothesised fixed sugar concentration at the cell level. The main originality of the model lies in the fact that all the differentiation process are assumed to start at the same date but do not progress at the same pace. Thus, when cell-wall thickness reach a certain threshold, the cell stops enlargement; and when cell-wall lignification is complete, the cell dies. However, the model also assume that carbon allocation to the wood increases exponentially from the beginning to the end of the growing season {see for example Fig. 5 in Carteni et al. 2018}.

In opposition to this common belief, our results revealed that the amount of material deposited per cell was almost constant along most of the ring, but decreased dramatically in the last 25 % of the ring to reach minimal values for the last latewood cells, where wood density was maximal. Because of this, changes in the amount of wall material per cell, and in the kinetics of wall deposition, only explained 33 % of the changes in wall thickness, and 25 % of the changes in wood density. This result forces us to completely rethink the way in which xylogenesis creates tree-ring structure in conifers.

In particular, our results emphasized the crucial role of the decrease in cell size in driving changes in wall thickness and wood density. Cell size reduction contributed to 67 % of the increase in wall thickness and to 75 % of the increase in wood density along the ring. These results can be explained by the geometric effect of cell size diminution. Namely, more or less constant amounts of wall material are placed into smaller and smaller lumen volumes, resulting in increasing wall thickness, cell to wall ratio and wood density. Therefore, counter-intuitively, along a conifer ring, increasing cell wall thickness and wood density do not reflect higher wood biomass allocation; but they mostly occur because the volumes in which the biomass is allocated decrease. Therefore, the main driver of wall thickness and density changes along a ring is the cell enlargement process.

Most experimental studies on the kinetics of tracheid differentiation show that the typical decrease in cell size along the tree ring parallels an overall decrease in the duration of cell enlargement {Wodzicki, 1971; Skene, 1972; Horacek et al., 1999}, whereas the rate of cell enlargement presents fewer variations and more irregular patterns {Wodzicki, 1971; Skene, 1972}. By providing an unambiguous assessment of the relative contributions of the duration and rate of enlargement to changes in cell size, our results now quantitatively establish the duration of enlargement as the main driver of cell size decrease. The changes in the duration of enlargement explained 75 % of the changes in cell size along the ring, whereas the changes in

the rate explained the remaining 25 %. It is interesting to note that several authors have also related the tapering of tracheid diameter along the stem to changes in the duration of enlargement {Denne, 1972; Dodd & Fox, 1990; Anfodillo et al., 2012}.

To visually demonstrate the role of the duration and rate of cell enlargement and wall deposition on tree-ring structure, we used the results of previous sensitivity analyses to simulate the formation of virtual radial files [Fig. 6.7]. Only the change in the duration of enlargement over the growing season is able to create 'realistic' virtual rings whose structure is like those typically observed for natural conifer tree rings [Fig. 6.7a-b]. By contrast, the other kinetic parameters lead to unrealistic tree-ring structures [Fig. 6.7c-e]. A change in the rate of enlargement, with other parameters fixed, produces mainly transition wood; a change in the duration of wall deposition reproduces the wall thickness increase, but cells have a constant size; a change in the rate of cell wall deposition inverts earlywood and latewood.

The same reasoning can explain variations in wood structure among the species. Scots pine, in comparison with Norway spruce and silver fir, exhibits a sharper transition between earlywood and latewood {Schweingruber, 1990; Schoch et al., 2004}. This species-specific anatomical feature also originates in enlargement duration particularities. Indeed, in pine, a prominent decline in the enlargement duration characterized transition-wood cells, whereas the decrease was more gradual in fir and spruce. Thus, using only the duration of the enlargement phase, we were able to simulate the species-specific EW-LW transitions for pine and spruce [Fig. 6.7b]. For fir however, the duration of enlargement was not sufficient to simulate the gradual transition, which can be explained by the greater importance of the enlargement rate in this species.

53 — Figure 6.7 | Schemes of the different tree rings formed when the different parameters of the kinetics of tracheid development are changed one at a time. Figure from Cuny et al. {2014}.

6.3.5 | Conclusions about kinetics of tracheid differentiation and conifer tree-ring structure

In Cuny et al. {2014}, we developed further and extended the use of the descriptive models of wood formation dynamics presented in Cuny et al. {2013}, in order to provided novel quantifications of wood formation kinetics that explain the development of the typical, widely observed, conifer tree-ring structure. Importantly, we refute the long-lasting assumption that the increase in wall thickness and wood density along the ring is driven by the fixation of more biomass during the wall-thickening process. Instead, we demonstrate that the amount of wall material per cell is almost constant along a ring. Consequently, changes in cell-wall thickness and wood density are principally driven by changes in cell size. Moreover, we estimated that the duration of cell enlargement contributes to 75 % of the changes in cell size, whereas the rate contributes to the other 25 %.
The mechanistic framework developed in Cuny et al. {2014} to understand the formation of the typical conifer tree-ring structure will now provide the basic tools for unravelling how climatic variations and more generally, environmental factors lead to deviations from the 'normal' tree-ring structure.

6.4 | The seasonal variations of the environmental factors influence the kinetics of tracheid differentiation and the resulting tree-ring structure in conifers

6.4.1 | The seasonal cycles of the environmental factors in the Vosges mountains

The climatic conditions of the Donon area followed a seasonal cycle representative of a cool, temperate region.
We observed very similar seasonal patterns between the different years and studied sites of the Donon area. Daylength followed a symmetric bell curve, reaching a maximum of about 16 h at the summer solstice (June 21) and dropping to a minimum of 8 h at the winter solstice (December 21). Light radiation followed the course of daylength, but with large day-to-day variations. By contrast, the daily air temperatures followed a bell curve slightly skewed to the right, culminating at 17°C ± 3.2°C during summer (July– August) and dropping at 1.7°C ± 4.5°C during winter (December–January).
At the Abreschviller site, the cambium temperature measured in one silver fir and one Scots pine tree during 2008 and 2009 perfectly followed air temperature, demonstrating that the air temperature measured for the three sites and used for further analyses can be considered a good indicator of cambium temperature (for more details see Cuny & Rathgeber {2016} Supplemental Fig. S4).
Precipitations were quite regularly distributed over the year (112 ± 61 mm per month on average), with two slightly drier periods in April and May and in September and October. Because of these abundant and regular rains, the relative extractable water (REW) in the soil was usually maintained at a high level (86 %

± 21 %) during the vegetation period. It was generally lower in May and June, due to the rapid increase in temperatures and the slight decrease in precipitations. During the 3 years of monitoring, dry spells (REW below 40 %) only occurred at the lowest site (Walscheid) and were of weak intensity (for more details see Cuny & Rathgeber {2016} supplemental Fig. S1 and Table S1).

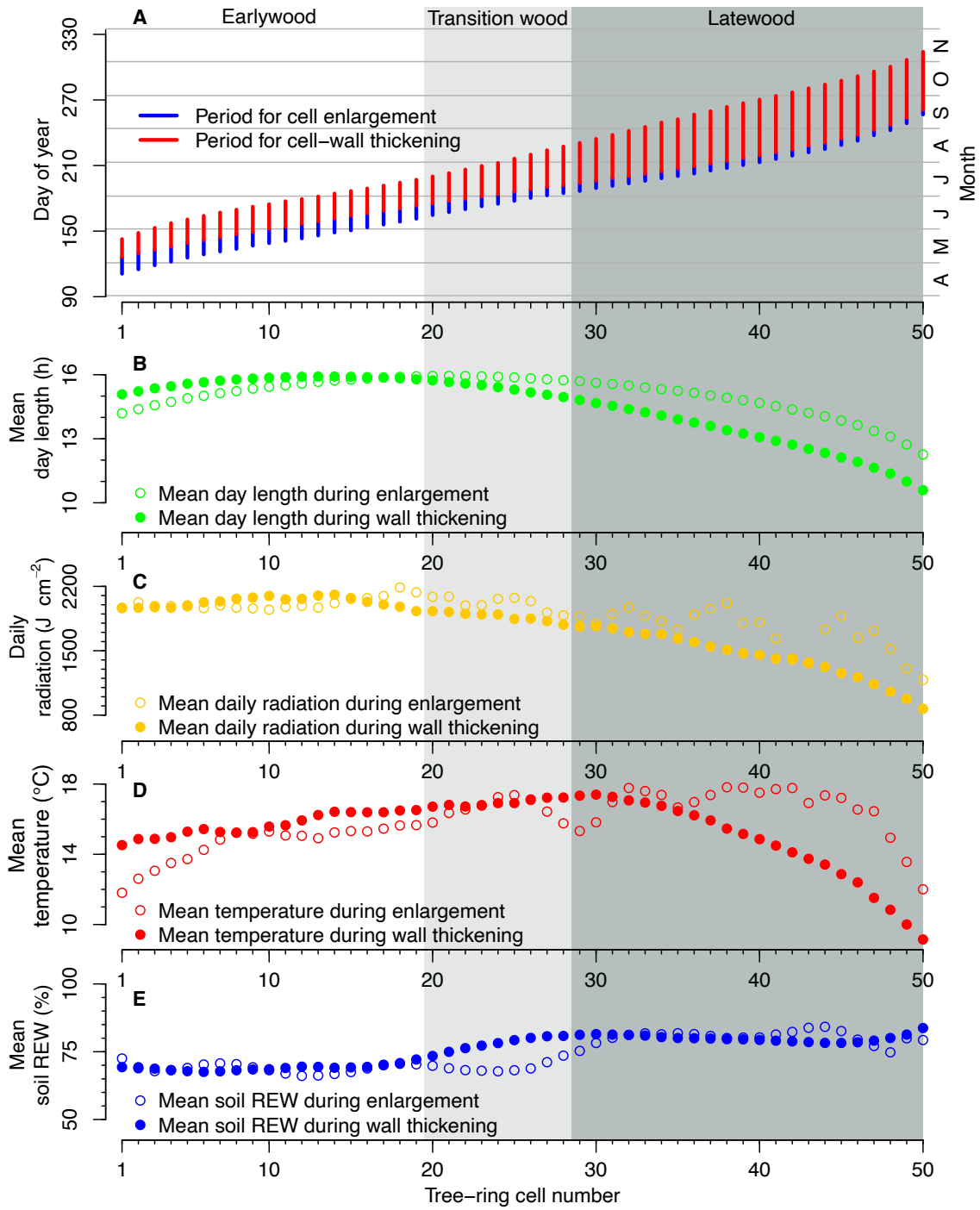
6.4.2 | Climatic conditions experienced by tracheids during cell differentiation

Overall, in the Donon area, the complete formation of the tree rings lasted from mid-April to mid-November, with similar timings of cellular development observed between the three species, sites, and years.

Early-wood cells developed from mid-April to mid-July; transition-wood, from mid-June to mid-August; and late-wood, from the beginning of July to mid-November. However, each cell in a tree ring experienced a unique "cocktail" of environmental conditions [Fig. 6.8], potentially shaping its final dimensions in a unique way, since each of its differentiation phases occurred within a specific time window.

The daylength and daily light radiation were maximal during earlywood formation, before decreasing regularly during transition wood and latewood formation [Fig. 6.8B-C]. In contrast, the temperatures experienced during differentiation increased slightly for the successive cells of the rings until latewood, in which it decreased abruptly [Fig. 6.8D]. In particular, the temperature for wall thickening increased slightly during earlywood formation (+2°C on average from the first to the last earlywood cells), whereas it decreased abruptly during latewood formation, passing from 17.3°C ± 0.2°C for the first latewood cells to 8.6°C ± 0.6°C for the last ones.

Because of the abundant and regular precipitations at the studied sites, all successive cells of the ring experienced relatively high soil REW during their differentiation [Fig. 6.8E]. Surprisingly, the soil water availability was higher (REW = 80 ± 3 %) during the enlargement of the late-wood cells, than during the enlargement of the early- and transition-wood cells (REW = 69 ± 3 %).



54 — Figure 6.8 | Timings of cell differentiation along with environmental conditions experienced by the tracheids during their differentiation.
For each successive cell along the ring, shown are the timing of cell enlargement and cell wall thickening **(A)** and the mean environmental conditions experienced by the studied trees during the periods of cell enlargement and cell wall deposition, with daylength **(B)**, cumulative daily light radiation **(C)**, temperature **(D)**, and soil REW (E). Average values for the three species, sites, and years (2007–2009) are shown. Figure from Cuny et al. {2016}.

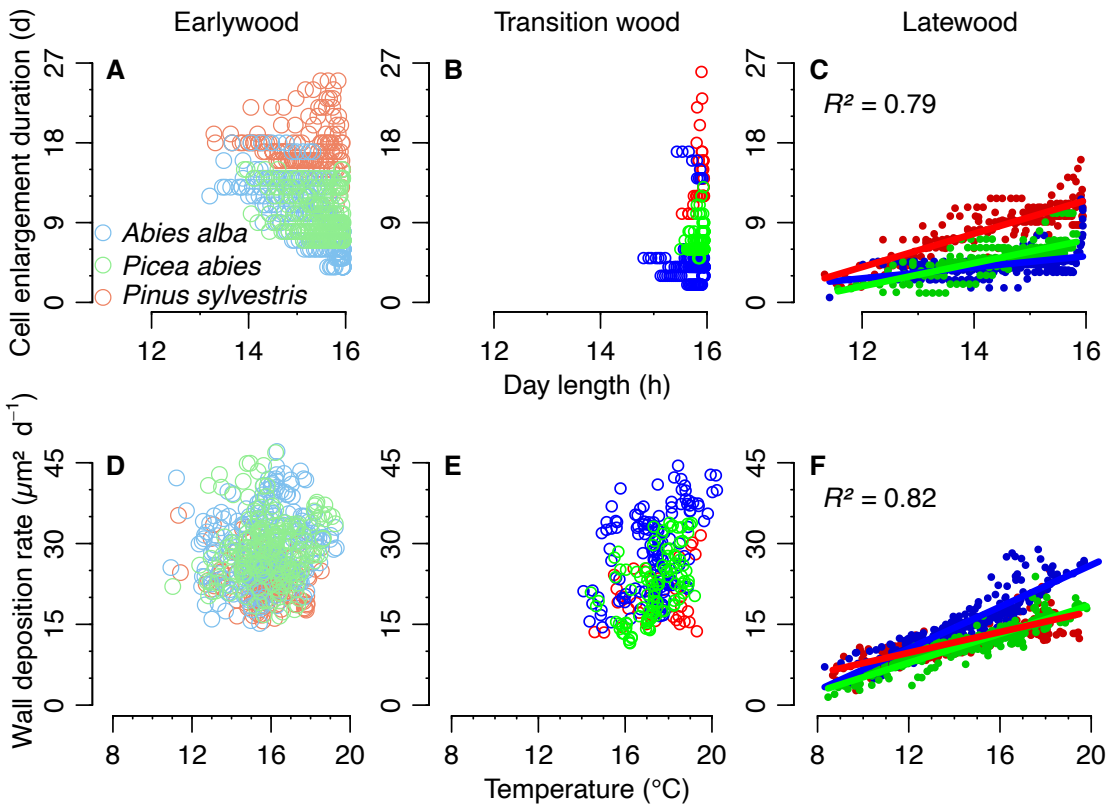
6.4.3 | Influence of the climatic factors on the kinetics of xylem cell differentiation

Whatever the considered type of wood cells, we did not find any significant effects of the considered climatic factors (temperatures, precipitations and radiations) prevailing during cell enlargement on the seasonal changes in the kinetics of cell enlargement [Fig. 6.9]. However, we observed a significant, positive and species-specific effect of daylength on the duration of latewood cell enlargement ($P < 0.001$; adjusted $r2 = 0.79$; $n = 697$).

In contrast, we found highly significant effects of light radiation and temperature on the rate of latewood cell wall deposition. Yet, the temperatures and radiations prevailing during cell wall thickening were highly correlated in our data set ($P < 0.0001$; adjusted $r2 = 0.84$; $n = 697$), but the construction of a statistical model expressing the daily rates of wall deposition as a function of temperature, radiation, and soil REW reveals a highly significant and positive effect of temperature, with radiation and soil REW containing no additional information.

Indeed, highly significant, species-specific, linear, and positive relationships were found between the mean daily temperatures experienced during wall thickening and the mean daily rate of wall deposition during latewood formation ($P < 0.001$; adjusted $r2 = 0.82$; $n = 697$) but not during early- and transition-wood formation [Fig. 6.9, D–F]. The highest temperature sensitivity of the wall deposition rate was observed in silver fir (slope = 1.94), then in Norway spruce (slope = 1.34), while the lowest sensitivity was found in Scots pine (slope = 0.96).

Whatever the process considered, the relationships were always better when using the environmental factors prevailing during the corresponding stage of differentiation. For example, the species-specific relationship found between the temperature and the wall deposition rate remained significant but was largely weakened ($P < 0.001$; adjusted $r2 = 0.14$; $n = 697$) when using the temperature experienced by the cells when they were in the cambial zone.



55 — Figure 6.9 | Influences of daylength on cell enlargement duration and of temperature on cell wall deposition rate.
A to **C**, Relationship between the daylength experienced during cell enlargement and the cell enlargement duration in early- **(A)**, transition- **(B)**, and late-wood **(C)**. **D** to **F**, Relationship between the temperatures experienced during wall thickening and the wall deposition rate in early- **(D)**, transition- **(E)**, and late-wood **(F)**. Each point represents a cell along a tree ring of one of the three species at one of the three studied sites and during 1 year in the period 2007 to 2009. In **C** and **F**, the lines represent species-specific linear relationships. Figure from Cuny et al. {2016}.

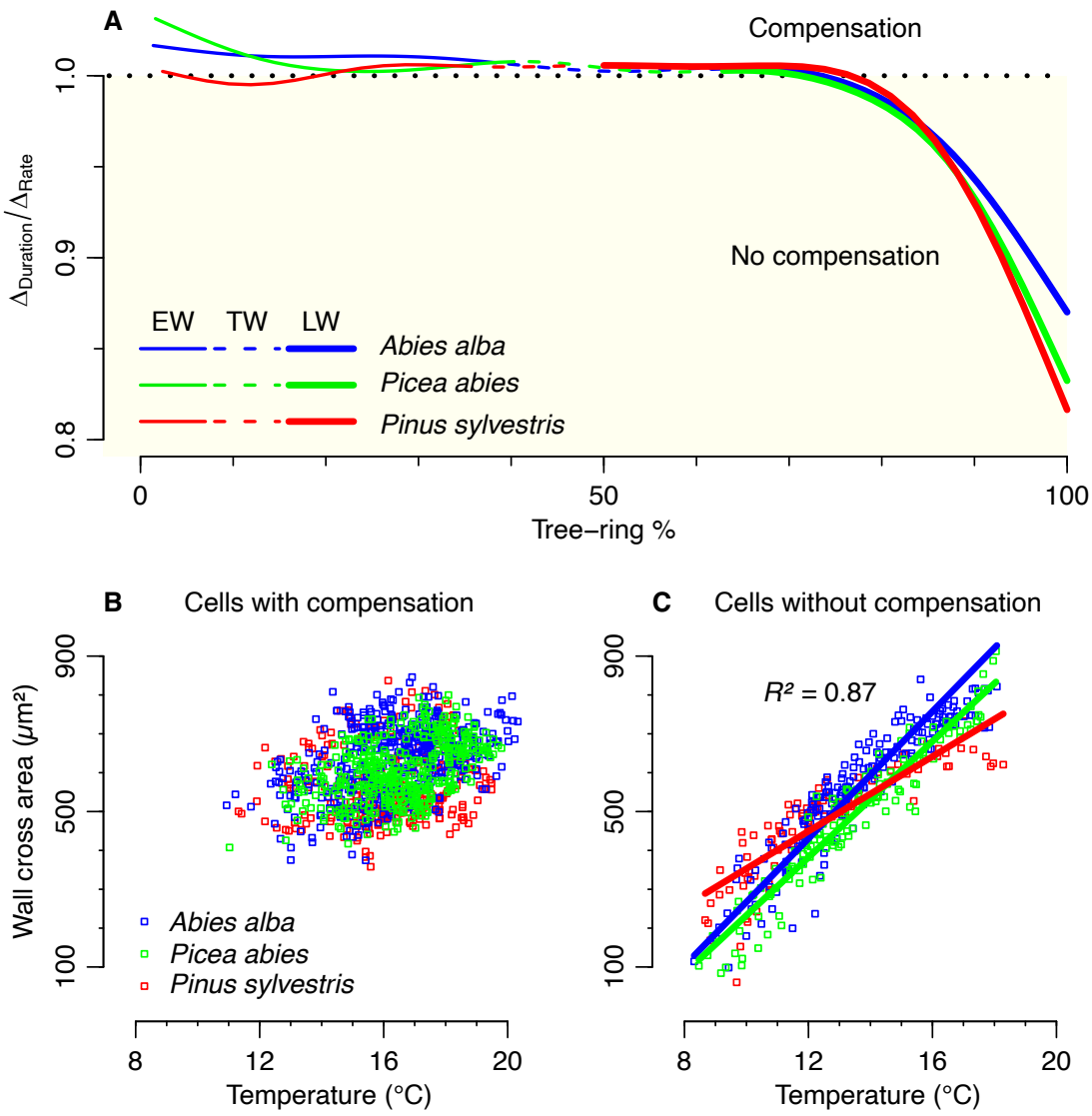
6.4.4 | Mechanisms of registration of the climatic signal in conifer tree-ring structure

For the majority of the cells along the ring, the variations in the rate and duration of wall deposition were inversely proportional: variations in the rate of wall deposition were accompanied by opposite variations of the same magnitude in process duration [Fig. 6.10]. Along the growth ring, as the rate of cell wall deposition decreases, the duration of wall deposition increases in the same proportion.

Because of this 'compensation effect', the amount of wall material incorporated by the tracheids exhibited little change along a large part of the tree-ring. Only in the second part of the late-wood did the compensation effect decrease to become minimal for the very last tracheids [Fig. 6.10A]. Then, in these last tracheids, variations in the rate of wall deposition resulted in equivalent variations in the wall cross area.

Because of the above described compensation effect, the influences of the temperatures on the rate of wall deposition were not translated into amounts of deposited wall material during most of the growing season; and thus, were not

recorded in the tree-ring structure [Fig. 6.10B]. Indeed, for cells presenting a strong compensation, influences of temperature on wall deposition rate were not traced in the wall cross area; whereas for cells presenting a weak compensation, highly significant, species-specific, linear, and positive relationships were found between the wall cross area and the temperature during the period of wall formation ($P < 0.001$; adjusted $R2 = 0.87$; $n = 398$) [Fig. 6.10C].
Consistent with the species-specific temperature sensitivities found for the wall deposition rate, the highest temperature sensitivity of the wall cross area was observed in silver fir (slope = 81.7), then in Norway spruce (slope = 74.6), while the lowest sensitivity was found in Scots pine (slope = 48.1).



56 — Figure 6.10 | Mechanisms of temperature signal registration in tree-ring structure in the Vosges mountains.
A, Compensation effect in the kinetics of wall deposition. **B**, Influence of temperature on final wall cross area for cells with a compensation effect. **C**, Influence of temperature on final wall cross area for cells without a compensation effect. In **A**, for each species, the line represents the ratio between the changes in the duration of wall thickening and the changes in the rate of wall deposition along the tree ring (means for the three sites and years). Different line types distinguish early-wood (EW), transition-wood (TW), and late-

wood (LW). The horizontal dotted line represents the value of a perfect compensation effect. In **B** and **C**, each point represents a cell along a tree ring of one of the three species at one of the three studied sites and during 1 year in the period 2007 to 2009. In **C**, the lines represent species-specific linear relationships, and the R^2 value is for the whole model. Figure modified from Cuny et al. {2016}.

6.4.5 | Is there a sensitive transition zone between the cambial and enlargement zones?

A long-lasting source of debate about the influences of environmental factors on tree-ring formation concerns the place and the moment at which external signals could be sensed. By analogy with the root apex {Baluška et al., 2010}, some authors suspect the existence of a transition zone located between the cambial and enlargement zones {Vaganov et al., 2011}. Such a transition zone would act as a sensor of environmental conditions and would translate them as adaptive responses during further stages of xylem cell differentiation. However, our results show that the relationships between the kinetic parameters and the climatic factors were improved when the selected 'time window' corresponded to the related xylogenesis processes, and not to the transition stage. Therefore, as defended by other authors {e.g., Wodzicki, 1971; Antonova and Stasova, 1993, 1997}, we argue that each xylogenesis sub-process is particularly sensitive to the environmental conditions prevailing at the time of its occurrence.

Our work does not definitively refute the theory of the existence of a transition zone, nor even the idea that a differentiated wood cell can store matter and/or information as it passes through this transition zone (e.g., sugar, hormones); but it shows that the climatic conditions experienced by the forming cells during their successive differentiation stages have a direct influence on the results of these processes.

6.4.6 | Does soil water availability trigger earlywood – latewood transition?

In contrast with our hypothesis that soil water availability (REW) is the driver of the seasonal changes in cell enlargement kinetics and the associated impressive decrease in cell dimension along tree rings, we did not find any relationship, either between REW and the rate or duration of cell enlargement, or between REW and the final diameter of tracheids. In fact, in the Vosges mountains, the narrow latewood cells even benefited from higher water availability to enlarge than the large earlywood cells [Fig. 6.8E]. Such results refute the long lasting belief that water conditions systematically trigger latewood formation {Kramer, 1964; Deleuze and Houllier, 1998}. By contrast, they are in agreement with observations that narrow latewood cells also are formed in irrigated trees {Zahner et al., 1964; Rossi et al., 2009b}.

6.4.7 | Influence of temperature on the cell wall deposition process

We hypothesized that the seasonal changes in cell wall deposition and resulting cell wall area were related to temperature. We actually found that the daily rate of wall deposition was strongly and positively related to the temperature, but only during latewood formation [Fig. 6.9 D-F]. Such contrasting sensitivities of earlywood and latewood cells to temperature agree with dendroclimatologists' observations that latewood conveys a much stronger climatic signal than earlywood {Lebourgeois, 2000; Wimmer and Grabner, 2000; Wang et al., 2002}. They could also be linked to the large changes in developmental signalling associated with latewood formation {Uggla et al., 2001} and reveal the strong interactions between environment and development during tree-ring formation. Moreover, these relationships were species specific: the higher slope was observed in silver fir, which is known to have the highest climatic sensitivity of the three species, whereas the lowest slope was observed in Scots pine, which is known as the least sensitive species {Lebourgeois et al., 2010}.

The nature of the influence of temperature is complex, as it can affect all the processes influencing photosynthate availability (e.g., photosynthesis, respiration, storage, transport) as well as the processes directly responsible for wall thickening (e.g., cellulose and lignin biosynthesis, cell wall material deposition). However, most results in the literature suggest a direct effect of temperatures on wall-thickening processes, which, for example, are inhibited at temperatures still favorable for photosynthesis {Körner, 1998, 2003; Donaldson, 2001; Rossi et al., 2008; Simard et al., 2013}. Lignification notably appears to be the most sensitive to temperature {Donaldson, 2001}, in particular during latewood formation {Gindl et al., 2000}.

We found a tight coupling between the duration and the rate of wall deposition {Wodzicki, 1971; Doley, 1979; Antonova and Stasova, 1993, 1997}, and we highlighted its crucial involvement in terms of the response of trees to their environment as well as its consequences in terms of tree-ring structure. Throughout the growing season, increasing durations of wall thickening counterbalanced decreasing wall deposition rates, except at the end of the season, during the formation of the last latewood cells. The direct consequence of this compensation is to prevent climate to leave traces in tree-ring structure via its influence on the rate of wall deposition. Thus, despite the strong influence of the temperatures on the wall deposition rate, the actual amount of deposited material per cell was relatively constant over the ring and related to the temperature only in the very last latewood cells, when the compensation effect of the duration ceased.

6.4.8 | Environmental versus developmental control of tree-ring formation

The results of our work allow us to draw a picture of which and how environmental factors influence the seasonal changes in xylogenesis processes creating the typical tree-ring structure for conifers grown in the temperate zone when no particular environmental stresses are involved.

Cell Enlargement

We did not find any clear influence of climatic factors on enlargement kinetics and associated cell size, suggesting that the regular decrease in cell size along a tree ring is primarily under developmental control and not linked to changes in climatic conditions. Indeed, we showed that the final tracheid diameter depends primarily on the duration of enlargement, whereas the rate of enlargement exhibits few changes during the season. We associated the high importance of the enlargement duration with a developmental control, in which phyto-hormones would be of primary importance.

Auxin, for example, is distributed across the cambial and enlargement zones according to a steep gradient, which serves as a positional signal for the developing cells {Uggla et al., 1996, 1998; Tuominen et al., 1997; Sundberg et al., 2000}. According to its position along the gradient, a cell has the ability to divide or to enlarge without dividing. However, the slope of the gradient increases during the season, implying: (1) a reduction of the width of the enlargement zone; (2) a decrease of cell enlargement duration; (3) and thus a decrease in cell diameter (if the rate remains about constant) {Uggla et al., 1998, 2001}. This strong developmental control may be coupled with daylength. We actually found a significant positive effect of daylength on cell enlargement duration [Fig. 6.9, A–C], but only during latewood formation. However, such a positive influence of daylength on cell enlargement and the resulting cell diameter has been experimentally documented for a long time, with long days inducing large diameters by promoting the synthesis of molecular substances (notably auxin) that stimulate enlargement {Larson, 1962; Richardson, 1964; Wodzicki, 1964; Balatinecz and Farrar, 1968}.

Such a strong developmental control does not fully prevent climatic influences, as well as complex inter-plays between developmental and environmental factors, particularly in more stressful environments. Indeed, the auxin gradient can undergo rapid modifications in response to extreme climatic events such as freezing temperatures {Schrader et al., 2003}. Moreover, many observations argue for strong influences of water availability on cell enlargement and the resulting diameter under harsh conditions. For example, the formation of intra-annual density fluctuations, characterized by the presence of narrow latewood-like tracheids within the earlywood or large earlywood-like tracheids within the latewood, has been attributed to unusual hydric conditions, notably drought for earlywood intra-annual density fluctuations {Wimmer et al., 2000; Bouriaud et al., 2005; Hoffer and Tardif, 2009}. In fact, an accelerating decline in cell sizes happens below some critical value of water potential {Von Wilpert, 1991}. Cell enlargement is physically inhibited and its rate collapses {Nonami and Boyer, 1990, 1990}; hence, the rapid reduction in diameter when a drought occurs {Abe et al., 2003; Rossi et al., 2009b}. This is why differences in the average cell diameter are generally observed when comparing sites or years with contrasting water conditions {Gruber et al., 2010}. However, ou works demonstrate that the huge reduction in cell size along tree rings does not necessarily results from changing water conditions.

The regular decrease in cell size is a major determinant of the whole conifer tree-ring structure, as it is the primary driver of the increase in wall thickness, and so of the transition from earlywood to latewood and of the tree-ring density profile.

Tree-ring width vs. structure

In opposition to ring width, which is very sensitive to environmental variations {Briffa et al., 2002; Esper et al., 2002}, tree-ring structure appears much more conservative. This echoes the observations from genetic studies that cell diameter, cell wall thickness, and tree-ring density are highly heritable while tree-ring width is not {Zhang and Morgenstern, 1995; Lenz et al., 2010}. So, while tree-ring width is largely related to environmental conditions, we believe that tree-ring structure is mainly related to developmental control and genetic variability. An important exception concerns the tag affixed by the temperature on the latest latewood cells, for which the variation of the rate of wall deposition was no more counterbalanced by its duration.

This late breakage of the compensatory mechanism at work in the wall material deposition process appears as a clue to explain why maximum latewood density, a parameter of the tree-ring structure localized at the end of latewood, is one of the most valuable tree-ring proxies for temperature reconstruction {Hughes et al., 1984; Briffa et al., 1998; Barber et al., 2000}. As the wall thickening takes almost 2 months for the last cells, the final part of the tree-ring structure possibly integrates several months of environmental influence, explaining why the maximal latewood density is usually correlated with the temperature on monthly to seasonal time scales {Hughes et al., 1984; Briffa et al., 1998}.

Finally, we speculate that recent warming may have decreased the temperature constraints on the cell wall deposition rate in latewood and the proportion of cells able to register the temperature signals, contributing to the explanation of the reduced sensitivity of maximal wood density to temperature over the last century {Briffa et al., 1998}.

6.4.9 | Conclusions and perspectives

In this chapter, we assessed how the seasonal variations in climatic factors influence the spectacular changes in tracheid dimensions characterizing the typical conifer tree-ring structure. We found consistent results for three conifer species grown under natural conditions in a temperate forest. Our results bring new insights on the developmental regulation and climatic sensitivity of tree-ring formation.

We showed strong and positive effects of temperature on the wall deposition rate in latewood cells, but not in early- and transition-wood cells, revealing the complex interactions between plant development and environment during tree-ring formation. On the other hand, we didn't observe any climatic influence on cell enlargement. Surprisingly, in the Vosges mountains, the narrow latewood cells benefited from higher water availability to enlarge than the large earlywood cells. This absence of a clear climatic determinism of the decreasing tracheid diameter along the ring, a crucial determinant of the whole tree-ring structure, argues for a tight developmental control of the kinetics of wood cell differentiation.

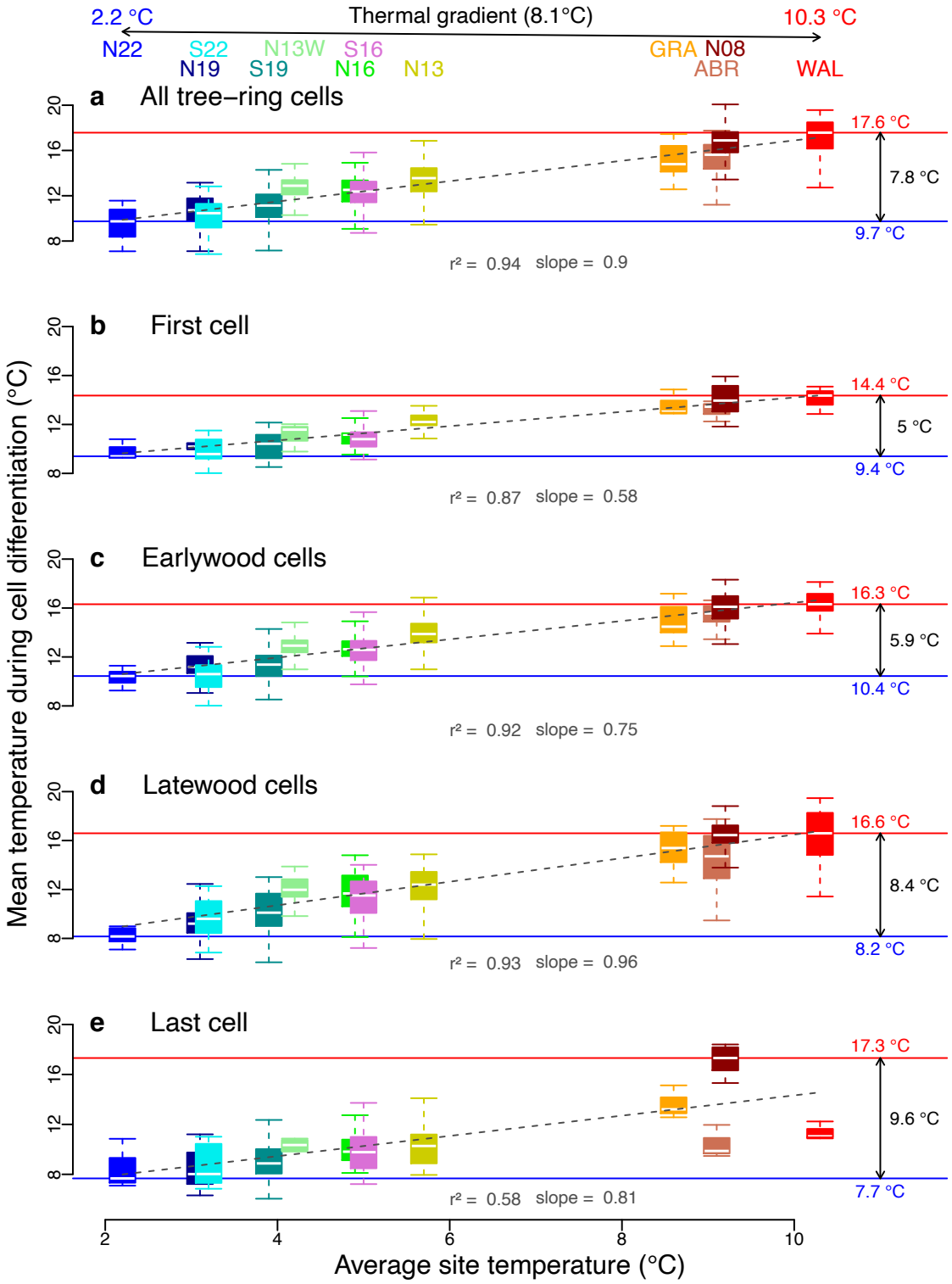
Our work also unravels the fine mechanisms by which climatic factors are recorded into the tree-ring structure. We notably revealed the existence of a compensatory mechanism that prevents the preservation of temperature signals in tracheid cell walls. Thus, we found that the climatic influences on the rate of wall deposition were offset by the compensation in the duration of wall deposition. Only for the

last latewood cells, because the duration leveled off, did the temperature influences on the wall deposition rate directly translate into the resulting amount of wall material deposited into the cells. Thus, the lack of compensation for the last latewood cells appears as a clue to explain the supremacy of maximum latewood density as a proxy for past climatic conditions in general, and temperature in particular.

6.5 | Couplings in cell differentiation kinetics mitigate air temperature influence on conifer tree-ring structure and wood functions

6.5.1 | Air temperature during cell development can varied widely between thermal environments

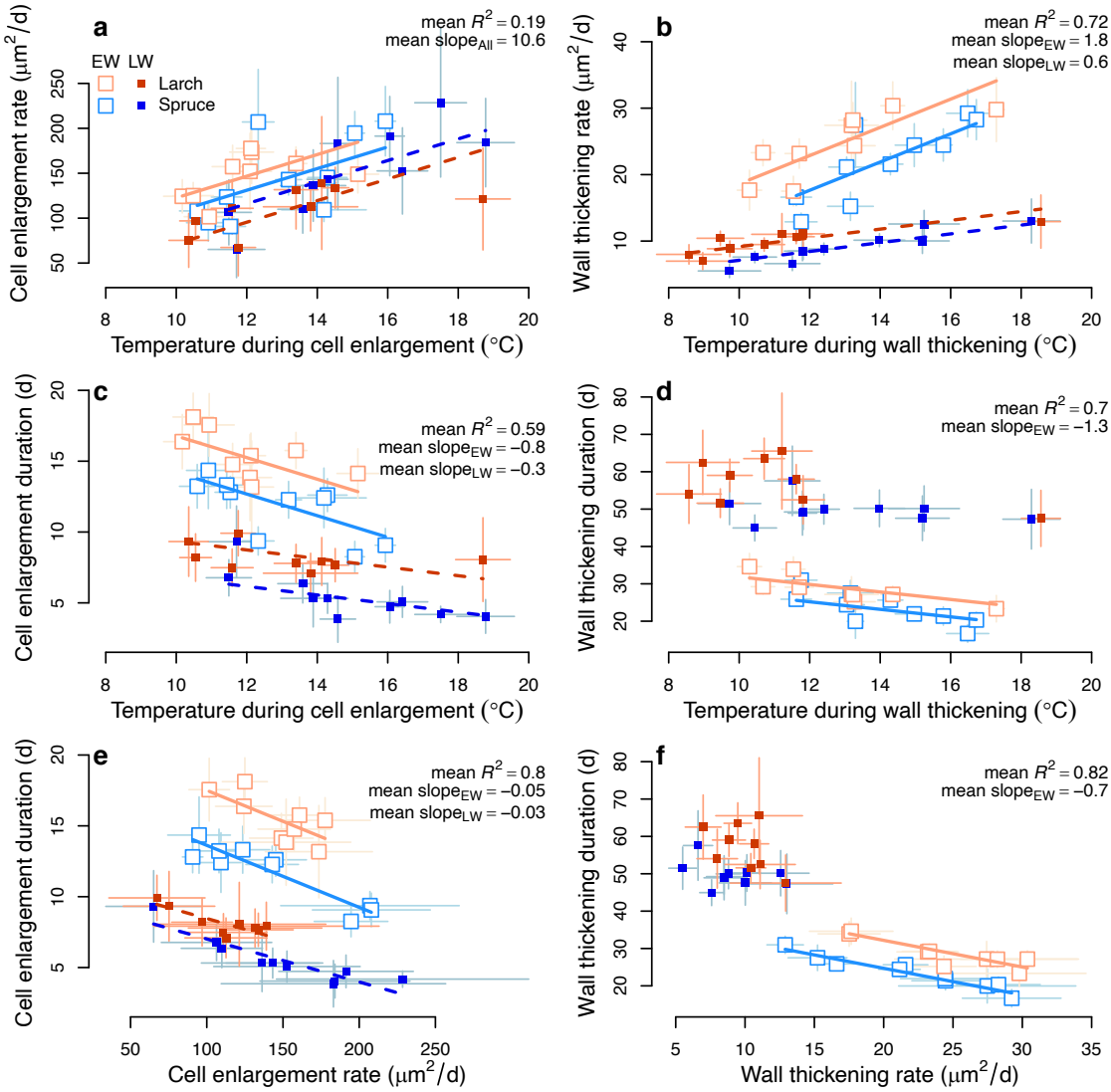
Our observations at the Donon-Lötschental elevation gradient show that despite the high plasticity observed in phenology, xylem cell differentiation operated at different air temperatures along the gradient [Fig. 6.11], even for the cells formed at the margin of the growing season (i.e., the first and last tracheid in the ring). For example, the air temperature during differentiation of the first cells varied by 5°C across sites [Fig. 6.11b]. Similarly, a 6°C range was observed for the air temperature experienced by earlywood cells [Fig. 6.11c], whereas thermal differences exceeded 8°C for latewood cells and approach 10°C for the last cells in a ring [Fig. 6.11d, e]. These observations of air temperature differences, which generally increased when moving from early- to late-wood cells even if when considering the maximum or minimum daily temperatures (data not shown), indicate that the delayed phenological onset at cold sites only partially buffered the gradient observed in mean annual temperature.



57 — Figure 6.11 | Air temperature experienced during xylem cell differentiation along the Donon-Lötschental gradient.
The figure shows the mean air temperature experienced by xylem cells during their differentiation at the 12 sites along the thermal gradients considering **(a)** all tree ring cells, **(b)** the first cell of the tree-ring, **(c)** earlywood cells, **(d)** latewood cells, and **(e)** the last cell of the tree-ring. Each boxplot represents the data included in one of the above mentioned categories for the two species at one site over the monitoring period. The dashed lines represent the relationships between the mean air temperature experienced during xylem cell differentiation and the average site temperature. Figure from Cuny et al. {2018}.

6.5.2 | Air temperature strongly influenced cell development kinetics

Our observations on the Donon-Lötschental elevation gradient revealed highly significant associations between air temperature and the kinetics of cell differentiation processes [Fig. 6.12], whereas the water conditions had no effects (analysis not shown). For both larch and spruce, we found strong, positive, linear relationships between the mean air temperature experienced during a process and the rates of this process [Fig. 6.12a-b]. However, we also observe strong, negative, linear relationships between air temperature and the durations of the processes [Fig. 6.12c-d]. In fact, rates and durations were tightly linked [Fig. 6.12e-f]. Towards colder sites, decreasing rates were associated with increasing durations. For cell enlargement, this coupling operated similarly in early- and late-wood [Fig. 6.12e]. In contrast, for wall thickening, coupling breaks down in latewood [Fig. 6.12f].

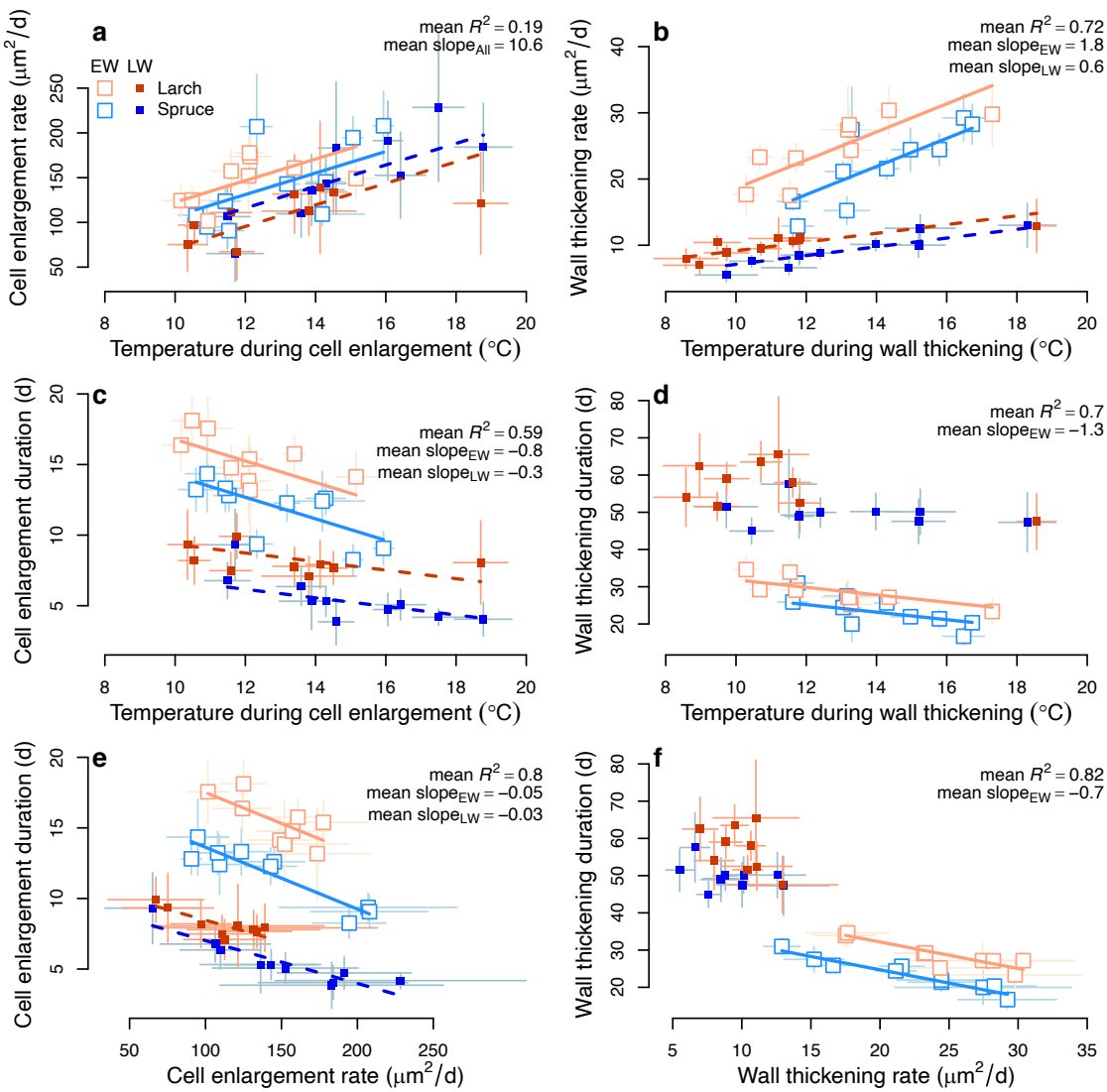


58 — Figure 6.12 | Influence of air temperature on xylem cell kinetics along the Donon-Lötschental gradient.
Relationships between the mean air temperature experienced during process realization and process kinetics, including **(a)** cell enlargement rate, **(b)** wall thickening rate, **(c)**

cell enlargement duration, and **(d)** wall thickening duration. Relationships between rate and duration of **(e)** cell enlargement and of **(f)** wall thickening. Each point represents the site and species average for early- (EW) or late-wood (LW) cells (nsites = 19), with the corresponding 95 % confidence interval. Lines represent the relationships for early- (solid light lines) and late-wood (dashed dark lines) considering all tree and year combinations, including trees observed over several years (ntree * year = 424). The mean R2 and the mean slope of the relationships are calculated as the mean of all R2 and all slopes obtained by bootstrapping 1,000 times the same linear model where only one combination tree and year is randomly selected from each tree (ntree * year = 76) to avoid repeated measures. The mean slopes are given separately for early- (slopeEW) and late-wood (slopeLW) when they are different; otherwise, a single mean slope is given (slopeAll). Figure from Cuny et al. {2018}.

6.5.3 | The plasticity observed in cell differentiation kinetics mitigated air temperature influence on tree-ring structure

The observed compensation between rates and durations of xylogenesis processes mitigated air temperature effects on tree-ring structure. Yet thermal conditions still had a significant influence on final cell dimensions [Fig. 6.13], but this influence was modest compared with the observed effects on the kinetics, when comparing slopes of relationships on normalized data (data not shown). Thus, we found consistent anatomical differences across the elevation gradient: tracheids had lower cross-sectional cell area [Fig. 6.13a], wall area [Fig. 6.13b], lumen area [Fig. 6.13c], and wall thickness [Fig. 6.13d] towards colder sites. Moreover, owing to the breakdown in rate-duration coupling at the end of wall thickening process, we observed a particularly high thermal sensitivity of wall area and thickness in latewood [Fig. 6.13.b, d].



59 — Figure 6.13 | Influence of air temperature on xylem cell dimensions along the Donon-Lötschental gradient.

Relationships between the mean air temperature experienced during process realization and the resulting cell dimension, including **(a)** cell cross-sectional area, **(b)** the wall cross-sectional area, **(c)** the lumen cross-sectional area, and **(d)** the wall thickness. Each point represents the site and species average for early- (EW) or late-wood (LW) cells (nsites = 19), with the corresponding 95 % confidence interval. Lines represent the relationships for early- (solid light lines) and late-wood (dashed dark lines) considering all tree and year combinations, including trees observed over several years (ntree * year = 424). The mean R2 and the mean slope of relationships are calculated as the mean of all R2 and all slopes obtained by bootstrapping 1,000 times the same linear model where only one combination tree and year is randomly selected from each tree (ntree * year = 76) to avoid repeated measures. The mean slopes are given separately for early- (slopeEW) and late-wood (slopeLW) when they are different; otherwise a single mean slope is given (slopeAll). Figure from Cuny et al. {2018}.

6.5.4 | The control of the kinetics of cell differentiation processes

Concerning the control of the kinetics of cell differentiation processes, the rate and the duration have usually been considered separately. On the one hand, the control of the rate has been associated with a direct influence of temperature on metabolism {Balducci et al., 2016; Cuny et al., 2015; Cuny & Rathgeber, 2016; Mellerowicz et al., 1992; Proseus, Ortega, & Boyer, 1999; Proseus, Zhu, & Boyer, 2000}.

Cell enlargement implies numerous enzyme reactions (e.g., cutting chemical bonds to loosen the wall, synthesizing, transporting, delivering, and inserting new wall polymers) with high activation energies and thus likely being very sensitive to temperature (Proseus et al., 1999; Proseus et al., 2000), whereas the processes involved in wall thickening (e.g., cellulose and lignin biosynthesis, transport, and deposition) are inhibited at temperatures still favourable for photosynthesis (Körner, 1998; Körner, 2003; Simard et al., 2013).

On the other hand, the regulation of process duration has more commonly been associated with hormonal signalling {Schrader et al., 2003; Tuominen, Puech, Fink, & Sundberg, 1997; Uggla, Moritz, Sandberg, & Sundberg, 1996}. A morphogenetic gradient of auxin concentration would shape the zonation of the developing xylem and govern process durations by providing positional information to differentiating cells {Tuominen et al., 1997; Uggla et al., 1996}.

By revealing a tight coupling between the rate and the duration of xylogenesis processes, our results urge for a more integrated approach to understanding xylogenesis and its control, with the necessity to consider together all components of the dynamics of the system and their interactions. Indeed, to date, the mechanisms coordinating the durations and rates of cell differentiation processes remain unknown. For wall thickening, a mechanism linking the rate of secondary wall deposition to the date of apoptosis may explain the observed coupling {Cuny & Rathgeber, 2016; Groover & Jones, 1999}. But why and how this coupling is broken during latewood formation is still unexplained {Cuny et al., 2014}.

With regard to coupling, the question arises as to why conifers maintain such consistent tree-ring structure (earlywood, latewood) instead of, for example, only adjusting the number of cells in a ring. This strategy makes sense when viewed from a functional standpoint, where a moderate change in tracheid dimensions can trigger a dramatic change in hydraulic functioning. For example, assuming tracheids behave as capillary tubes, the conductivity scales with the lumen diameter to the fourth power {Hagen-Poiseuille law; Sutera & Skalak, 1993}. Hence, a 2-fold decrease in lumen diameter implies a 16-fold decrease in lumen conductivity. In other words, it would take 16 cells to obtain the conductivity of a single cell having a twice wider lumen.

6.5.5 | Implications of the observed kinetics compensatory mechanisms in terms of wood functions

We used the relationships we found between temperature and cell differentiation kinetics to simulate the average dimensions and associated properties of tracheids at two theoretical sites (cold and warm) representing a 5 °C gradient. To assess the implications of the observed kinetics regulation, we then performed this exercise for the cold site but using durations of the warm site.

We estimated that, without adjustment in the durations of cell differentiation processes, trees growing at the cold site would produce earlywood tracheids with approximately 25 % smaller lumen areas than at warm site, implying nearly 50–60 % reduction in conductivity. In reality, the adjustments in duration of expansion allow producing cells with only 10 % times smaller lumen areas and less than 15 % reduction in conductivity.

Simulations also revealed that duration adjustments allow increasing cell wall reinforcement and hydraulic safety of tracheids at the cold site. This makes sense with the necessity to increase resistance to frost-induced embolism by avoiding wall collapse under negative pressure {Charra-Vaskou et al. 2016}.

Despite the compensatory mechanisms at play in the kinetics of cell development processes, anatomical differences were observed between the different thermal environments: Trees growing at colder sites produced xylem cells having smaller dimensions (cell area, wall area, wall thickness, and lumen area).

Moreover, because of the breakdown of the coupling between rates and durations of wall thickening, latewood tracheids were particularly sensitive to temperature. Such results contribute to explaining why the maximal wood density — a dendrochronological parameter tightly connected to the anatomy of latewood tracheids — is particularly well related to temperature conditions and so useful as a proxy for paleoclimate reconstructions (Briffa et al., 1998; Frank & Esper, 2005; Hughes, Schweingruber, Cartwright, & Kelly, 1984).

Our works reveal and quantify the strong response of xylogenesis kinetics to thermal environment. The compensation observed between rates and durations of tracheid differentiation notably appears as an essential mechanism that allows conifers to cope with environmental change. In addition to its role in dealing with seasonal climatic variations {Cuny & Rathgeber, 2016} and mitigating the impacts of unusual extreme climatic events such as drought {Balducci et al., 2016}, we show that this rate-duration compensation preserves the characteristic tree-ring structure optimized for mechanical stability and water transport across a wide range of thermal conditions.

6.6 | Compensatory mechanisms in tracheid differentiation kinetics mitigate the effect of drought and warming on conifer tree-ring structure

6.6.1 | Environmental condition during drought and warming experiment

In this drought and warming experiment, we monitored environmental conditions, water potential, gas exchange and xylogenesis of about 1,200 black spruce saplings grown in three different greenhouses. In the first greenhouse (T0) the temperature followed the natural external variations, in the second one the temperature was increased of about 6°C during the day (T+day), while in the third one the temperature was increased of about 6 °C during the night (T+night). In addition, in all the three green houses, two water treatments were applied: the wet treatment (W), in which soil water content was maintained above 80 % of the field capacity; and the drought treatment (D), in which irrigation was withheld for 25 days in June. So, in total there were six combinations of treatments: three thermal conditions × two irrigation regimes.

At the beginning of this experiment, the average daily air temperature in the control greenhouse was about 7 °C and increased to 12 °C in spring (April–May). Daily mean temperatures followed similar seasonal trends during the whole growing season, but T+day and T+night treatments were 4.5 and 5.2 K warmer on average than the T0. Maximal temperatures in T0 (around 24 °C) were reached in July. The temperature then gradually decreased to a minimum of 3.8 °C in October. During the water deficit period, temperature in T0 ranged between 14 °C and 22 °C.

During the water deficit period, the volumetric water content (VWC) decreased for all non-irrigated treatments, whatever the thermal conditions, reaching 0 at the end of the period. After the resumption of irrigation, the water content increased quickly, reaching field capacity again in about two weeks. Saplings from D*T0 treatment spent about 5 days in conditions where VWC was null; saplings from D*T+night treatment, 10 days; and saplings from D*T+day treatment, 20 days.

6.6.2 | Effect of drought and warming on water potential, gas exchange and carbon assimilation

From April to May, before the drought treatments, all saplings showed optimal water status (predawn leaf water potential), gas exchange (stomatal conductance), and carbon assimilation (maximum photosynthesis rate).

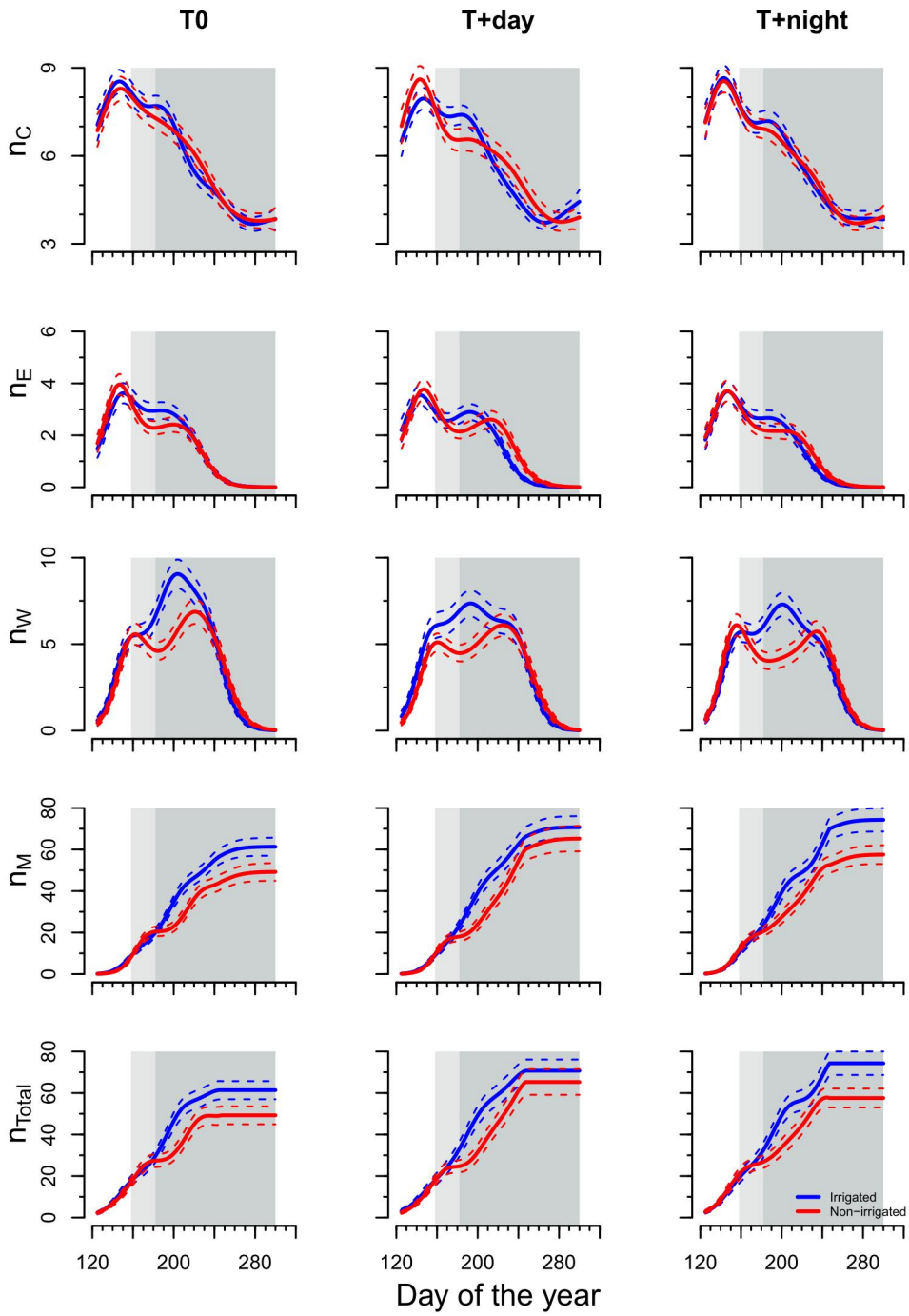
About 20 days after withholding irrigation, the predawn leaf water potentials (Ψpd) of non-irrigated saplings were much lower than those of the irrigated saplings (-1.1 MPa in D*T+day, - 1.3 MPa in D*T0 and -2.2MPa in D*T+night, respectively; versus 0.0 in irrigated treatments).

Stomatal conductance (gs) declined all along the growing season (with gs going from 0.012 to 0.06 mol m-2 s-1). The drought treatments leaded to a brutal decrease (with gs going from 0.013 to 0.03 mol m-2 s-1), followed by a return to normal trend.

Carbon assimilation (Amax) presented U-shaped trends over the growing season for all the treatments (with mean values around 7 µmol CO2 m-2 s-1). However, drought amplified these trends to such a point that assimilation was minimal or even stopped (Amax = 0.51 ± 0.90; 0.41 ± 0.50; -0.03 ± 1.00 µmol CO2 m-2 s-1 for D*T0, D*T+day, D*T+night)
After the resumption of irrigation, the timing of the recovery of saplings was significantly affected by the thermal treatments. The return of pre-dawn leaf water potential, gas exchange and carbon assimilation to normal values took about 1 week in T0, 2 weeks in T+day, and 4 weeks in T+night treatments.

6.6.3 | Effect of drought and warming on wood formation dynamics

The number of cells in the cambial zone followed similar classical bell-shape curves skewed to the left whatever the thermal treatment, while the drought spell significantly reduced the number of cells in the cambial zone, especially for T+day [Fig. 6.14].
The same is true for the enlargement zone, with an even more marked decrease in the number of cells at the end of the water stress period, especially for T+day.
The number of cells in the wall thickening zone exhibited classical bell-shape curves skewed to the right for all the three irrigated treatments; but bimodal curves, with a marked dip at the end of the drought period for all the three non-irrigated treatments.
The accumulation of xylem cells in the mature zone was speeded up by the increase of temperatures and slowed down by the occurrence of water stress.
Finally, the total number of xylem cells produced during the whole growing season was positively influenced by the warming and negatively influenced by the drought. However, it is worthy to mention important interactions since the irrigated, night-warming treatment is the most favourable one, while the non-irrigated, night-warming treatment is the less favourable one (total number of cell per treatment: W*T+night > W*T+day > D*T+day > W*T0 > D*T+day > D*T0).

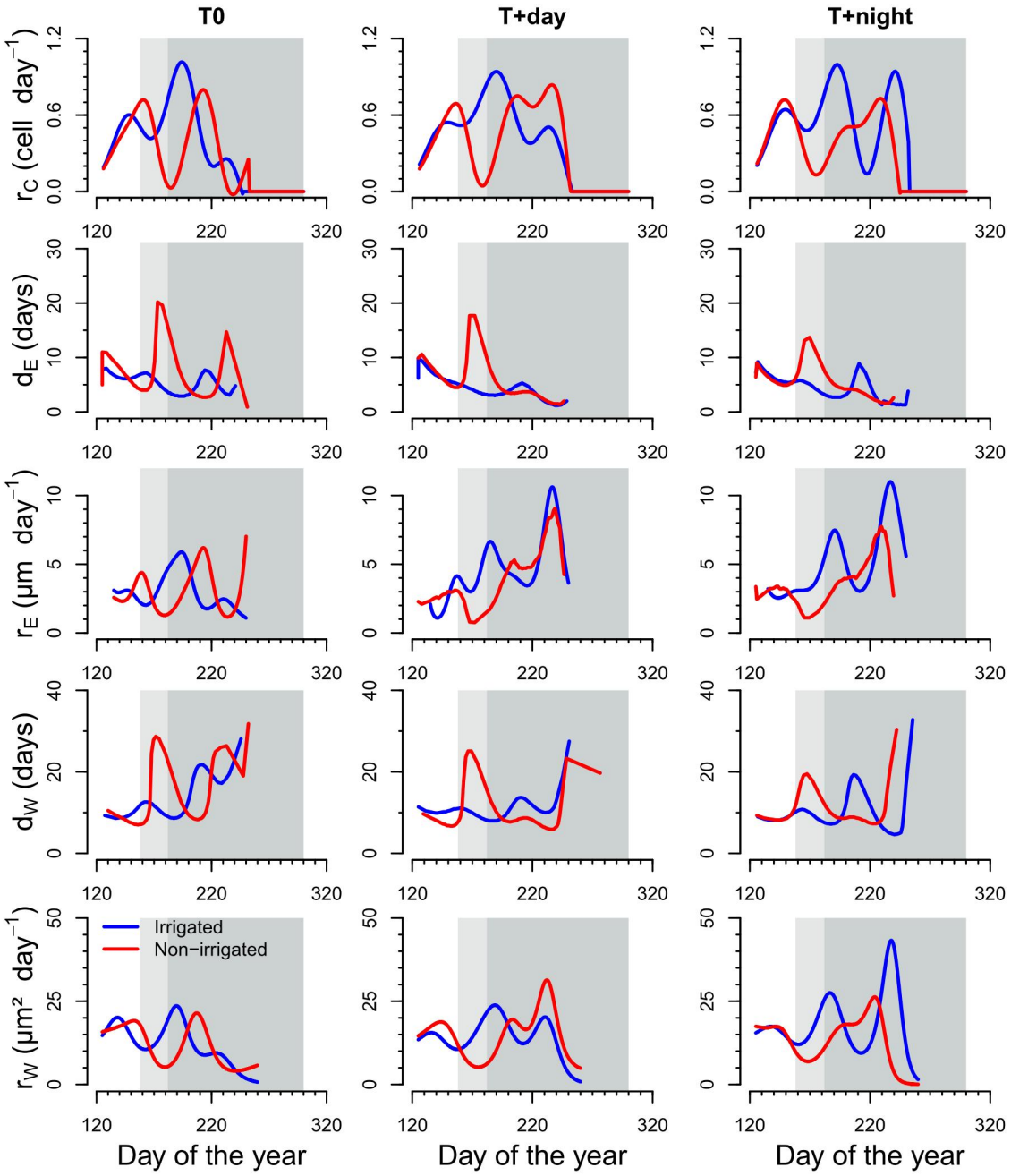


60 — Figure 6.14 | Radial number of cambial (nC), enlargement (nE), wall thickening (nW), mature (nM) and total cells (nTotal) in black spruce saplings before, during and after the water deficit period (light grey).
Blue and red curves indicate the mean number of cells in irrigated and non-irrigated saplings, respectively. Dotted blue and red curves delimit the 90 % confidence intervals for irrigated and non-irrigated saplings, respectively. Figure from Balducci et al. {2016}.

6.6.4 | Effect of drought and warming on tracheid differentiation kinetics

Rate of cell production

In general, the rate of cell production (rC) was slightly increased by the warming. The rate of cell production in non-irrigated saplings exhibited a bimodal pattern marked by a strong and significant decrease throughout the period without irrigation, some saplings even stopped cell production at the end of the drought spell [Fig. 6.15]. The collapse of the cell production rate during the drought spell explains the stagnation of the total number of cell during this period [Fig. 6.14].



61 — Figure 6.15 | Rate of cell radial production (rC) in the cambial zone, rates of enlargement (rE) and rate of wall deposition (rW), duration of enlargement (dE) and duration of wall deposition (dW) as computed from generalized additive models (GAMs).

Blue and red curves indicate the duration and rate in irrigated and non-irrigated saplings, respectively. White, light grey and dark grey background correspond to before, during and after the water deficit period, respectively. Figure from Balducci et al. {2016}.

Rate and duration of cell enlargement

In general, the durations of cell enlargement (dE) of non-irrigated saplings were higher than those of irrigated ones, while the rates of cell enlargement (rE) were lower.

The dE of irrigated saplings presented few changes during the growing season whatever the thermal treatment, decreasing progressively from about 10 days in May to 3 days in August. On the other hand, the dE of non-irrigated saplings increase of about threefold (from 5-6 to 15-20 days) during the drought period, but returned to their pre-drought values a couple of weeks after the resumption of irrigation [Fig. 6.15]. The rE followed opposite trends with progressively increasing trends for irrigated saplings and threefold abrupt decrease during the drought period for non-irrigated saplings.

The increase in dE was more important in D*T0 than in D*T+day and in D*T+night, while the decrease in rE was less pronounced in D*T0 than in D*T+day and in D*T+night, as if temperature was able to counter-balance the negative effect of drought on rE.

Rate and duration of wall thickening

In general, the durations of wall thickening (dW) of non-irrigated saplings were higher than those of irrigated ones, while the rates of wall thickening (rW) were lower.

The dW of irrigated saplings presented exponential increasing trends throughout the growing season, increasing from about 10 days in May to 30 days in August. On the other hand, the dW of non-irrigated saplings increased of about threefold (from 8-10 to 20-30 days) during the drought period, but returned to their pre-drought values a couple of weeks after the resumption of irrigation [Fig. 6.15]. The rW followed opposite trends with progressively decreasing trends for irrigated saplings and threefold abrupt decrease during the drought period for non-irrigated saplings.

The increase in dW was more important in D*T0 than in D*T+day and in D*T+night, while the decrease in rE was less pronounced in D*T0 than in D*T+day and in D*T+night, as if temperature was able to counter-balance the negative effect of drought on rW.

Tracheid differentiation kinetics overview

The different kinetics parameters (rC, dE, rE, dW, rW) were all affected by the drought treatment and presented strong responses of similar amplitudes. However, the timings of these responses seemed slightly delayed, with dW and rW reacting first, followed by dE and rE, and then by rC.

6.6.5 | Effect of drought and warming on tracheid morphology and tree-ring structure

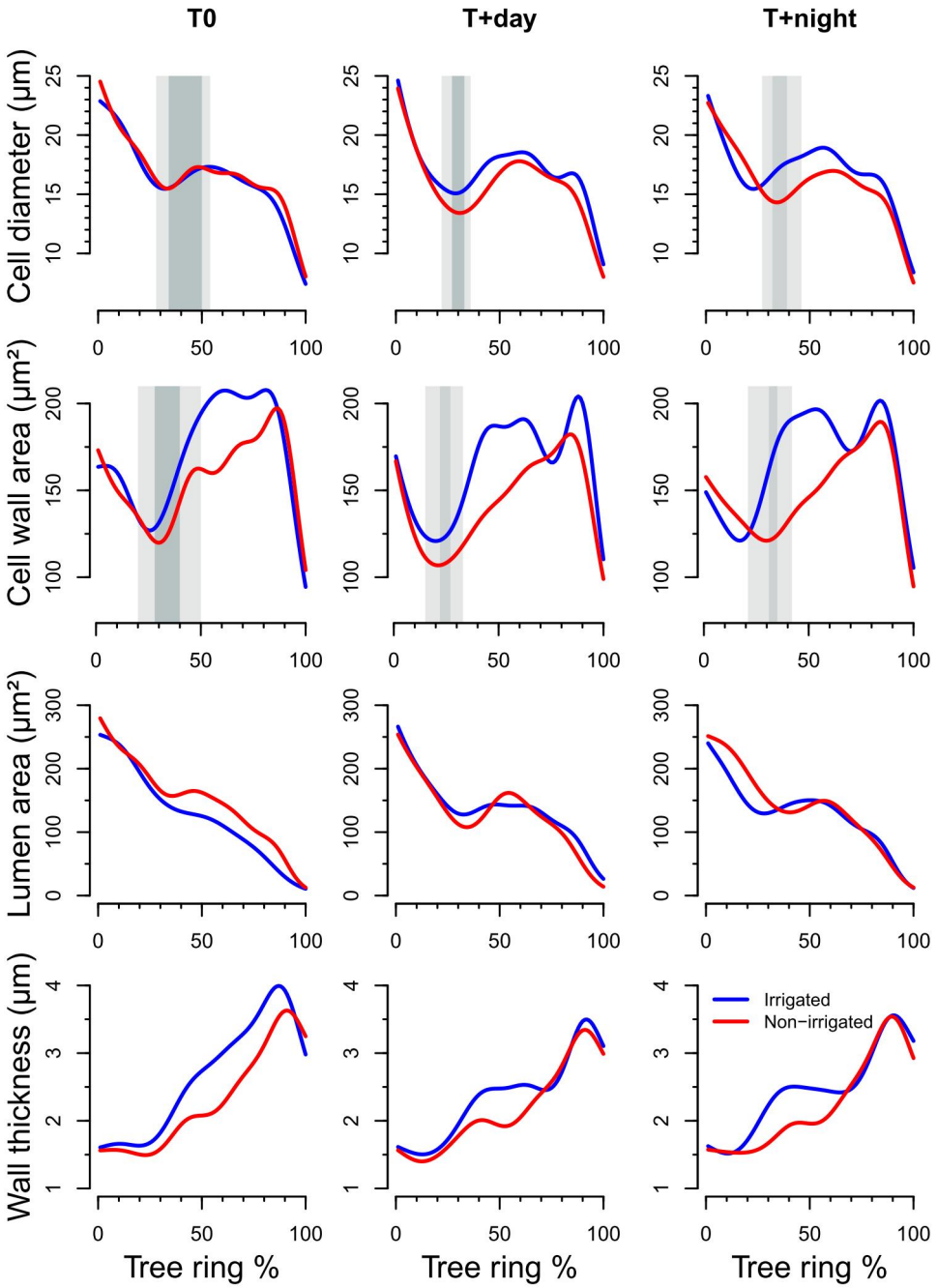
Cell number

The most important effects of our experimental manipulations of water and temperature condition are visible on the total number of cells produced by the saplings during the growing season. Saplings from the D*T0 treatment produced 48 cells on average (-21 %), saplings from the D*T+night: 54 (- 11 %), saplings from the W*T0: 61 (control), saplings from the D*T+day: 63 (+ 3 %), saplings from the W*T+day: 69 (+ 13 %) and saplings from the D*T+day: 72 (+ 18 %).

Cell diameter

Mature xylem cells in irrigated saplings were only 4 % larger, on average, than in non-irrigated saplings. However, these differences are not very informatives because of the strong trends in cell radial diameters (CRD) all along the ring from the first early wood cells (about 25 µm for all treatments) to the last latewood cells (about 5 µm for all treatments).

However, significant decreases in CRD (of about 3-4 µm) are visible for tracheids differentiated under water stress (and after) for T+day and T+night treatments [Fig. 6.16].



62 — Figure 6.16 | Cell features of the tracheids produced by irrigated and non-irrigated black spruce saplings at three thermal conditions.
T0 control temperature; T+day, 6 °C higher daytime temperature; T+night, 6 °C higher night-time temperature, before, during and after the water deficit period. Blue and red curves are for irrigated and non-irrigated saplings, respectively. Dark grey corresponds to cells that did their entire enlargement (for curve of cell diameter) or their entire wall thickening (for curve of wall cross area) during water deficit. Light grey corresponds to cells with at least 50% of their enlargement duration (for curve of cell diameter) or wall thickening duration (for curve of wall cross area) during water deficit. Figure from Balducci et al. {2016}.

Cell wall area

Mature tracheids in irrigated saplings exhibited 12 % more cell wall area (CWA), on average, than those in non-irrigated saplings.
Irrigated and non-irrigated saplings presented two distinct complex patterns of variations that started to be different during the drought period [Fig. 6.16]. Indeed, drought strongy reduced CWA area during the non-irrigated period and after. The differences between irrigated and non-irrigated saplings increased from T0, to T+night and T+day treatments.

Cell lumen area

Cell lumen area (CLA) presented strong monotonic decreasing trends all along the tree-rings. The drought period was hardly visible on these trends since CRD decreases counter-balanced CWA drops [Fig. 6.16].
However, CLA values were slightly but significantly different between the two irrigation treatments. Indeed, because they contained much more wall material without being much larger, cells of irrigated saplings had a lumen area 5 % lower on average than cells of non-irrigated saplings.

Cell wall area and thickness

Similarly, to CLA, Cell wall thickness (CWT) presented strong monotonic increasing trends all along the tree-rings, with slight differences between the irrigation treatments visible during the drought period [Fig. 6.16].
However, CWT also differed slightly but significantly according to the irrigation treatments. Indeed, because they contained more wall material for almost the same size, cells from irrigated saplings had walls about 11 % thicker than those from non-irrigated saplings.

6.6.6 | Influence of temperature and water stress on wood cell production

The interruption of irrigation for 25 days strongly reduced the cell production rate in saplings regardless of the thermal conditions. At the end of the period without watering, the rate of cell production was reduced by 86 % in non-irrigated saplings. These results confirm previous experimental studies showing that cell division is strongly affected by water deficit {Rossi et al., 2009; de Luis et al., 2011}. In situ observations also show that conifers in dry environments tend to produce fewer cells of smaller diameters {Camarero et al., 2010; Vieira et al., 2013}.
Some species, like *Juniperus przewalskii*, *Juniperus thurifera*, *Pinus halepensis*, *Pinus sylvestris* and *Pinus ponderosa* are even documented to stop cambial activity earlier when drought is too severe {Camarero et al., 2010, Gruber et al., 2010; Eilmann et al., 2011; Ren et al., 2015, Ziaco et al. 2016}. However, in our study, the rate of cell production didn't return to zero during the drought period, even at the end of the period and for D*T0. Black spruce saplings maintained a minimal rate of cell division during the drought, allowing a faster increase in cell production when favourable conditions resume.
Indeed, we showed in this study that the cambium is highly plastic and can resume strong cambial activity as soon as water conditions become favourable again for

growth. This echoes in situ observations of the formation of intra-annual density fluctuations in some species like *Pinus halepensis* {de Luis et al., 2011}.
Finally, in our study, warming treatments had always a positive influence on cell division rates even for non-irrigated saplings. Rates were nearly doubled in the night-warming treatment, suggesting that most of the cell production may occur during the night-time {Saveyn et al., 2007; Turcotte et al., 2011}.

6.6.7 | Unravelling compensatory mechanisms in cell differentiation kinetic parameters under drought and warming treatments

Presentation of the compensatory mechanisms

The non-irrigated saplings responded to water deprivation by considerably decreasing the rates of all xylogenesis sub-processes (i.e. cell division, cell enlargement and secondary wall formation), but these reductions in rates were compensated by proportional increases in the durations of the corresponding sub-processes. While the rates of enlargement and thickening decreased by about threefold during the drought period, the durations of these two sub-processes increased by two to threefold, mitigating in large proportion the negative impact of water stress on cell morphology and tree-ring structure.
The compensatory mechanisms, we unravel in this study, seem to be very general since we observed them in all the three xylogenesis sub-processes (i.e. cell division, cell enlargement and secondary wall formation), and for all the studied conifer species {Cuny et al. 2016, 2018; Balducci et al. 2016}. This suggest that the couplings between the rates and the durations of the xylogenesis sub-processes directly come from the functioning of the wood formation system (i.e. the cambial zone plus the developing xylem).

The compensation between the rate and the duration of cell enlargement — The 'conveyor belt' hypothesis

Adopting a cinematic view, the developing-xylem zone can be seen as a conveyor-belt: the cells that are produced by the cambium are then 'transported' through the successive differentiation zones by the 'developing-xylem conveyor-belt'. It is important to note that in this conceptual view the differentiating xylem cells are at the same time the 'engine, the belt, and the parcels' of the carousel!
During drought, the speed of the 'developing-xylem carousel' slows down because the growth rates of the cells decrease due to water stress. If the widths of the successive differentiation bands on the carousel are not modified, then the time spend by a cell to cross the band will increase proportionally to the reduction of the speed of the carousel.
This image of the 'developing-xylem conveyor-belt' helps to see how the rates and durations of wood-formation sub-processes are intrinsically linked together.

The morphogenetic gradient and the effect of phytohormones

In the developing xylem, the width of the successive differentiation zones, and thus the time spend by the differentiating cells in these zones are determined by

morphogenetic gradients of phytohormones {e.g. Bhalerao & Fischer 2017}. For example, auxin is distributed along a steep concentration gradient across the division and enlargement zones and is proposed to act as a morphogen for this region {Bhalerao & Fischer 2014, Uggla et al., 1996; Tuominen et al., 1997; Sundberg et al., 2000}.
However, auxin also stimulates cell growth by inducing extracellular acidification, which activates expansins, proteins that loosen the cell wall to allow enlargement {Cosgrove, 2000; Perrot-Rechenmann, 2010}.
Then, increasing the level of auxin, for example, should increase the width of the enlargement zone, but, because of the stimulating effect of auxin on cell growth (in the enlargement and division zones), it should also increase the 'speed' at which cells are 'crossing' this zone, resulting in the formation of cells of the same dimensions, but produced more rapidly.
The 'conveyor-belt hypothesis' can explain why, under water stress, the duration of xylem cell enlargement increases proportionally to the diminution of its rate, assuming that water stress has a direct effect on the cell growth rate through the reduction of turgor pressure, but without modifying the auxin morphogenetic gradient and its control on the cell fate and potential growth rate.

The coupling between the rate and duration of cell wall thickening — The 'protease intoxication' hypothesis

In contrast to cell enlargement, the compensatory mechanism between the duration and rate of cell wall thickening is at work, not only in the adaptation to drought stress events {Balducci et al. 2016}, but also to low temperatures {Cuny et al. 2018}. Even during the growing season, the decrease in the deposition rate is most of the time offset by the increase in duration {Cuny et al. 2016}. This results in an almost constant amount of wall material deposited thorough the growing season, except for the last quarter of the ring {Cuny et al. 2014}.
However, the 'conveyor-belt hypothesis' cannot fully explain the strong coupling between the duration and the rate of cell wall thickening. During our drought experiment, for example, the diminution of the wall deposition rate is only indirectly associated with the augmentation of the deposition duration thanks to the concomitant diminution of the cell growth rate (i.e. the speed 'developing-xylem conveyor-belt'). Thus another mechanism should be at play to explain such a thigh coupling between cell wall thickening rate and duration.
Groover & Jones {1999} revealed a biological mechanism coordinating secondary wall synthesis and programmed cell death that could provide an explanation of the observed coupling. During the secretion of wall material, a protease accumulates in the extra-cellular matrix. Once a critical concentration of this protease is reached, apoptosis is triggered and secondary wall formation stops. According to this mechanism, the diminution in the rate of wall deposition observed thorough the growing season is accompanied by a decreased excretion of protease in the extracellular matrix. Thus, as the growing season progresses, more time will be required for protease to reach the critical level, delaying apoptosis and increasing proportionally the duration of wall deposition. For the last latewood cells, however, the coupling between rate and duration is broken {Cuny et al. 2016}. At this stage, the deposition rate may be too slow to allow the concentration of protease to reach

the critical threshold. A secondary mechanism may take over to trigger apoptosis, such as sucrose concentration or reactive oxygen species {Bollhöner et al., 2012}. Such a complex and dynamic system highlights the need for detailed models patterning plant cell development (including xylogenesis) to take into account the intertwined effects of regulatory agents in the different developmental stages {Harashima & Schnittger, 2010} (but see section 6.7 for a detailed presentation of wood formation models).

The functional consequences of the compensatory mechanisms

Thanks to the compensatory mechanisms involved in xylem differentiation kinetics the typical conifer tree-ring structure is maintained even under severe drought stress. Overall, the loss of biomass production was relatively limited, with wood from non-irrigated saplings being about 5 % lighter than wood from irrigated ones (620 vs 660 kg.m-3 respectively; see Balducci et al. {2015} for the detailed description of wood density measurements on the saplings) and tree-rings from non-irrigated saplings being about 18 % narrower than those from irrigated saplings (55 vs. 67 cells respectively). The loss of efficiency in mechanical support or water conduction of the tree-rings from non-irrigated saplings is also limited and mainly due to the reduction in the total number of cells.
Finally, this study highlights the contrasting sensitivity of tree-ring width and structure, the former being highly plastic and the latter very conservative.

6.6.8 | Coordination in cell differentiation kinetics sub-processes under drought and warming treatments

Extension of the 'conveyor belt' hypothesis

The 'conveyor-belt' hypothesis is also generally used to provide a time-frame for the coordination of the different sub-processes of xylogenesis under drought. When water stress occurs, stem water potential drops, leading cell growth and cell division rates to fall down; thus, newly produced cells no longer 'push' older developing cells out of their differentiating zones. Consequently, developing cells get 'stuck' in their current differentiation stages (enlarging cells experiencing low growth rates and thickening cells low deposition rates) and waiting for better conditions to resume normal differentiation. This reasoning is commonly used to explain how xylogenesis creates intra-annual density fluctuations (bands of narrow thick-walled tracheids) under water stress.

Cell growth rate maintenance under drought

Indeed, adequate turgor pressure maintenance is required in all the cell differentiation processes, from cell division to the deposition of cell walls, and especially for cell growth {Proseus et al., 2000; Pantin et al., 2011}. Génard et al. {2001} estimated the minimal turgor pressure necessary for irreversible cell wall expansion (the wall yielding threshold) at about 0.9 MPa. In the absence of water deficit, this threshold is normally reached after sunset when the water flows back from the mature wood to the developing xylem cells and the cambium {Steppe et al., 2015}. In this study pre-dawn leaf water potential were between -0.5 and -0.6 MPa in irrigated treatments, and between -1.09 and -2.28 MPa in non-irrigated

treatments indicating severe water stress in non-irrigated saplings at the end of the drought period. This strong diminution of water potential most probably push differentiating xylem cell turgor pressure under the wall yield threshold, preventing cell growth.
Plant cells respond to low external water potential by decreasing their own osmotic potential, accumulating solutes (e.g. sugars, ions and proteins) inside their vacuoles in order to maintain, as much as possible adequate turgor pressure {Major & Johnsen, 1999; Proseus et al., 2000}. In black spruce, for example, the accumulation of raffinose, an osmotically active sugar, start when the water potential fall below -1 MPa {Deslauriers et al., 2014}. This value possibly indicating the threshold at which water starts to flow from the living cells to the mature xylem because of the reduction of turgor pressure. Thus, turgor pressure regulation in plant cell is deeply connected with sugar allocation and management.

Wall deposition rate maintenance under drought

We saw in C2TM experiment that water stress lead to a rapid decrease in gas exchange, which lead in turn to a strong reduction of photosynthetic activity, which results in a strong diminution in carbon availability {Deslauriers et al. 2014, 2016}. We know that photo-assimilates play a central role in xylogenesis, since sugars are the main resource for cell wall construction {Ainsworth & Bush, 2011; Giovannelli et al., 2007; Pantin et al., 2013; Deslauriers et al., 2014}. Then, the negative effects of water stress on carbon assimilation may indirectly reduce the rate of cell wall deposition and explain the compensatory effect between rate and duration in cell wall formation. However, we do not yet know, how sugars from reserves can be used to compensate for the deficient assimilation under drought.
Nevertheless, during severe water stress, sugars are more likely to be directly involved in osmoregulation and cannot fully sustain the demande related to cell wall deposition process {Pantin et al., 2013; Deslauriers et al., 2014}.

Xylogenesis sub-processes coordination under drought

Counterintuitively — From an eco-physiological point of view, if no other regulations are considered, water stress should hit first the wall deposition process, reducing its rate, because the sugar destined to wall construction are used for osmotic regulation instead. The process of cell division should be hit in second since it involves both primary cell wall production and cell growth. The process of cell enlargement should be hit last since it only involves cell growth. Figure 6.15 do not refute this theory.
Then water stress should affect biomass sequestration into tree-ring even more than radial growth s.s. This is what we observed when we assed that tree-rings from non-irrigated saplings were 10 % narrower, than control ones, exhibiting in addition a reduction of 5 % of their density.

6.7 | Modelling the kinetics of tracheid differentiation in conifers

6.7.1 | Introduction

The 'stability' of the conifer tree-ring structure

The anatomical structure of conifer tree rings, with their succession of large earlywood cells and narrow latewood cells, demonstrates a strikingly stable organisation under contrasting conditions {Balducci et al. 2016; Kiorapostolou et al. 2018; Cuny et al. 2018}. Over one growing season, xylem radial growth follows a typical Gompertz curve, whose parameters depends on internal and external factors {Camarero et al. 1998; Rossi et al. 2003; Cuny et al. 2012}.

Wood formation monitoring through microcore samplings reveals that, during the growing season, the developing xylem generally displays a spatial pattern composed of: (1) a division zone (or cambial zone sensu stricto), where cells grow and divide; (2) an enlargement zone, where the newly formed xylem cells grow without dividing; (3) a maturation zone, where non-growing cells undergo secondary wall deposition and lignification; and (4) a mature zone, composed of dead, fully functional xylem cells {Wilson 1984; Rathgeber et al. 2016}. Over the growing season, the width of each zone follows a specific bell-shape curve {Cuny et al. 2013, 2014, 2016, 2018; Balducci et al. 2016}.

The stability over the growing seasons of these dynamic patterns, and of the resulting tree-ring structure, suggests a tight internal control of xylem formation.

The morphogenetic-gradient hypothesis

Spatial organisation could be established through the flow of biochemical signals between the phloem and the xylem. Indeed, the role played by such signals in the control of wood formation is well-documented {see a review by Fischer et al. 2019}. The radial distribution of auxin, the most studied phytohormone, has been measured in several species and at different times and positions inside the developing wood during the growing season {Tuominen et al. 1997; Uggla et al. 1996, 1998, 2001}, revealing a concentration peak around the cambial zone that varies during the season. Based on these observations, some authors put forward the 'morphogenetic-gradient hypothesis', according to which the auxin concentration profile prescribes the width of each differentiation zone and, eventually, the final sizes of produced xylem cells {Sundberg et al. 2000; Bhalerao and Bennett 2003}.

However, it has been shown using dynamical modelling that while the morphogenetic-gradient hypothesis accounts for the shape of the xylem growth curve and for the seasonal dynamics of the developing xylem zonation, it fails at explaining the dimensions of the produced tracheids {Hartmann et al. 2017}. As long as it is assumed that a single signal sets both division and enlargement identities (which is the core of the morphogenetic-gradient hypothesis), tracheid dimension patterns do not follow the typical conifer tree-ring structure that is commonly observed.

Process-based models of tree-ring formation

In parallel, several models of tree-ring formation have focused on carbon and water resources {Deleuze and Houllier 1998; Vaganov et al. 2006, 2011; Hölttä et al. 2010; Wilkinson et al. 2015; Drew and Downes 2015; Schiestl-Aalto et al. 2015}. But they all aim at establishing relationships between environmental conditions, radial growth and wood density, while paying little attention to the biological mechanisms involved at the cellular level.

More recently, Carteni et al. {2018} developed an original functional approach and proposed a mechanism linking the seasonal variations in sugar availability in the cambium to the anatomical structure of tree rings. This model convincingly reproduces the typical conifer tree-ring structure, but do not fully represent the biological mechanisms behind tracheid differentiation since primary and secondary wall deposition are not distinguished. Another strong limitation of the model is the absence of spatial aspects, making it unable to capture the coordination of the xylogenesis processes at the tissue level.

A mechanistic framework to model tree-ring formation dynamics

While carbon and water are indispensable ingredients of wood formation, a growing body of experimental works points at the driving role played by hormones and peptides {Etchells et al. 2015; Immanen et al. 2016; Gursanscky et al. 2016; Brackmann et al. 2018; Han et al. 2018; Smetana et al. 2019}. Moreover, the stability in space and time of wood formation process, despite fluctuating environmental conditions, underline the importance of intrinsic regulatory mechanisms. Since the full picture of this regulation remains unclear, dynamical models are needed to disentangle the role played by each signal in xylogenesis.

Additional inspiration could come from results obtained in the root apical meristem {Beemster and Baskin 1998; Bishopp et al. 2011; Muraro et al. 2013, 2014}, which is more easily accessible to experimental investigations. Bishopp et al. {2011} showed that a crosstalk between auxin and cytokinin specifies developmental zones, with cytokinin promoting auxin polar transport. Based on this finding, Muraro et al. {2013, 2014} developed models of vascular patterning in Arabidopsis thaliana roots.

In this section, we present the last development of the XyDyS (Xylogenesis Dynamics Simulator) modelling framework, which was initially created to test the morphogenetic-gradient hypothesis {Hartmann et al. 2017}. The new XyDyS version assigns xylem cell identity based on two interacting biochemical signals.

6.7.2 | Model description

Core of the model

Taking advantage of the symmetry of the xylem tissue, we only consider a single radial file of differentiating cells. The radial file is composed of cells that (within a given growing season) either differentiate into tracheids (possibly after one or several division cycles) or remain undifferentiated in the cambium at the end of the season. We focus on a single growing season and the formation of one tree ring. Spatially, the first boundary of the system ('the cambium boundary') is the interface with the part of the cambium, which differentiates into phloem. The second boundary ('the xylem boundary') is the interface with the mature xylem

produced during the previous year. Within a file, cells are indexed from i = 1, at the cambium boundary, to i = N(t), at the xylem boundary, N(t) being the number of cells in the radial file at time t. Each cell is geometrically characterized by its radial dimension, called 'length' Li(t). L(t) denotes the total length of the radial file at time t. For the initial condition, we suppose that there are initially N0 cambial cells in the radial file, all with the same length Linit.
Two signals coming from the cambium boundary and denoted here after by D and G flow through the radial file. Signal D is associated with cell division and could be identified as either the TDIF peptide or the cytokinin phytohormone, while signal G is associated with cell growth and is identified as auxin.

Apoplastic diffusion of signal D

The exact nature of signal D is not elucidated yet, but we assume that it diffuses in the apoplast, like peptides and cytokinins do.
The simplest model for signal diffusion is Fick's law {Crick, 1970}, with a constant decay rate. We also assume that signal D is not produced in the developing tissue but only comes from an external source at the cambium boundary. This is a 'source-diffusion-decay mechanism' as proposed by Wartlick et al. {2009} and Grieneisen et al. {2012}. Given the very slow growth of the developing xylem, dilution and advection (i.e. directed movement driven by tissue growth) can be neglected {Hartmann et al. 2017}. Then, the transport equation of signal D writes as
Equation (6.1)
D(x, t) denotes the concentration of signal D at position x and time t, δD denotes its diffusion coefficient and μD its decay rate. The space variable x is defined such that the cambium boundary of the file is located at x = 0 and the xylem boundary at x = L(t).
Equation 6.1 can be solved analytically. It is useful to introduce a characteristic length associated with the diffusion-decay process, expressed as:
Equation (6.2)
When the file becomes long compared to λ, the concentration profile reaches a stationary exponential shape, given by the equation:
Equation (6.3)
Finally, Di denotes the average concentration of signal D in cell i.

Symplastic polar transport of signal G

To describe the flow of signal G, we use a model of auxin fluxes similar to the 'unidirectional transport mechanism' from Grieneisen et al. {2012}. Where PIN carrier proteins are present, auxin is polarly transported from one cell to another. In addition to this active transport, there is a residual constitutive permeability to auxin, which is the same between every consecutive cell. The auxin flux Fi,i+1 from cell i to cell i+1 depends on the concentration of auxin in cell i and on the amount of PIN in cell i oriented toward cell i+1 {Grieneisen et al. 2012}. This writes as
Equation (6.4)
where Gi(t) is the concentration of signal G in cell i, pi,i+1 is the amount of PIN proteins in cell i oriented toward cell i+1, and q is the constitutive permeability to auxin. Moreover, we assume that PIN proteins are always oriented toward the

xylem, i.e. $p_{i,i-1} = 0$. Therefore, auxin fluxes toward the cambium boundary rely only on constitutive permeability, i.e. $F_{i,i-1}(t) = qG_i(t)$.
If one considers cell i, entering fluxes from cells i-1 and i+1 are respectively $F_{i-1,i}$ and $F_{i+1,i}$, and exiting fluxes toward cells i-1 and i+1 are respectively $F_{i,i-1}$ and $F_{i,i+1}$. Considering also decay, and dilution due to cell growth, the concentration of signal G in cell i is governed by the following equation:
Equation (6.5)
μG is the decay rate of signal G, and is the growth rate of cell i {Moulia and Fournier 2009}, defined by:
Equation (6.6)
If fluxes are decomposed into polar and passive components, equation 5 becomes:
Equation (6.7)

Cell identity assignment

In the classical morphogenetic-gradient model {Bhalerao and Fischer 2014; Hartmann et al. 2017}, cell identities are set by a single signal, with two concentration threshold values: a division threshold Tdiv, and an enlargement threshold Tenl, with Tdiv > Tenl . But this way of assigning identities leads to unrealistic patterns in mature tracheid diameters {Hartmann et al. 2017}. Therefore, we propose an amended hypothesis in which two distinct signals control cell identities [Fig. 6.17]. Where the concentration of signal D is higher than the division threshold, cells are able to divide. Similarly, where the concentration of signal G is higher than the enlargement threshold, cells enlarge. Put in a more formal way, for a given cell:

- If $D_i \geq T_{div}$, the cell enlarges and is able to divide;
- if $D_i < T_{div}$ and $G_i \geq T_{enl}$, the cell enlarges but is not able to divide;
- if $G_i < T_{enl}$, the cell no longer enlarges.

Moreover, we assume that auxin efflux carriers (PIN proteins) are present only in cells that are able to divide (i.e. $p_{i,i+1} > 0$ only if $D_i \geq T_{div}$). In these cells, the amount of PIN proteins, $p_{i,i+1}$, is assumed to be proportional to the auxin concentration in cell i.

Cell growth and division

Although the mechanical force for cell enlargement comes from turgor pressure, this process is controlled by cell wall extensibility {Cosgrove, 2005}. We assume that auxin also acts on wall extensibility, and thus controls the growth rate of those cells which are able to enlarge. The simplest guess is a direct proportionality between the growth rate and the concentration of signal G {Hartmann et al. 2017}. However, this relationship implies exponential growth for constant levels of auxin, which tends to amplify inhomogeneities in cell sizes. Therefore, we propose here that larger cells display a weaker growth response to auxin, in the form of an inverse proportionality to cell size:
Equation (6.8)
Cell division follows a simple geometrical criterion. If a cell has an identity that allows division when reaching a critical length defined as twice its initial length Linit, it divides.

Boundary conditions

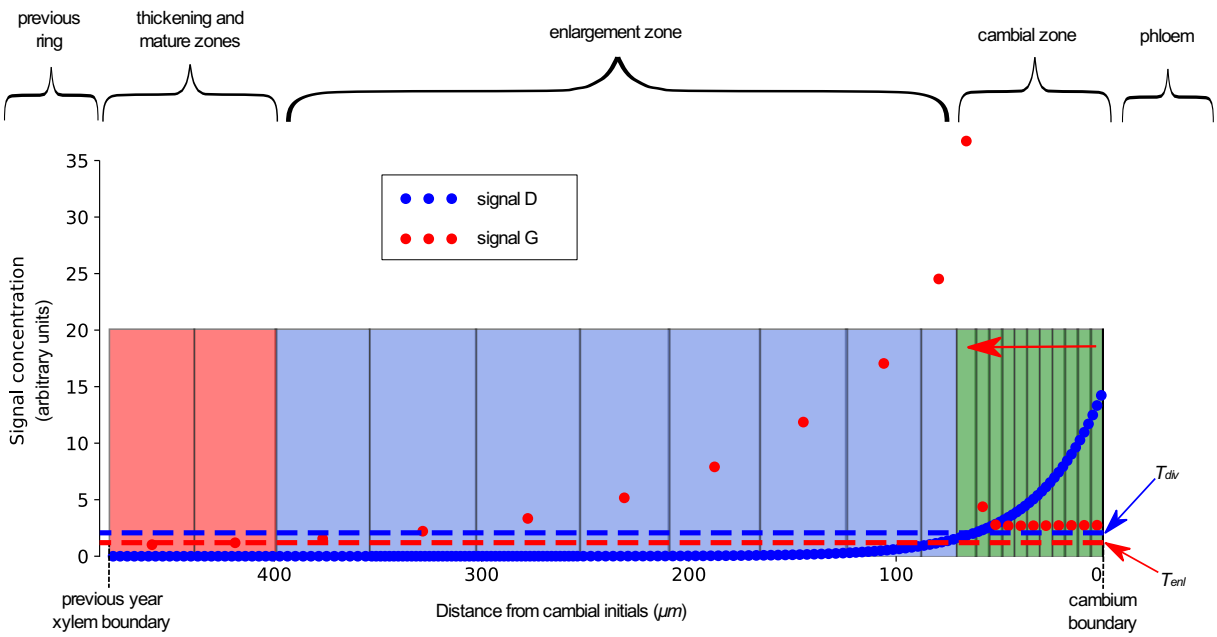
The concentrations of signals D and G are imposed at the cambium boundary of the file, and are given as entries of the simulations. The concentration of signal D at the cambium boundary is assumed to increase slightly during the first weeks of the season, and then decreases slowly and linearly. The cambium-boundary concentration of signal G is assumed to start from zero at the very beginning of the season, then rapidly increases during the first weeks of the season to reach a maximum, and then progressively decreases as the season goes. This reflects the sudden flush of auxin coming from the shoots during bud break.
Finally, we assume that the xylem acts as an impermeable barrier to molecules of signals D and G. Accordingly, a zero-flux boundary condition is imposed for both signals at the xylem boundary.

Implementation and visualization of the simulations

Experimentally, the descriptions of the differentiation zones are based on visual criteria. In order to be able to compare the outputs of XyDyS models with real data, we apply similar criteria a posteriori on model outputs, setting 'apparent statuses' to virtual cells:

- Cambial cells are defined as growing cells that are smaller than two times the diameter of a newly created cell (Li < 2Linit).
- Enlarging cells are growing cells larger than two times the diameter of a newly created cell (Li > 2Linit)
- Wall-thickening and mature cells have stopped their growth [Fig. 6.17].

F. Hartmann has developed a graphical user interface dedicated to XyDyS models on Traits and TraitsUI libraries.



63 — Figure 6.17 | Schematic layout of a XyDyS simulation.
Signals D and G form concentration gradients (respectively blue and red dots), which impose cell identities and growth rates. Cells with a concentration of signal D above the division threshold (T_{div}) have the ability to divide. Cells with a concentration of signal G above the enlargement threshold (T_{enl}) are growing, with a growth rate related to the concentration of signal G. Carrier proteins (materialized by the red arrow) transporting

signal G toward the xylem are present only in cells that have the ability to divide. The zonation is based on post-simulation cell identity determination. Cambial zone in green is composed of small growing and dividing cells. Enlargement zone in blue is composed of large growing cells (at least twice larger than cambial cells). Thickening zone and mature zone in red is composed of non-growing cells. Figure from Harman et al. {in preparation}.

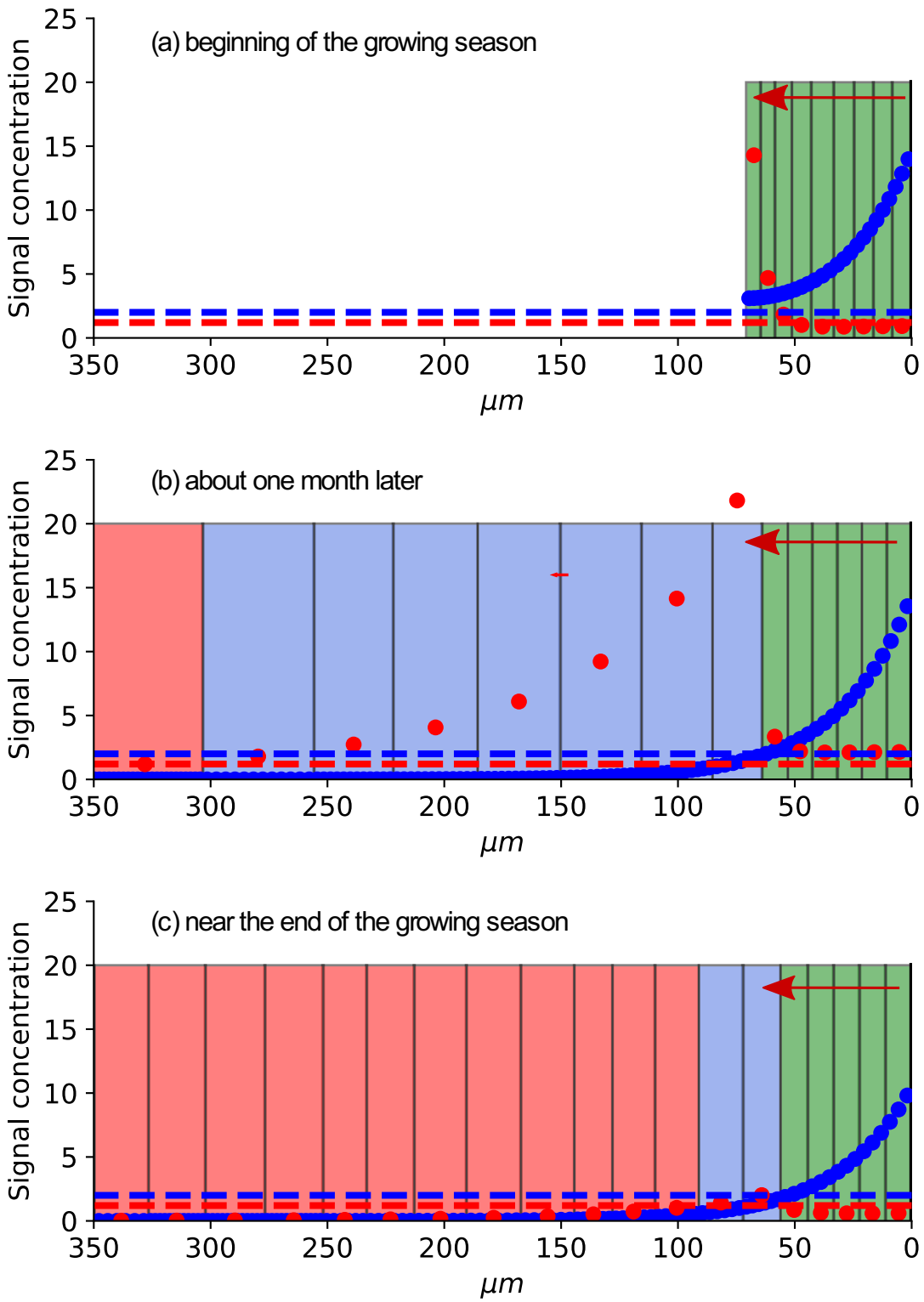
6.7.3 | Results

The cross-talk between signal D and G leads to the progressive establishment of a stable auxin gradient

At the beginning of the growing season, the length of the radial file is shorter than the characteristic length (λ) of the signal D, which is then filling in the cells of the file with a high concentration [Fig. 6.18]. Therefore, all cells are dividing and transport auxin toward the xylem. As a consequence, signal G accumulate in the cells close to the xylem boundary, which thus present a high growth rate.

When the radial file become larger than λ, the concentration profile of signal D progressively reached the stationary exponential shape given by Eq. 3. From this time on, polar transport was limited to a few dividing cells and the concentration of signal G peaked around the boundary between the cambium and the enlarging zone.

The gradient of signal G is then stable and the height of the peak depend only on the concentration of signal G at the cambium boundary. This shows that active polar transport, regulated by another signal, can account for the peaked distribution of auxin observed in the developing xylem.



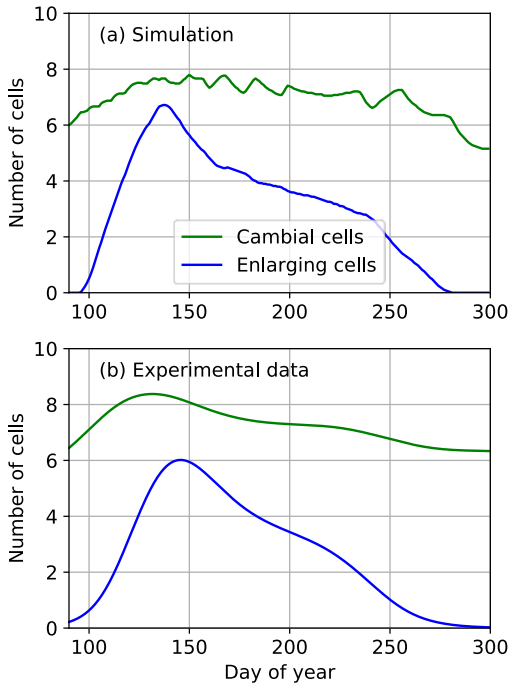
64 — Figure 6.18 | Evolution of the gradients of the two biochemical signals along the growing season.
(a) At the beginning of the growing season, signal D (blue dots) is above the division threshold in every cell. Signal G (red dots) is transported toward the xylem and accumulates at the xylem end of the cell file. **(b)** about one month later, when the file has grown longer, both signals reach a stationary gradient. The concentration of signal G peaks around the boundary between the cambial and enlargement zones. **(c)** Toward the end of the growing season when the supply of signal decreases, growth is nearly over. Figure from Harman et al. {in preparation}.

The cross-talk between signal D and G controls the zonation of the developing xylem over the growing season

The width of the cambial zone is mainly controlled by signal D. At the beginning of the growing season, all cells belong to the cambium and the cambial zone expanded rapidly since signal D was high in every cell. This caused an early 'burst' in the number of cambial cells and, after a lag, in the number of enlarging cells. Such a rapid increase had also been observed in monitoring studies {Cuny et al. 2014, 2018; Balducci et al. 2016}. As the concentration profile of signal D stabilises into a stationary gradient, the number of cambial cells reaches a plateau. This plateau depends only on the concentration of signal D imposed at the cambium boundary and on the characteristic length, λ. Since λ was assumed to be constant (because the diffusion coefficient and decay rate of signal D are themselves constant), the width of the cambial zone is entirely driven by the concentration of signal D at the cambium boundary.

Regarding the enlargement zone, the width of the gradient of signal G is the main driver. For a given value of decay rate, this width increased with the height of the concentration peak, which in turn depended on the concentration of signal G at the cambium boundary and on the number of polar transporters in dividing cells. Since the number of transporters is proportional to the local concentration of signal G, the width of the enlargement zone is entirely driven by the concentration of signal G imposed at the cambium boundary.

The patterns of variations that we imposed on the concentrations of signals D and G at the cambium boundary, lead to realistic variations in cell numbers of the cambial and enlargement zones. Comparison with experimental data from Cuny et al. {2014} displays good agreement across the annual ring [Fig. 6.19]. This proves that the distribution of the differentiation zones in the developing xylem can be adequately controlled by the cross-talk between two biochemical signals.



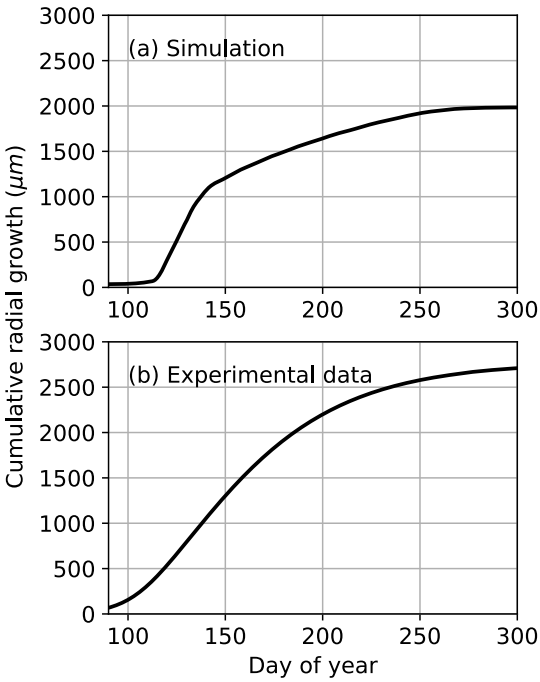
65 — Figure 6.19 | Evolution of the number of cambial and enlarging cells over a growing season.

(a) As simulated by the current version of the XyDyS model. **(b)** From observations on *Abies alba* in the Vosges Mountains (France) reported in Cuny et al. {2014}. Figure from Harman et al. {in preparation}.

The cross-talk between signal D and G engenders in a realistic pattern of stem radial growth

The global growth rate of the cell file is directly related to the total quantity of signal G in the xylem tissue. Three factors determine this quantity: (1) The concentration of signal G imposed at the cambium boundary; (2) the number of cells contributing to the polar transport of signal G (i.e. the number of dividing cells, which is controlled by the gradient of signal D); and (3) the amount of polar transporters in each of these cells (pi,i+1), which was itself directly proportional to the local concentration of signal G. As a consequence, the global growth rate of the cell file is principally controlled by the concentration of signal G at the cambium boundary, and to a lesser extent, by the concentration of signal D at the same boundary.

The current version of the model is able to simulate a realistic xylem growth over the growing season. It can be compared, for example, with measurements on *Pinus sylvestris* from Michelot et al. {2012}. In our simulations, we did not try to match the final cumulative growth, which depends on many factors, so only the general shape of the curves should be compared. Although the agreement is not perfect, the simulated curve reproduces qualitatively the slow start, the progressive acceleration, the stable linear part, and the final progressive cessation of growth [Fig. 6.20].



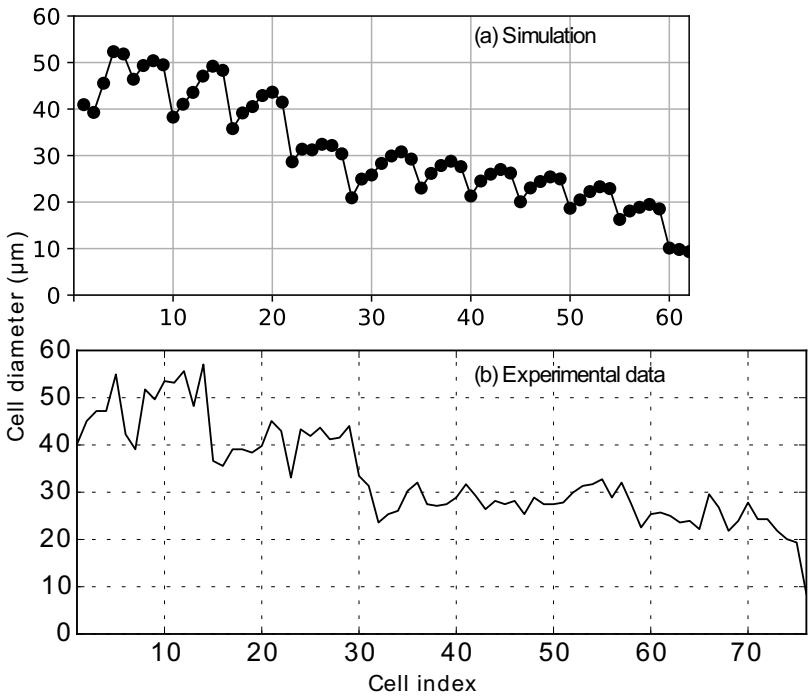
66 — Figure 6.20 | Cumulative radial growth of a tree ring.
(a) as simulated by XyDyS, and **(b)** as fitted from microcore measurements on *Pinus sylvestris* growing in Fontainebleau forest, close to Paris (France) and reported in Michelot et al. {2012}. Figure from Harman et al. *{in preparation}*.

The cross-talk between signal D and G engenders in a realistic tree-ring structure

The final size of each tracheid is proportional to the height of the G signal concentration peak at the time the cell lost its ability to divide. In other words, the unloading of signal G in the cambium, along with the magnitude of active polar transport, controlled the final cell sizes. The strong supply of signal G at the beginning of the growing season resulted in large earlywood cells. The progressive decrease in auxin supply through the end of the growing season lead to narrow latewood cells.

Another issue with the morphogenetic-gradient hypothesis was that it predicted regular spatial oscillations of high amplitudes in final cell sizes. The size-dependence between auxin concentration and growth rates introduced in Eq. 8 greatly improved this problem. This hypothesis did not produce smooth variations in cell sizes along a ring, but rather moderate-amplitude irregularities that can also be found in experimental data [Fig. 6.21].

Taken together, these results show that our two-signals model, associated with realistic temporal variations in the boundary conditions, can reproduce the typical conifer tree-ring structure in terms of cell size variations.



67 — Figure 6.21 | Evolution of tracheid radial diameters along a mature tree ring.

(a) as simulated by XyDyS; and **(b)** as measured on a microcore of *Pinus sylvestris* growing in the Vosges Mountains (France) —data courtesy from Henri Cuny. Figure from Harman et al. {in preparation}.

6.7.4 | Discussion

Short summary of previous results

In Hartmann et al. {2017} we have shown using a modelling approach that the morphogenetic-gradient hypothesis, which is based on one single signal, is not able to explain the creation of the anatomical structure of conifer tree-rings.
We thus propose a new model involving two biochemical signals. The first signal is associated with cell division. It could be identified either as the peptide TDIF, which is known to enter the cambium from the phloem and to be involved in vascular stem cell maintenance {Hirakawa et al. 2008; Etchells et al. 2015}. Another good candidate is the plant hormone cytokinin, whose regulatory effects on cambial activity has been reported in aspen {Nieminen et al. 2008}.
The second signal is associated with cell growth and is identified as auxin. Indeed, auxin is known to stimulate cell growth in many tissues, including stems {Perrot-Rechenmann 2010}, and to inhibit secondary cell wall deposition {Johnsson et al. 2018}. Our new model based on the cross-talk between to signals originating from the phloem reproduce well the main features of the intra-annual dynamics of conifer wood formation over a growing season, i.e. the shape of the radial growth curve, the temporal evolution of the width of the differentiation zones, and the anatomical structure of the resulting tree-ring. It also provided an explanation for the pattern of auxin distribution in the developing xylem.

Emergence of a hierarchical control of treacheid features

In our model, the final radial size of xylem cells is principally controlled by the supply of auxin into the cambium. Counter-intuitively, such a control was not possible in the model based on the classical morphogenetic-gradient hypothesis {Hartmann et al. 2017}. It became possible only by decoupling cell growth and division through (1) the introduction of a second signal; and (2) the introduction of a feedback of auxin on its own transport. Thanks to these two new features, the final radial diameter of a tracheid was essentially set by its auxin content at the time it exits the cambial zone. Surprisingly, this result supports the idea of hierarchical control proposed by Vaganov et al. {2011}.
Our assumption that auxin polar lateral transport plays a significant role in wood formation is based on an experimental study on aspen by Schrader et al. {2003}. In particular, they observed higher expression of PIN genes in dividing xylem cells than in expanding ones. This is why we assumed that PIN proteins responsible for lateral auxin transport are only present in dividing cells. However, there is no spatially-resolved direct measurement of the concentration and localisation of PINs in the cambium. Our hypothesis that PINs are polarised toward the xylem is hence speculative.
Another crucial assumption of our model is the auxin-dependence of PIN synthesis. Although there is no direct evidence for such a dependence in the cambium, it is strongly supported by experiments on apical meristems {Vieten et al. 2005}.
Further experimental works are needed to get a better understanding of polar auxin transport in the developing xylem and assess our assumptions.

Damping of oscillations in xylem cell final size

In Hartmann et al. {2017}, we reported large oscillations of tracheid final sizes along a simulated tree-ring. We have shown here that these oscillations can be strongly attenuated by assuming that cell growth response is size-dependent. This assumption is based on the observation that larger cells have a lower density of DNA in their cytoplasm, and thus have a lower capacity to sustain growth. This is only true provided there is no endoreplication in cambial cells, as suggested by Mellerowicz and Riding's {1992} works.

Further support for weaker growth response in larger cells comes from observations in sepal epidermis, where smaller cell lineages grow faster than smaller ones, resulting in a homogenization of cell sizes {Tsugawa et al. 2017}. Similar observations are reported by Willis et al. {2016} in the apical meristem of *Arabidopsis thaliana* where, after an asymmetrical division, the smallest daughter cell grows at a faster rate than the largest one.

Although attenuated, fluctuations in final cell sizes were still present in our new simulations. They were, however, similar in amplitude to fluctuations observed in actual tracheidograms (and which are generally overlooked by wood anatomists).

Numerous cellular processes also involve a stochastic component {Meroz and Bastien 2014; Meyer et al. 2017}, and this aspect could be investigated in the future. However, it is interesting to note that the oscillations we observed in our simulations are absolutely not due to the presence of a stochastic component in the model, but emerge directly, in a mechanistic way, from the dynamics of xylem formation. Therefore, our results underline the fact that not all heterogeneities in cell features are attributable to random processes.

Indeed, in our model, the division process is triggered by a purely deterministic criterion related to the size of the growing cell. This way of doing reflect the probable existence of a cell size checkpoint located at the G1-S transition of the cell cycle {Schiessl et al. 2012}. Moreover, analyses of cell size distribution along the growth zone of developing roots {Beemster and Baskin 1998} and leaves {Fiorani et al. 2000} suggest that all the cells in a given meristem divide in half at about the same length. However, in the apical meristem, Willis et al. {2016} found that the critical-size criterion could not fully account for the observed cell-cycle statistics. Thus, this criterion is likely to be a first-order approximation. Future modelling works could explore whether introducing stochasticity in this process could help better reproduce wood anatomical structure.

Perspective and future development of the model

Currently XyDyS do not model xylem cell maturation. However, despite this cell differentiation phase involves many biochemical processes (i.e. secondary wall deposition, cell-wall lignification, programmed cell death), it could be controlled in first instance by the same two biochemical signals. Indeed, it has been observed that the amount of secondary wall material is about the same in each mature xylem cell along a tree ring, except for the very last latewood cells {Cuny et al. 2014}. This observation could be used to compute secondary wall thickness from cell size. Besides, temperature seems to play little role in wall thickness, since forming tracheids compensate a decreased rate of differentiation by an extended duration, except for the last cells of the latewood {Cuny et al. 2018}. In a second

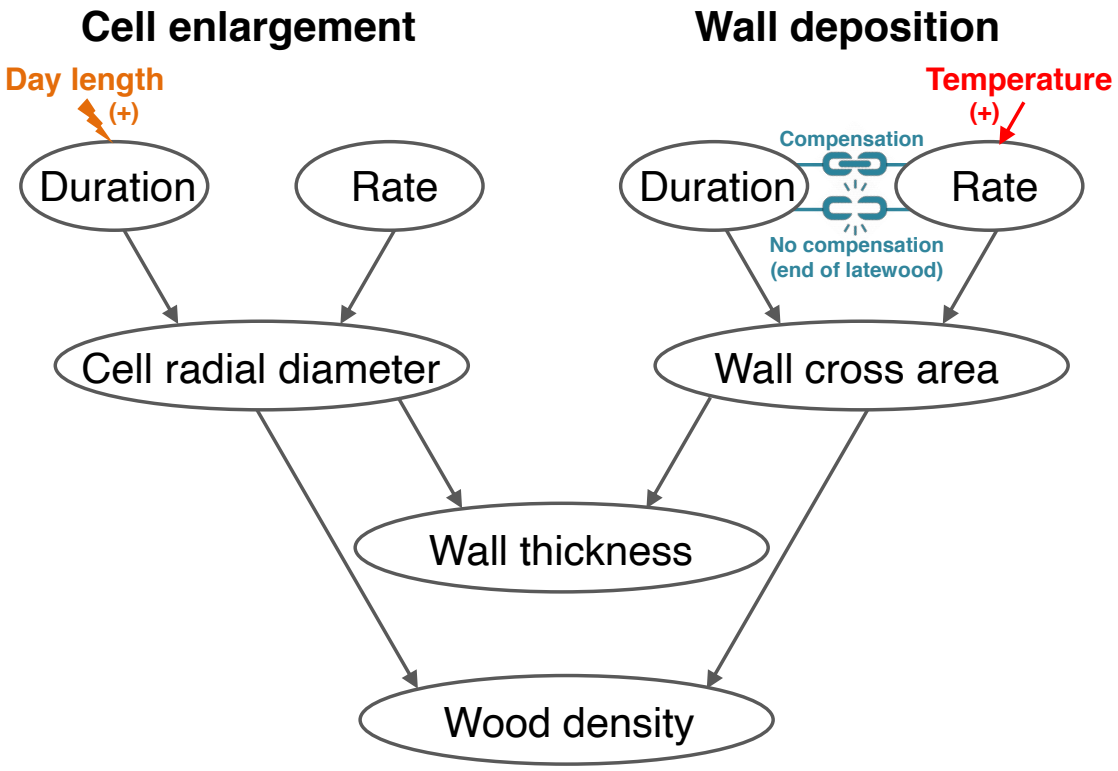
instance, it could be interesting to test the role of sugar as a third signal, building on the works from Carteni et al. {2018}.
In this work on wood formation modelling, we decided focusing on internal biochemical signals, excluding the direct effects of environmental factors. However, in the real world, the seasonal course of these two signals is undoubtedly related to environmental conditions. However, the nature of these relationships are not yet well known. Also, tree-scale models are needed to connect signal sources (e.g., growing leaves, fine roots, cambium) to sinks. Moreover, it is known yet that temperature and water availability also alter the capacity of xylem cells to respond to internal signals {Begum et al. 2018; Schrader et al. 2003}. For this first approach, we made the implicit hypothesis that environmental conditions were not limiting. Further developments of our model could consider how water stress affects wood formation dynamics. Ignoring temperature effects also limits the scope of our model. For instance, we do not model the timing of the onset of cambial activity, which is likely to be triggered by temperature {Begum et al. 2012, 2018; Delpierre et al. 2019}. Similarly, growth cessation in autumn may involve responses to day length {Baba et al. 2011}, temperature {Begum et al. 2016}, or even drought {Ren et al. 2019; Ziaco et al. 2016, 2018}. Thus, two simple approaches could be use in the future to introduce the effects of the environmental factors in our model. The first approach consist in relating the amount of the two biochemical signals used in the model to the seasonal course of the main environmental factors (e.g. photoperiod, temperature and precipitation). The second approach consist in relating the sensitivity of the developing xylem to the biochemical signals to the main environmental factors (e.g. temperature).
Here we considered that the spatial organisation of the cambium relies only on biochemical signals. However, it is possible that the mechanical pressure exerted by the bark is also involved in developing xylem organisation, by setting a radial polarity field {Yeoman and Brown 1971}. While biochemical signals are likely to play a major role in controlling cell differentiation, division, and growth rate during the growing season, mechanics probably also provides cues to cambial cells. Future, more advanced models of cambial activity and wood formation should embrace both biochemical, environmental and mechanical signalling.

6.8 | Conclusion and perspectives

6.8.1 | Synthesis

In the second section of this chapter, we presented our approach to describe and quantify accurately the kinetics of xylem cell differentiation using a conceptual model of wood formation dynamics and generalized additive models.
In the third section, we provided novel quantifications of wood formation kinetics that explain the development of the typical, widely observed, conifer tree-ring structure [Fig. 6.22]. Importantly, we refute the long-lasting assumption that the increase in wall thickness and wood density along the ring is driven by the fixation of more biomass during the wall-thickening process. Instead, we demonstrated that the amount of wall material per cell is almost constant along a ring. We also shown that the duration of cell expansion decreases along the ring (from about 3 to 1 week), while the duration of cell-wall deposition increase (from 1 to 2 month), and the rate of cell-wall deposition decrease. Consequently, changes in cell-wall

thickness and wood density are principally driven by changes in cell size. Moreover, we estimated that the duration of cell enlargement contributes to 75 % of the changes in cell size, whereas the rate contributes to the other 25 %.
In the fourth section, we explored the relationship between tracheid differentiation kinetics and environmental factors to show that temperature and water stress have significant influences on the rate of cell expansion and cell wall deposition. However, these influences only remain visible in the very last latewood cells of a tree-ring.
In the fifth and sixth section of this chapter we show how couplings between the rate and the duration of cell differentiation kinetics allow trees to adjust the xylogenesis process to the changing environment conditions to produce typical conifer tree-ring structure. We also show how compensatory mechanisms allow trees to adapt to cold temperature at high elevation or to water stress in arid conditions.
Finally, in the last section of this chapter, we present XyDyS the modeling framework we developed to simulate tracheid differentiation kinetics and resulting tree-ring structure. This approach allows us to demonstrate that the classical morphogenetic gradient theory was not working and that at least two distinct biochemical signals (e.g., auxin and cytokine or TDIF) must form gradients of concentration over the developing xylem to organise cell division in the cambium and cell differentiation in the developing xylem, and creates realistic conifer tree-ring structure.
The kinetic model presented here has been established on three major European conifer species, whose typical tree-ring structure is representative of most conifers {Schweingruber, 1990; Schoch et al., 2004}. We believe that the conceptual framework provided by this model can be applied to many species and other environments. However, further investigations are needed to understand how the relationships between cell development kinetics and wood anatomy are modified for conifer species with narrow latewood (e.g., Pinus cembra, Juniperus species) or rings formed under stressful conditions (e.g. xeric site, drought year, insect attacks, late frosts) and typically associated with wood anatomical anomalies (e.g. light latewood, false rings).



68 — Figure 6.22 | Graphical model of which and how environmental factors influence xylogenesis processes and the resulting cell dimensions during latewood formation of conifer tree rings.
For daylength, the lightning bolt symbolizes an indirect effect on the duration of cell enlargement, whereas for temperature, the arrow symbolizes a direct effect on the rate of wall deposition. Signs of the relationships are given below in pa- rentheses. For wall deposition, the chain links symbolize the coupling between the duration and the rate of the process; this coupling is broken during the formation of the last latewood cells. Figure modified from Cuny et al. {2014}.

6.8.2 | Perspectives

We started to develop our conceptual model and the associated GAM approach since 2012, and published the methods and the first results in 2013 {Cuny et al. 2013}. Since this time, we developed the method further {Andriantenaina et al. 2019; Perez-de-Lis in prep.} and applied it to other species (e.g. Picea Marianna, Larix decidua) {Balducci et al. 2016, Cuny et al. 2018}. This method start to be applied now by other teams {e.g., Butto et al. 2019; Palomo et al. 2018}, but not always correctly. Thus, integrating into CAVIAR may help its correct diffusion in good conditions.
Integrating the kinetic approach into CAVIAR will also allow to explore large data base (GloboXylo) for ecological patterns.
Another research line we started to develop in collaboration with colleagues from SCION (New Zealand) is a better description of the kinetics of the lignification process using confocal microscopic analysis {Dickson et al. 2016}.
Up to now the kinetic approach has been restricted to conifer tracheids, a grand challenge of the coming years is to apply it to angiosperm tracheids, vessels and parenchyma cells, from the less problematic diffuse porous species to the more difficult ring porous species.

Also, this approach may be applied to the differentiation of phloem cells, for conifer species first and then for angiosperm species.

6.9 | References

This reference list is incomplete and not properly formatted

Agusti J, Greb T (2013) Going with the wind–adaptive dynamics of plant secondary meristems. Mech Dev 130(1):34–44. doi:10.1016/j.mod.2012.05.011

Baba K, Karlberg A, Schmidt J, Schrader J, Hvidsten TR, Bako L, Bhalerao RP (2011) Activity-dormancy transition in the cambial meristem involves stage-specific modulation of auxin response in hybrid aspen. Proc Natl Acad Sci U S A 108:3418-23. doi: 10.1073/pnas.1011506108

Baker RE, Maini PK (2007) A mechanism for morphogen-controlled domain growth. J Math Biol 54:597–622. doi:10.1007/s00285-006-0060-8

Balducci L, Cuny HE, Rathgeber CBK, Deslauriers A, Giovannelli A, Rossi S (2016) Compensatory mechanisms mitigate the effect of warming and drought on wood formation. Plant Cell Environ 39(6):1338–1352. doi:10.1111/pce.12689.

Barnett JR (1978) Fine structure of parenchymatous and differentiated Pinus radiata callus. Ann Bot 42(2):367–373.

Beemster GT, Baskin TI (1998) Analysis of cell division and elongation underlying the developmental acceleration of root growth in Arabidopsis thaliana. Plant Physiol 116(4):1515–1526. doi:10.1104/pp.116.4.1515

Begum S, Nakaba S, Yamagishi Y, Yamane K, Islam MA, Oribe Y, Ko J-H, Jin H-O, Funada R (2012) A rapid decrease in temperature induces latewood formation in artificially reactivated cambium of conifer stems. Ann Bot 110:875-885. doi:10.1093/aob/mcs149

Begum S, Kudo K, Matsuoka Y, Nakaba S, Yamagishi Y, Nabeshima E, Rahman MH, Nugroho WD, Oribe Y, Jin H-O, Funada R (2016) Localized cooling of stems induces latewood formation and cambial dormancy during seasons of active cambium in conifers. Ann Bot 117:465-477. doi:10.1093/aob/mcv181

Bennett T, Hines G, Leyser O (2014) Canalization: what the flux? Trends in Genetics 30(2):41–48. doi:10.1016/j.tig.2013.11.001

Bhalerao RP, Bennett MJ (2003) The case for morphogens in plants. Nat Cell Biol 5(11):939–43. doi:10.1038/ncb1103-939

Bhalerao RP, Fischer U (2014) Auxin gradients across wood – instructive or incidental? Physiol Plant 151(1):43–51. doi:10.1111/ppl.12134

Bishopp A, Help H, El-Showk S, Weijers D, Scheres B, Friml J, Benková E, Mähönen AP, Helariutta Y (2011) A mutually inhibitory interaction between auxin and cytokinin specifies vascular pattern in roots. Curr Biol 21(11):917–926. doi: 10.1016/j.cub.2011.04.017

Brackmann K, Qi J, Gebert M, Jouannet V, Schlamp T, Grünwald K, Wallner ES, Novikova DD, Levitsky VG, Agustő J, Sanchez P, Lohmann JU, Greb T (2018) Spatial specificity of auxin responses coordinates wood formation. Nature communications 9(1):875. doi:10.1038/s41467-018-03256-2

Brown CL, Sax K (1962) The influence of pressure on the differentiation of secondary tissues. American Journal of Botany 49(7):683–691. doi:10.1002/j.1537-2197.1962.tb14997.x

Camarero JJ, Guerrero-Campo J, Gutiérrez E (1998) Tree-ring growth and structure of Pinus uncinata and Pinus sylvestris in the central Spanish Pyrenees. Arctic and Alpine Research 30(1):pp. 1–10

Carteni F, Deslauriers A, Rossi S, Morin H, De Micco V, Mazzoleni S, Giannino F (2018) The physiological mechanisms behind the earlywood-to-latewood transition: A process-based modeling approach. Frontiers in plant science 9. doi: 10.3389/fpls.2018.01053

Cosgrove DJ (2005) Growth of the plant cell wall. Nat Rev Mol Cell Biol 6(11):850–861. doi:10.1038/nrm1746

Crampin EJ, Gaffney EA, Maini PK (2002) Mode-doubling and tripling in reaction-diffusion patterns on growing domains: A piecewise linear model. J Math Biol 44:107–128. doi:10.1007/s002850100112

Cuny HE, Rathgeber CBK, Lebourgeois F, Fortin M, Fournier M (2012) Life strategies in intra-annual dynamics of wood formation: example of three conifer species in a temperate forest in north-east France. Tree Physiol, 32, 612-625. doi: 10.1093/treephys/tps039

Cuny HE, Rathgeber CBK, KiessŽ TS, Hartmann FP, Barbeito I, Fournier M (2013) Generalized additive models reveal the intrinsic complexity of wood formation dynamics. J Exp Bot, 64, 1983-1994. doi: 10.1093/jxb/ert057

Cuny HE, Rathgeber CBK, Frank D, Fonti P, Fournier M (2014) Kinetics of tracheid development explain conifer tree-ring structure. New Phytol 203, 1231-1241 . doi:10.1111/nph.12871

Cuny HE. Fonti P, Rathgeber CB, von Arx G, Peters RL, Frank D (2018) Couplings in cell differentiation kinetics mitigate air temperature influence on conifer wood anatomy. Plant, Cell & Environment, Wiley Online Library

Deleuze C, Houllier F (1998) A simple process-based xylem growth model for describing wood microdensitometric profiles. J Theor Biol 193(1):99–113. doi:10.1006/jtbi.1998.0689

Delpierre N, Lireux S, Hartig F, Camarero JJ, Cheaib A, Čufar K, Cuny H, Deslauriers A, Fonti P, Gričar J, Huang J-G, Krause C, Liu G, de Luis M, MŠkinen H, del Castillo EM, Morin H, Nšjd P, Oberhuber W, Prislan P, Rossi S, Saderi SM, Treml V, Vavrick H, Rathgeber CBK (2019) Chilling and forcing temperatures interact to predict the onset of wood formation in Northern Hemisphere conifers. Global Change Biology 25:1089-1105. doi:10.1111/gcb.14539

Drew DM, Downes G (2015) A model of stem growth and wood formation in pinus radiata. Trees 29(5):1395–1413. doi:10.1007/s00468-015-1216-1

Etchells JP, Mishra LS, Kumar M, Campbell L, Turner SR (2015) Wood formation in trees is increased by manipulating pxy-regulated cell division. Curr Biol 25(8):1050–1055. doi:10.1016/j.cub.2015.02.023

Fiorani F, Beemster GT, Bultynck L, Lambers H (2000) Can meristematic activity determine variation in leaf size and elongation rate among four poa species? a kinematic study. Plant Physiol 124(2):845–856. doi:10.1104/pp.124.2.845

Grieneisen VA, Scheres B, Hogeweg P, Marée AFM (2012) Morphogengineering roots: comparing mechanisms of morphogen gradient formation. BMC Syst Biol 6:37. doi:10.1186/1752-0509-6-37

Gursanscky NR, Jouannet V, Grünwald K, Sanchez P, Laaber-Schwarz M, Greb T (2016) Mol1 is required for cambium homeostasis in arabidopsis. The Plant Journal 86:210–220. doi:10.1111/tpj.13169

Han S, Cho H, Noh J, Qi J, Jung HJ, Nam H, Lee S, Hwang D, Greb T, Hwang I (2018) Bil1-mediated MP phosphorylation integrates PXY and cytokinin signalling in secondary growth. Nature plants 4(8):605. doi:10.1038/s41477-018-0180-3

Hirakawa Y, Shinohara H, Kondo Y, Inoue A, Nakanomyo I, Ogawa M, et al. (2008) Non-cell-autonomous control of vascular stem cell fate by a CLE peptide/receptor system. Proc Natl Acad Sci U S A 105:15208–15213. doi : 10.1073/pnas.0808444105

Höltta¨ T, Ma¨kinen H, Nöjd P, Ma¨kela¨ A, Nikinmaa E (2010) A physiological model of softwood cambial growth. Tree Physiol 30(10):1235–1252. doi:10.1093/treephys/tpq068

Immanen J, Nieminen K, Smolander OP, Kojima M, Serra JA, Koskinen P, Zhang J, Elo A, Mähönen AP, Street N, Bhalerao RP, Paulin L, Auvinen P, Sakakibara H, Helariutta Y (2016) Cytokinin and auxin display distinct but interconnected distribution and signaling profiles to stimulate cambial activity. Current Biology 26(15):1990–1997. doi:10.1016/j.cub.2016.05.053

Johnsson C, Jin X, Xue W, Dubreuil C, Lezhneva L, Fischer U (2018) The plant hormone auxin directs timing of xylem development by inhibition of secondary cell wall deposition through repression of secondary wall NAC-domain transcription factors. Physiol Plantarum 165, 673-689. doi: 10.1111/ppl.12766

Kiorapostolou N, Galiano-PŽrez L, von Arx G, Gessler A, Petit G (2018) Structural and anatomical responses of Pinus sylvestris and Tilia platyphyllos seedlings exposed to water shortage. Trees, 32, 1211-1218, doi:10.1007/s00468-018-1703-2

Li Z, Cui K (1988) Differentiation of secondary xylem after girdling. IAWA Journal 9(4):375–383. doi:10.1163/22941932-90001099

Meroz Y, Bastien R (2014) Stochastic processes in gravitropism. Frontiers in Plant Science 5, 674. doi:10.3389/fpls.2014.00674

Meyer HM, Teles J, Formosa-Jordan P, Refahi Y, San-Bento R, Ingram G, Jšnsson H, Locke JCW, Roeder AH (2017) Fluctuations of the transcription factor ATML1 generate the pattern of giant cells in the Arabidopsis sepal. eLife, 6. doi:10.7554/eLife.19131

Michelot A, Simard S, Rathgeber CBK, Dufrêne E, Damesin C (2012) Comparing the intra-annual wood formation of three European species (Fagus sylvatica, Quercus petraea and Pinus sylvestris) as related to leaf phenology and non-structural carbohydrate dynamics. Tree Physiol 32, 1033-1045. doi:10.1093/treephys/tps052

Moulia B, Fournier M (2009) The power and control of gravitropic movements in plants: a biomechanical and systems biology view. J Exp Bot 60(2):461–486. doi:10.1093/jxb/ern341

Muraro D, Byrne H, King JR, Bennett M (2013) The role of auxin and cytokinin signalling in specifying the root architecture of arabidopsis thaliana. J Theor Biol 317(0):71–86. doi:10.1016/j.jtbi.2012.08.032

Muraro D, Mellor N, Pound MP, Help H, Lucas M, Chopard J, Byrne HM, Godin C, Hodgman TC, King JR, Pridmore TP, Helariutta Y, Bennett MJ, Bishopp A (2014) Integration of hormonal signaling networks and mobile micrornas is required for vascular patterning in arabidopsis roots. Proc Natl Acad Sci U S A 111(2):857–62. doi:10.1073/pnas.1221766111

Nieminen K, Immanen J, Laxell M, Kauppinen L, Tarkowski P, Dolezal K, TŠhtiharju S, Elo A, Decourteix M, Ljung K, Bhalerao RP, Keinonen K, Albert VA, Helariutta Y (2008) Cytokinin signaling regulates cambial development in poplar. Proc Natl Acad Sci U S A 105:20032-7. doi: 10.1073/pnas.0805617106

Rathgeber CB, Cuny HE, Fonti P (2016) Biological basis of tree-ring formation: a crash course. Frontiers in plant science, Frontiers 7, 734. doi:10.3389/fpls.2016.00734

Perrot-Rechenmann C (2010) Cellular Responses to Auxin: Division versus Expansion. Cold Spring Harb Perspect Biol 2. doi: 10.1101/cshperspect.a001446

Rossi S, Deslauriers A, Morin H (2003) Application of the gompertz equation for the study of xylem cell development. Dendrochronologia 21(1):33–39. doi:10.1078/1125-7865-00034

Schiessl K, Kausika S, Southam P, Bush M, Sablowski R (2012) Jagged controls growth anisotropy and coordination between cell size and cell cycle during plant organogenesis. Curr Biol 22(19):1739–1746. doi:10.1016/j.cub.2012.07.020

Schiestl-Aalto P, Kulmala L, Mäkinen H, Nikinmaa E, Mäkelä A (2015) Cassia – a dynamic model for predicting intra-annual sink demand and interannual growth variation in scots pine. New Phytologist 206(2):647–659. doi:10.1111/nph.13275

Schrader J, Baba K, May ST, Palme K, Bennett M, Bhalerao RP, Sandberg G (2003) Polar auxin transport in the wood-forming tissues of hybrid aspen is under simultaneous control of developmental and environmental signals. Proc Natl Acad Sci U S A 100(17):10096–101. doi:10.1073/pnas.1633693100

Smetana O, MŠkilŠ R, Lyu M, Amiryousefi A, S‡nchez Rodr'guez F, Wu M-F, SolŽ-Gil A, Leal Gavarr—n M, Siligato R, Miyashima S, Roszak P, Blomster T, Reed JW, Broholm S, MŠhšnen AP (2016) High levels of auxin signalling define the stem-cell organizer of the vascular cambium. Nature, 565, 485-489. doi:10.1038/s41586-018-0837-0

Sundberg B, Uggla C, Tuominen H (2000) Cambial growth and auxin gradients. In: Savidge R, Barnett J, Napier R (eds) Cell and Molecular Biology of Wood Formation. BIOS Scientific Publishers, pp 169 – 188

Tsugawa S, Hervieux N, Kierzkowski D, Routier-Kierzkowska AL, Sapala A, Hamant O, Smith RS, Roeder AHK, Boudaoud A, Li CB (2017) Clones of cells switch from reduction to enhancement of size variability in arabidopsis sepals. Development 144(23):4398–4405. doi:10.1242/dev.153999

Tuominen H, Puech L, Fink S, Sundberg B (1997) A radial concentration gradient of indole-3-acetic acid is related to secondary xylem development in hybrid aspen. Plant Physiol 115(2):577–585. doi:10.1104/pp.115.2.577

Uggla C, Magel E, Moritz T, Sundberg B (2001) Function and dynamics of auxin and carbohydrates during earlywood/latewood transition in scots pine. Plant Physiol 125(4):2029–2039. doi:10.1104/pp.125.4.2029

Uggla C, Mellerowicz EJ, Sundberg B (1998) Indole-3-acetic acid controls cambial growth in scots pine by positional signaling. Plant Physiol 117(1):113–121. doi:10.1104/pp.117.1.113

Uggla C, Moritz T, Sandberg G, Sundberg B (1996) Auxin as a positional signal in pattern formation in plants. Proc Natl Acad Sci U S A 93(17):9282–9286

Vaganov EA, Anchukaitis KJ, Evans MN (2011) Dendroclimatology: Progress and Prospects. chapter How Well Understood Are the Processes that Create Dendroclimatic Records? A Mechanistic Model of the Climatic Control on Conifer Tree-Ring Growth Dynamics, pp 37–75 Springer Netherlands, Dordrecht doi:10.1007/978-1-4020-5725-0 3

Vaganov EA, Hughes MK, Shashkin AV (2006) Growth dynamics of conifer tree rings: images of past and future environments. Ecological studies. Springer

Vieten A, Vanneste S, Wisniewska J, Benkov‡ E, Benjamins R, Beeckman T, et al. (2005) Functional redundancy of PIN proteins is accompanied by auxin-dependent cross-regulation of PIN expression. Development. 132:4521–4531. doi:10.1242/dev.02027.

Willis L, Refahi Y, Wightman R, Landrein B, Teles J, Huang KC, Meyerowitz EM, Jönsson H (2016) Cell size and growth regulation in the arabidopsis thaliana apical stem cell niche. Proceedings of the National Academy of Sciences 113(51):E8238–E8246. doi:10.1073/pnas.1616768113

Wilson BF (1984) The Growing Tree. University of Massachusetts Press

Yeoman, MM, Brown R (1971) Effects of Mechanical Stress on the Plane of Cell Division in Developing Callus Cultures. Ann Bot (35):1102-1112. doi:10.1093/oxfordjournals.aob.a084545

CHAPTER 7 | INTRA-ANNUAL DYNAMICS OF WOODY CARBON SEQUESTRATION

7.0 | Synthesis

7.0.1 | Related papers

Cuny H, Rathgeber CBK, Frank D, Fonti P, Mäkinen H, Prislan P, Rossi S, Martinez del Castillo E, Campelo F, Vavrčík H, Camarero JJ, Bryukhanova MV, Jyske T, Griçar J, Gryc V, de Luis M, Vieira J, Čufar K, Kirdyanov AV, Oberhuber W, Treml V, Huang J-G, Li X, Swidrak I, Deslauriers A, Liang E, Nöjd P, Gruber A, Nabais C, Morin H, Krause C, King G, Fournier M. 2015. Woody biomass production lags stem-girth increase by over one month in coniferous forests. *Nature Plants* 1: 15160: 1–4.

7.0.2 | Summary

Wood is the main terrestrial biotic reservoir for long-term carbon sequestration, and its formation in trees consumes around 15% of anthropogenic carbon dioxide emissions each year. However, the seasonal dynamics of woody biomass production cannot be quantified from eddy covariance or satellite observations. As such, our understanding of this key carbon cycle component, and its sensitivity to climate, remains limited. Here, we present high-resolution cellular based measurements of wood formation dynamics in three coniferous forest sites in northeastern France, performed over a period of 3 years. We show that stem woody biomass production lags behind stem-girth increase by over 1 month. We also analyse more general phenological observations of xylem tissue formation in Northern Hemisphere forests and find similar time lags in boreal, temperate, subalpine and Mediterranean forests. These time lags question the extension of the equivalence between stem size increase and woody biomass production to intra-annual time scales. They also suggest that these two growth processes exhibit differential sensitivities to local environmental conditions. Indeed, in the well-watered French sites the seasonal dynamics of stem-girth increase matched the photoperiod cycle, whereas those of woody biomass production closely followed the seasonal course of temperature. We suggest that forecasted changes in the annual cycle of climatic factors may shift the phase timing of stem size increase and woody biomass production in the future.

7.0.3 | Key words

Carbon sequestration, Cambium activity, Xylem development, Wood production, Tree radial growth, Climate changes

7.1 | Introduction

The atmospheric carbon dioxide concentration undergoes a seasonal cycle in the Northern Hemisphere with a 6–19 p.p.m. amplitude {Graven et al. 2013}, thereby representing intra-annual net fluxes greater than annual anthropogenic

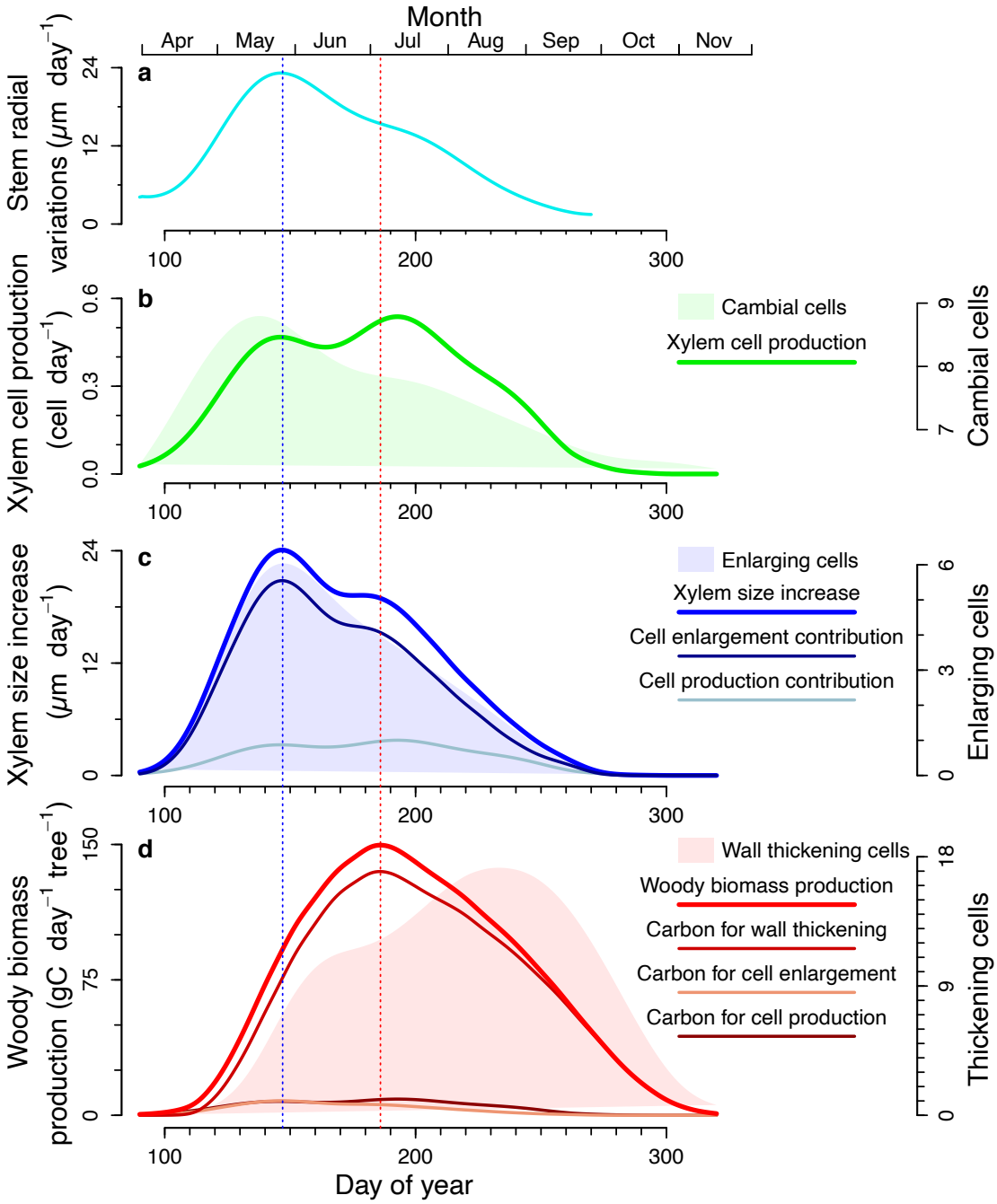
emissions. This cycle is predominantly driven by the annual rhythm of terrestrial plant activity, including the balance between carbon assimilation by photosynthesis, release by respiration and sequestration through biomass formation. Wood formation is the primary biological process through which carbon is durably sequestered in woody plants, and is thus a major contributor to the net forest carbon sink of about 2.5 petagrams of carbon per year that mitigates climate change {Pan et al. 2011}. However, large uncertainties remain in the response of the global carbon cycle to continuing climate change {Moss et al. 2010}. For example, increased seasonal amplitudes in the Northern Hemisphere carbon dioxide concentration over the last decades suggest large ecological changes in northern forests and a major shift in the global carbon cycle {Graven et al. 2013}. In situ observations are required to better identify the intra-annual dynamics of the biological processes controlling carbon cycling in terrestrial ecosystems, and to accurately quantify their influence on the global carbon budget.

Since the late 1990s, 'eddy covariance' measurements have emerged as the primary technique to evaluate net carbon fluxes between a forest canopy and the atmosphere, from sub-hourly to yearly time scales {Baldocchi 2003}. Inside a forest ecosystem, carbon allocation among the different biomass components (for example leaves, stems, roots, soil) can be estimated using repeated inventories {Barford et al. 2001}, including measurements of tree size changes {Etzold et al. 2011} and litter fall {Davidson et al. 2002}, and/ or assessment from phenocams {Richardson et al. 2009} or remote sensing {Dong et al. 2003}. At fine time scales, joint assessments of carbon fluxes in forest ecosystems using eddy covariance and tree size measurements often do not yield compatible perspectives on intra-annual ecosystem carbon dynamics {Granier et al. 2008; Zweifel et al. 2010}. This is not surprising, as none of these methods allow a direct quantification of when carbon is sequestered in the wood or mechanistic insights on the physiological processes driving woody net primary production. Moreover, external measurements of tree girth are not only recording xylem tissue formation, but are also influenced by the formation and degradation of bark tissues and stem water status {King et al. 2013; Zweifel et al. 2010}. A detailed, process-oriented understanding of woody biomass production is therefore a timely and necessary endeavour to improve our knowledge of terrestrial carbon cycle and its interactions with climate.

Here, we investigate the seasonal dynamics and climatic sensitivity of tree radial growth and woody biomass production by performing 3 years of weekly observations of wood formation for three temperate mixed coniferous forests (silver fir, Norway spruce and Scots pine) located in the Vosges Mountains in northeast France. Detailed measurements of the developing wood and resulting tree-ring structure in concert with accurate computation of cellular development kinetics {Cuny et al. 2014} allow us, for the first time, to quantify the magnitude and dynamics of carbon sequestration into tree stem on intra-seasonal time scales. Notably, by compiling and analysing more general data spanning the Northern Hemisphere on the timing of xylem tissue formation, we demonstrate the consistency of key findings across the major coniferous forest biomes.

7.2 | Main results

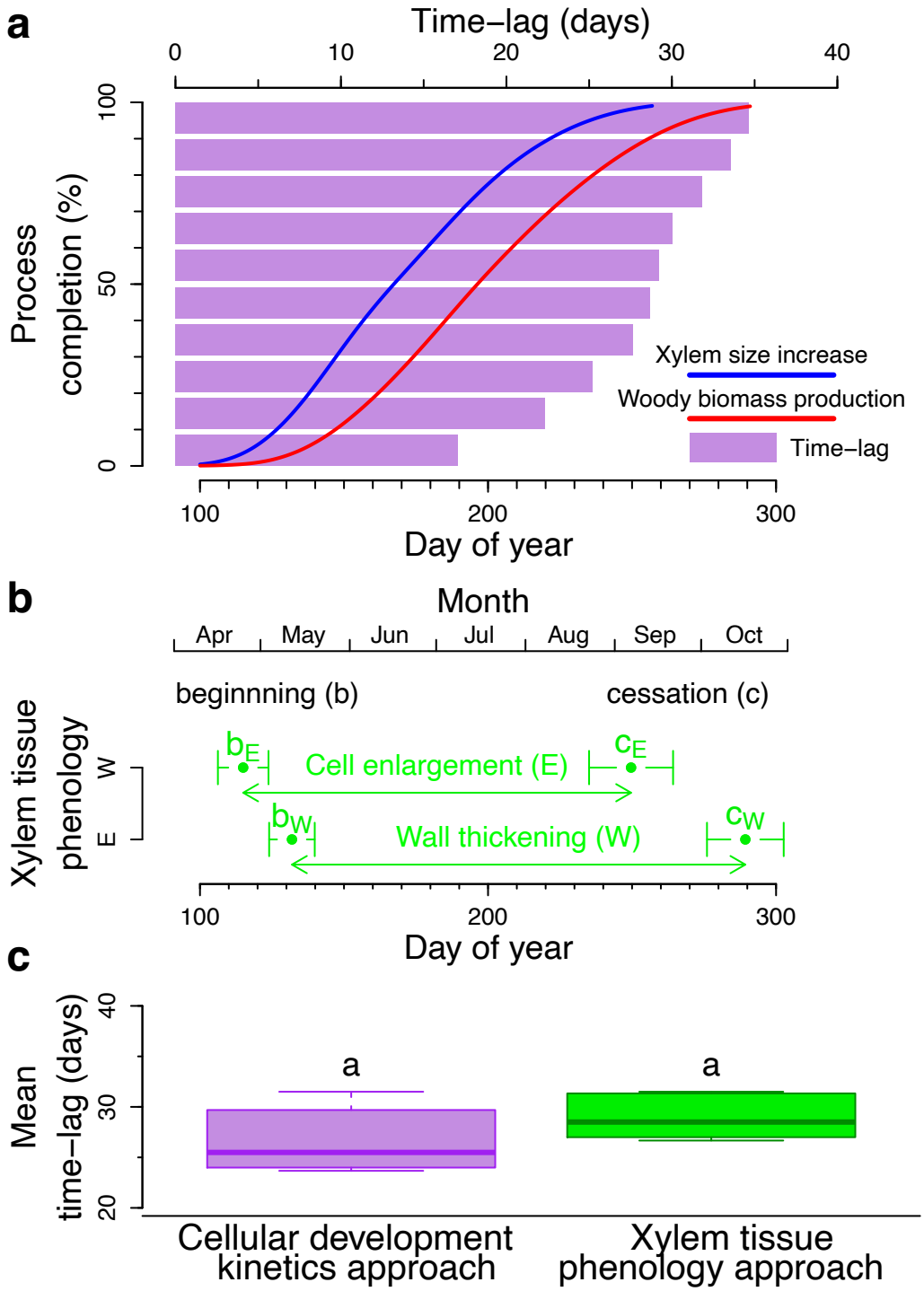
We find that wood formation processes spanned nearly 7 months, from mid-April to early November, in the Vosges Mountains [Fig. 7.1]. This period encompasses the production and differentiation of the new xylem cells through cambial cell divisions, cell enlargement and cell wall thickening (including secondary wall formation and lignification). The complex integration of these cellular processes results in both xylem size increase and woody biomass production. The rates of xylem cell production and xylem size increase peaked at the end of May, with xylem cell production also showing a secondary mid-summer local maximum [Fig. 7.1b]. In these consistently well-watered forests, the dynamics and magnitude of xylem size increase generally matched the seasonal changes of stem girth measured by dendrometers on the same trees [Fig. 7.1a,c]. Enlargement of the newly differentiating xylem cells was responsible for 80% of the xylem size increase, with the remaining 20% attributable to the production of the new xylem cells [Fig. 7.1c]. Consequently, the rate of xylem size increase peaked when most cells were in the enlarging phase, which furthermore coincided with development of large earlywood cells. In contrast, woody biomass production was mostly (90%) driven by cell wall thickening and followed a symmetric bell-shaped curve peaking in early July [Fig. 7.1d]. Contrary to long-held hypotheses that the maximal rate of woody biomass production occurs during latewood development, we find this takes place during the formation of the transition wood (between earlywood and late-wood), before the differentiation of the small and thick-walled late-wood cells had even begun.



69 — Figure 7.1 | Seasonal dynamics of stem-girth increase, xylem size increase and woody biomass production.

a, Stem-external radial variations. **b**, Rate of xylem cell production through cambial cell divisions and number of cambial cells (shading). **c**, Rate of xylem size increase, with isolated contributions of cell production and cell enlargement, together with the number of enlarging cells (shading). **d**, Rate of woody biomass production, which sums the carbon sequestered by wall thickening plus that from cell enlargement and cell production. Shading is number of wall thickening cells. Vertical blue and red dashed lines indicate time of maximal rates of xylem size increase and woody biomass production. Data are means for the three northeast France sites (Vosges mountains), with the three species (silver fir, Norway spruce and Scots pine) monitored during three years (2007–2009). Figure from Cuny et al. {2015}.

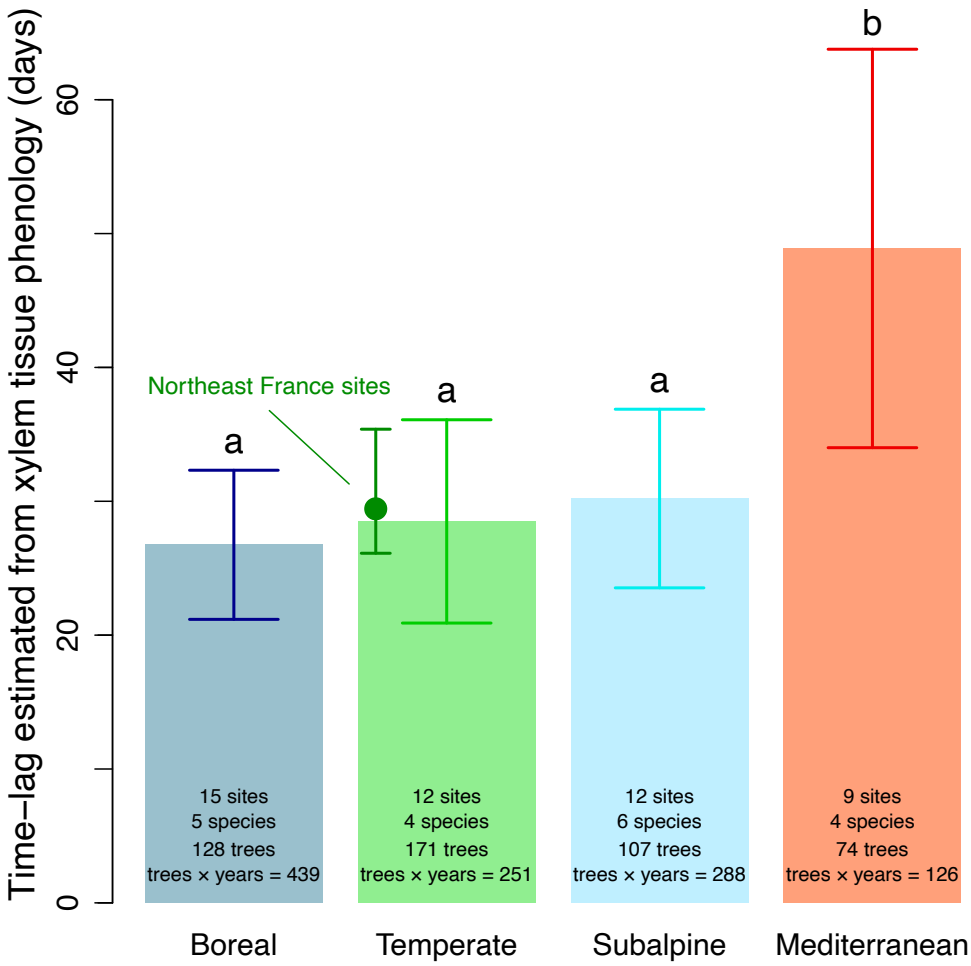
We observe a shift of 40 days between the maximal rates of xylem size increase and woody biomass production [Fig. 7.1c,d]. In mid- September, when xylem stopped increasing in size, woody biomass production was still at almost 50% of its maximal rate. Woody biomass production requires on average 26 days to reach the same relative advancement as xylem size increase [Fig. 7.2]. In this sense, 90% of the final tree-ring width was completed by early August, whereas only 70% of the final amount of carbon was permanently fixed into xylem cell walls [Fig. 7.2a].



70 — Figure 7.2 | Asynchrony of xylem size increase and woody biomass production, along with xylem phenology.
a, Time lag between seasonal dynamics of xylem size increase and woody biomass production when both processes are expressed in percentage of completion. **b**, Critical

dates of the phenology of xylem tissue formation used to estimate the mean time lag between xylem size increase and woody biomass production (mean time lag = ((bW − bE) + (cW − cE))/2). **c**, Similarity of results for mean time lag between xylem size increase and woody biomass production as derived from the integration of the kinetics of each developing xylem cell and from the assessment of the phenology of xylem tissue formation. In **a** and **b**, data represent the means (with associated standard deviations in b) for northeast France (over sites, species and years). In **c**, boxplots represent nine values of mean time lag (one value per site and year). Different letters above boxes indicate significant differences ($P < 0.05$, Wilcoxon rank-sum test, $n = 18$). Figure from Cuny et al. {2015}.

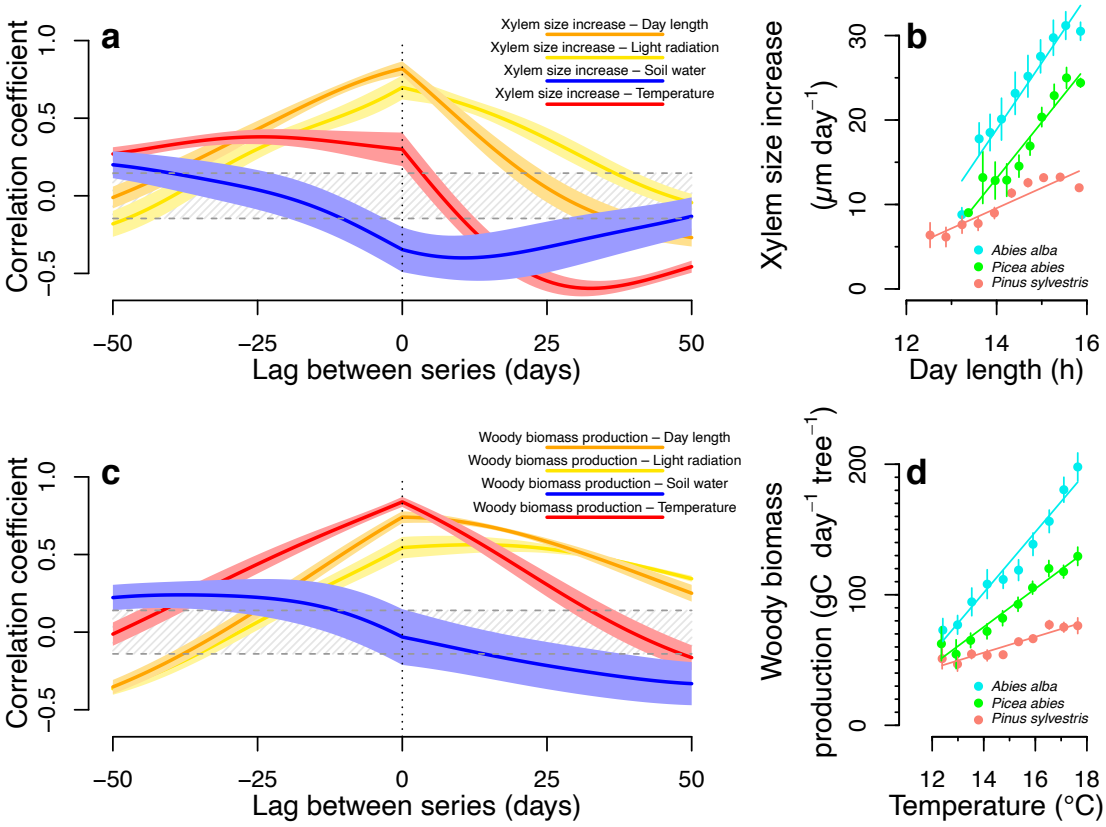
The asynchrony we observe at the tissue level between xylem size increase and woody biomass production is the complex integral of cellular characteristics, developmental phases and kinetics, and the ever-changing proportion of cells in these phases during tree-ring formation. In fact, growth in size and biomass are dissociated even at the cellular level. Immediately after its birth, a maturing xylem cell enlarges radially, stretching its thin primary wall without incorporating much carbon. Only once the final cell size is reached, the cell wall thickening starts and sequesters the majority of carbon fixed in the cell structure. Moreover, although cell enlargement is principally a rapid physical process, wall thickening involves time-consuming assembly of complex material {Cuny et al. 2014}. This difference systematically increases during the growing season, as the wide thin-walled earlywood cells require 12 days for enlargement and 22 days for wall thickening, whereas the narrow thick-walled latewood cells require 6 and 50 days, respectively {Cuny et al. 2014}. All these factors explain why growth in biomass lags behind growth in size at our sites in France.
To assess if the time lag between xylem size increase and woody biomass production is common to diverse coniferous forest biomes, we compiled phenological observations of xylem tissue formation from a network of 51 sites across the Northern Hemisphere (GloboXylo database). Although detailed cellular development kinetics are not available for these sites, our data from northeast France show that the periods devoted to xylem size increase, woody biomass production and the mean time lag between these two processes can be reasonably estimated by using the available observations on the timing of xylem tissue formation (that is beginning and cessation of cell enlargement and cell wall thickening periods) [Fig. 7.2b,c]. Accordingly, we observe approximately 1-month time lags for boreal (27±6days), temperate (28±8 days) and subalpine forests (30 ± 7 days), and nearly 50% longer for Mediterranean forests (49 ± 15 days) [Fig. 7.3]. The longer and more variable time lags observed in Mediterranean regions may be linked to longer growing seasons (related to favourable thermal conditions) and more complex growing patterns (related to summer water stress) that characterize such environments {Camarero et al. 2010}. These analyses allow us to conclude that the delay between xylem size increase and woody biomass production quantified for the French sites is common to the Northern Hemisphere coniferous forests.



71 — Figure 7.3 | Delay between xylem size increase and woody biomass production for the major coniferous forest biomes of the Northern Hemisphere. Different letters above bars indicate significant differences ($P < 0.05$, one-way analysis of variance with Tukey post-hoc test, $n = 213$). Figure from Cuny et al. {2015}.

The widespread asynchrony between xylem size increase and woody biomass production suggests a different sensitivity of these processes to climatic factors. We can test these notions by returning to the comprehensive French dataset. Cross-correlation and regression analysis between the seasonal course of the environmental conditions (day length, light radiation intensity, soil water content, temperature) and the intra-annual dynamics of xylem size increase and woody biomass production reveal consistent synchronizations for all three species: the intra-annual dynamics of xylem size increase had the strongest relationship with photoperiod [Fig. 7.4a,b], whereas woody biomass production was tightly coupled with the seasonal temperature course [Fig. 7.4c,d]. These results offer new insights into the adaptation of plants to their environment. Photoperiod has long been regarded as a stable indicator of the time of the year that controls many developmental responses in plants {Jackson 2009}. In extra-tropical trees, the onset of xylem production is controlled by temperature {Rossi et al. 2008; Oribe et al. 2001}, but our results support the idea that its dynamics then synchronizes with the photoperiod cycle to ensure cells will have sufficient time to finish their differentiation before winter {Rossi et al. 2006}. The synchronization we find

between woody biomass production and the seasonal cycle of temperatures is less well understood, but could be linked to the strong metabolic activity that supports cell wall thickening {Simard et al. 2013; Körner 2003}. Cell wall lignification in particular has been suggested to be the most temperature-sensitive process involved in tree carbon balance {Donaldson 2001}. Yet, caution is warranted in extrapolating these climatic associations from northeast France, a region characterized both by sufficient precipitation and strong seasonal changes in light and temperature, to other regions. While we expect comparable findings for similar biomes, a higher sensitivity to water availability may be expected in environments characterized by strong and regular mid-season soil water deficits, as in the Mediterranean.



72 — Figure 7.4 | Coordination of xylem size increase and woody biomass production with environmental factors.
a, Cross-correlation between the seasonal dynamics of xylem size increase and environmental factors. **b**, Relationships between the seasonal dynamics of xylem size increase and day length. **c**, Cross-correlation between the seasonal dynamics of woody biomass production and environmental factors. **d**, Relationships between the seasonal dynamics of woody biomass production and temperature. Data are means for the three northeast France sites (Vosges mountains), with the three species (silver fir, Norway spruce and Scots pine) monitored during 3 years (2007–2009). In **a** and **c**, the different curves represent the mean correlation coefficients between the different couples of series according to a time lag applied to one of the two considered series. Shaded area around lines represents 95% confidence interval. Hatched area represents coefficient values above significance level ($P > 0.05$). For **b** and **d**, each point represents the mean value of the process rate within a class of the environmental factor, and vertical bars represent 95% confidence interval. Figure from Cuny et al. {2015}.

7.3 | Discussion

The time lag and different climatic associations found for xylem size increase and woody biomass production agree with observations made by dendroclimatologists that cell size and cell wall thickness chronologies (linked to xylem size increase and woody biomass production, respectively) convey climatic signals from different parts of the season {Fonti et al. 2013}. Most generally, our findings contribute to the explanation of why different climatic fingerprints are widely observed in tree-ring width and maximum wood density {Briffa et al. 2002, Babst et al. 2014}. Finally, since global change involves shift in the seasonal climatic cycles {Stine et al. 2009}, the phasing of xylem size increase and woody biomass production might change. With projections of continuing climate change, all of these considerations suggest profound impacts on tree-ring structure {Cuny et al. 2014}, associated functional trade-offs {Lachenbruch et al. 2014}, and carbon fluxes in forest ecosystems {Barford et al. 2001; Doughty et al. 2015}.

Our results question the inter-changeable usage of size growth and biomass growth at intra-annual scales. Classically, in biomass studies 'growth' is used to indifferently define an irreversible increase in size or mass due to metabolic process {Barford et al. 2001; Etzold et al. 2011; Gough et al. 2008; Doughty et al. 2015; Babst et al. 2014}. As mature trees cannot be weighed easily, mass gain is usually assessed from stem diameter and/or tree height measurements via allometric equations {Barford et al. 2001; Etzold et al. 2011; Gough et al. 2008; Doughty et al. 2015; Babst et al. 2014}. Increases in size and in biomass are actually well related in space {Babst et al. 2014}, and at inter-annual time scales {Barford et al. 2001;Babst et al. 2014}, which has further fuelled the idea that seasonal dynamics of tree-girth changes equated directly woody biomass production {Barford et al. 2001; Etzold et al. 2011; Gough et al. 2008; Doughty et al. 2015; Babst et al. 2014}.

However, we demonstrate that, at the intra-annual scale, woody biomass production lags significantly behind xylem size increase, suggesting challenges to properly infer the seasonal dynamics of woody net primary production from external measurements of stem size change. At a minimum, this time lag needs to be considered, but estimates are further complicated by other factors such as changes in stem water status that cause reversible stem size fluctuations {King et al. 2013; Zweifel et al. 2010}. Moreover, we hypothesize that reduced agreement between cellular observations and external measurements of xylem production would be found at sites where the proportion of bark to the annual xylem increment is greater or where water storage undergoes stronger seasonal fluctuations. The revealed time lag also provides a mechanistic basis to understand the discrepancies observed between eddy covariance and tree size data. It notably contributes to explanations for the lack of relationships between net ecosystem productivity and tree size changes at short time scales {Gough et al. 2008; Zweifel et al. 2010}, and the positive forest carbon uptake when stem- girth increase is over in autumn {Granier et al. 2008}.

7.4 | Conclusion and perspectives

Finally, our work helps to better understand the seasonal balance of terrestrial carbon, providing insights and methods to link forest–atmosphere exchanges and woody carbon sequestration at intra-seasonal time scales. Modelling the dynamics of the processes that drive carbon fluxes in different ecosystem components is a key issue, especially for assessing how climate change impacts these ecosystems {Medvigny et al. 2009}. Our detailed mechanistic representation of when and how carbon is sequestered into the wood during the growing season provides crucial information on a major carbon flux and storage component of forest ecosystems. Such information is essential for further developing the process-based biosphere models to better constrain modern carbon budgets and to predict future carbon–climate interactions {Medvigny et al. 2009}.

This work opened a new research avenue for the PhD thesis of Anjy Andriantenaina (see section 8.2 for more information).

7.5 | References

Babst, F. et al. Above-ground woody carbon sequestration measured from tree rings is coherent with net ecosystem productivity at five eddy-covariance sites. New Phytol. 201, 1289–1303 (2014).

Baldocchi, D. D. Assessing the eddy covariance technique for evaluating carbon dioxide exchange rates of ecosystems: past, present and future. Global Change Biol. 9, 479–492 (2003).

Barford, C. C. et al. Factors controlling long-and short-term sequestration of atmospheric CO2 in a mid-latitude forest. Science 294, 1688–1691 (2001).

Briffa, K. et al. Tree-ring width and density data around the Northern Hemisphere: Part 1, local and regional climate signals. Holocene 12, 737 (2002).

Camarero, J. J., Olano, J. M. & Parras, A. Plastic bimodal xylogenesis in conifers from continental Mediterranean climates. New Phytol. 185, 471–480 (2010). 19.

Cuny, H. E., Rathgeber, C. B. K., Frank, D., Fonti, P. & Fournier, M. Kinetics of tracheid development explain conifer tree-ring structure. New Phytol. 203, 1231–1241 (2014).

Davidson, E. et al. Belowground carbon allocation in forests estimated from litterfall and IRGA-based soil respiration measurements. Agr. Forest Meteorol. 113, 39–51 (2002).

Donaldson, L. A. Lignification and lignin topochemistry—an ultrastructural view. Phytochemistry 57, 859–873 (2001).

Dong, J. et al. Remote sensing estimates of boreal and temperate forest woody biomass: carbon pools, sources, and sinks. Remote Sens. Environ. 84, 393–410 (2003). 14. Granier, A., Bréda, N., Longdoz, B., Gross, P. & Ngao, J. Ten years of fluxes and stand growth in a young beech forest at Hesse, North-eastern France. Ann. For. Sci. 65, 704–716 (2008).

Doughty, C. E. et al. Drought impact on forest carbon dynamics and fluxes in Amazonia. Nature 519, 78–82 (2015).

Etzold, S. et al. The carbon balance of two contrasting mountain forest ecosystems in Switzerland: similar annual trends, but seasonal differences. Ecosystems 14, 1289–1309 (2011).

Fonti, P. et al. Temperature-induced responses of xylem structure of *Larix sibirica* (Pinaceae) from the Russian Altay. Am. J. Bot. 100, 1332–1343 (2013).

Gough, C., Vogel, C., Schmid, H., Su, H.-B. & Curtis, P. Multi-year convergence of biometric and meteorological estimates of forest carbon storage. Agr. Forest Meteorol. 148, 158–170 (2008).

Graven, H. et al. Enhanced seasonal exchange of CO2 by northern ecosystems since 1960. Science 341, 1085–1089 (2013).

Jackson, S. D. Plant responses to photoperiod. New Phytol. 181, 517–531 (2009). 20. Rossi, S. et al. Critical temperatures for xylogenesis in conifers of cold climates. Global Ecol. Biogeogr. 17, 696–707 (2008).

King, G., Fonti, P., Nievergelt, D., Büntgen, U. & Frank, D. Climatic drivers of hourly to yearly tree radius variations along a 6 °C natural warming gradient. Agr. Forest. Meteorol. 168, 36–46 (2013).
Körner, C. Carbon limitation in trees. J. Ecol. 91, 4–17 (2003).
Lachenbruch, B. & McCulloh, K. A. Traits, properties, and performance: how woody plants combine hydraulic and mechanical functions in a cell, tissue, or whole plant. New Phytol. 204, 747–764 (2014).
Medvigy, D., Wofsy, S., Munger, J., Hollinger, D. & Moorcroft, P. Mechanistic scaling of ecosystem function and dynamics in space and time: Ecosystem Demography model version 2. J. Geophys. Res. 114, G01002 (2009).
Lal, R. Sequestration of atmospheric CO2 in global carbon pools. Energy Environ. Sci. 1, 86–100 (2008).
Moss, R. H. et al. The next generation of scenarios for climate change research and assessment. Nature 463, 747–756 (2010).
Oribe, Y., Funada, R., Shibagaki, M. & Kubo, T. Cambial reactivation in locally heated stems of the evergreen conifer Abies sachalinensis (Schmidt) Masters. Planta 212, 684–691 (2001).
Pan, Y. et al. A large and persistent carbon sink in the world's forests. Science 333, 988–993 (2011).
Richardson, A. D., Braswell, B. H., Hollinger, D. Y., Jenkins, J. P. & Ollinger, S. V. Near-surface remote sensing of spatial and temporal variation in canopy phenology. Ecol. Appl. 19, 1417–1428 (2009).
Rossi, S. et al. Conifers in cold environments synchronize maximum growth rate of tree-ring formation with day length. New Phytol. 170, 301–310 (2006).
Simard, S. et al. Intra-annual dynamics of non-structural carbohydrates in the cambium of mature conifer trees reflects radial growth demands. Tree Physiol. 33, 913–923 (2013).
Stine, A. R., Huybers, P. & Fung, I. Y. Changes in the phase of the annual cycle of surface temperature. Nature 457, 435–440 (2009).
Zweifel, R. et al. Link between continuous stem radius changes and net ecosystem productivity of a subalpine Norway spruce forest in the Swiss Alps. New Phytol. 187, 819–830 (2010).

CHAPTER 8 | FRONTIERS IN TREE-RING FORMATION DYNAMICS

8.0 | Synthesis

8.0.1 | Related papers

Andrianantenaina AN, Rathgeber CBK, Pérez-de-Lis G, Cuny H, Ruelle J. 2019. Quantifying intra-annual dynamics of carbon sequestration in the forming wood: a novel histologic approach. *Annals of Forest Science* 76, 62: 1–12.

Andrianantenaina AN, Rathgeber CBK, Pérez-de-Lis G, Longdoz B. Interplay between intra-annual dynamics of carbon fixation and sequestration within forming wood for spruce, beech and oak temperate forests. In preparation a.

Andrianantenaina AN, Rathgeber CBK, Pérez-de-Lis G. Tree-ring structure determines the coordination between the intra-annual dynamics of stem radial growth and carbon sequestration. In preparation b.

Fernández de Uña L, Rathgeber CBK., Andrianantenaina AN, Pérez-de-Lis G, Cuntz M. Intra-annual dynamics of xylem potential hydraulic conductivity in three tree species (European beech, Sessile Oak and Norway spruce) presenting contrasted tree-ring structures. In preparation a.

Fernández de Uña L, Rathgeber CBK, Cuntz M. Intra-annual dynamics of water flux in a temperate beech forest. In preparation b.

Pérez-de-Lis G, Rathgeber CBK, Ponton S. Can we chop a tree-ring into time-slices? What wood formation dynamics can bring to tree-ring sciences. In preparation.

Rathgeber CBK. 2017. Conifer tree-ring density inter-annual variability - anatomical, physiological and environmental determinants. *New Phytologist* 216: 621–625.

8.0.2 | Related projects

WatFlux — Modelling water flux in the xylem, from seasonal dynamics to long-term variations, from xylem cell maturation to forest ecosystems. LabEx ARBRE 2018-2019.

WoodCap — Seasonal dynamics of carbon flux in trees — from sequestration into wood back to capture by leaves. LabEx ARBRE 2015-2016.

WoodIsotop — Unravelling the influence of environment and development on intra-annual dynamics of wood formation and resulting tree-ring structure and isotope ratios. Labex ARBRE 2017-2019.

8.0.3 | Summary

In this chapter, we present three research avenues we are currently developing : (1) describing, quantifying and understanding the intra-annual dynamics of carbon fluxes in trees in the framework of the WoodCap project; (2) describing, quantifying and understanding the intra-annual dynamics of water transport in trees in the framework of the WatFlux project; and (3) describing, quantifying and understanding the creation of carbon and oxygen isotopic signals in tree-ring archives in the framework of the WoodIsotop project. Additionally, we also present our vision for a broad, integrated approach to tree-ring science and wood quality.

8.0.4 | Key words

Carbon sequestration, Climate changes, Isotopic signal, Tree radial growth, Water transport, Wood formation

8.1 | Introduction

We present in this chapter four research avenues we are currently working on. The three firsts are presented in the form of project research, while the last one is presented in the form of an opinion paper.

8.2 | Intra-annual dynamics of carbon fluxes in trees — The WoodCap project

8.2.1 | Context

Plant sequester nearly half of the 120 Pg of carbon captured annually by photosynthesis, contributing to uptake 15 % of anthropogenic CO2 emissions. Most of the carbon is sequestered into the wood, which represents about 80 % of the living terrestrial biomass. The biomass accumulation, in northern hemisphere woody plants, closely matches the seasonal cycles, with modifications that can strongly impact on the global carbon cycle. However, the seasonal dynamics of woody carbon sequestration remains poorly quantified, limiting our understanding of the terrestrial carbon cycle, and its sensitivity to on-going and future climate changes.

8.2.2 | Objectives

In order to better understand the process of carbon sequestration into stem wood, along with its environmental drivers, we propose to quantify the dynamics of the carbon flux, from its initial capture by the leaves, to its final sequestration into the wood. The aim of WoodCap project is to accurately assess the seasonal dynamics of gross primary productivity, non-structural carbohydrates, tree radial growth, and woody carbon sequestration. In addition, this work will allow us to explore the complex relationships between the seasonal dynamics of carbon pools and fluxes, and the course of the environmental factors.

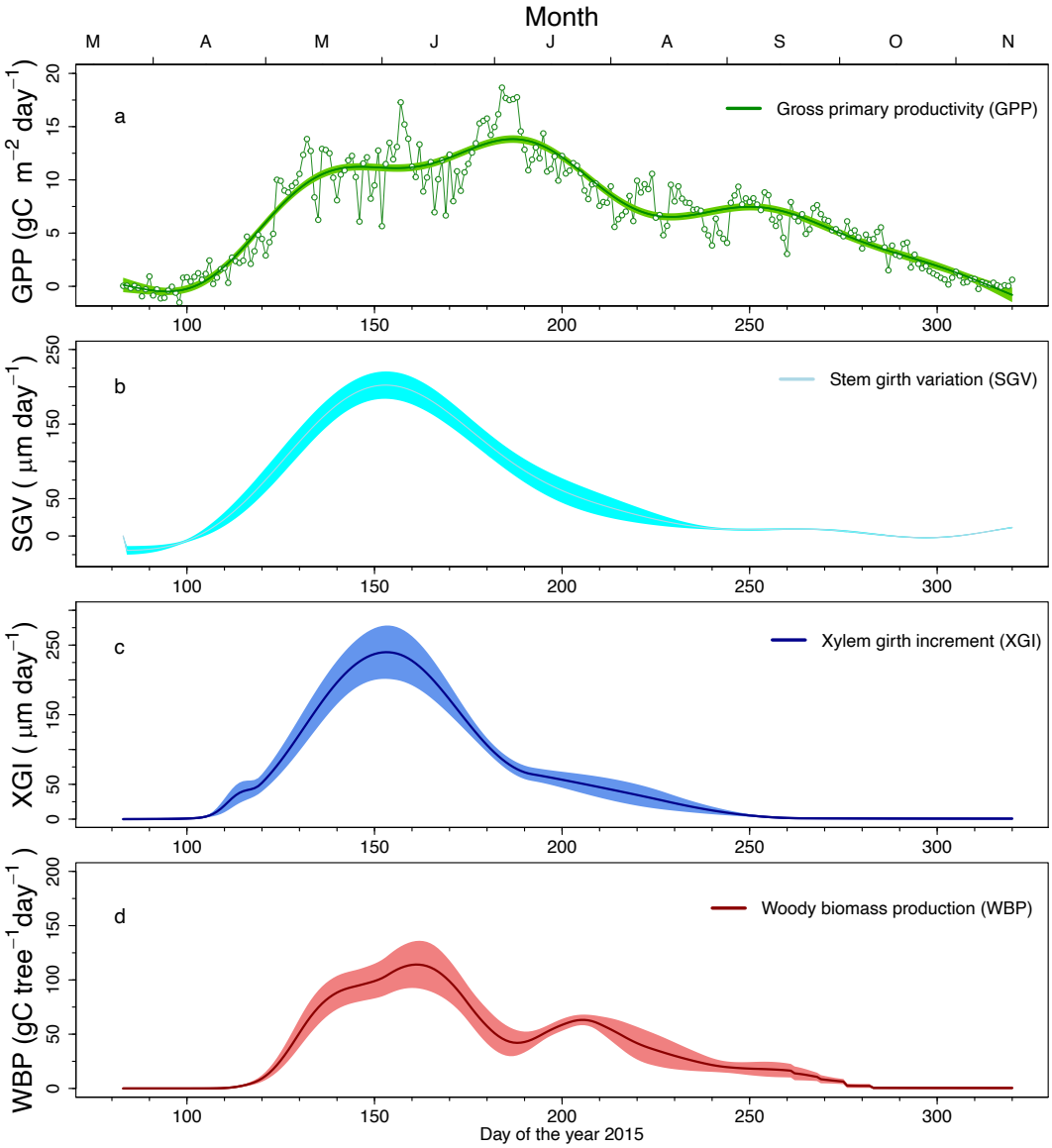
8.2.3 | Approaches

This project is based on the Hesse ICOS (Integrated Carbon Observation System) site (Moselle, France), where a tower measuring water and carbon fluxes has been installed in 1996 on a lowland beech forest growing on a grey-brown podzolic soil. The station includes more than 100 automatic sensors devoted to the monitoring of the evolution of the soil, atmospheric, and vegetation conditions. In addition to the regular measurements, tree radial growth, wood formation, and non-structural carbon concentration of seven beeches and seven oaks, growing in the "footprint" of the eddy covariance system, have been monitored, on a weekly basis, all along the 2015, 2016, 2017, 2018 and 2019 growing seasons.

8.2.4 | Preliminary results

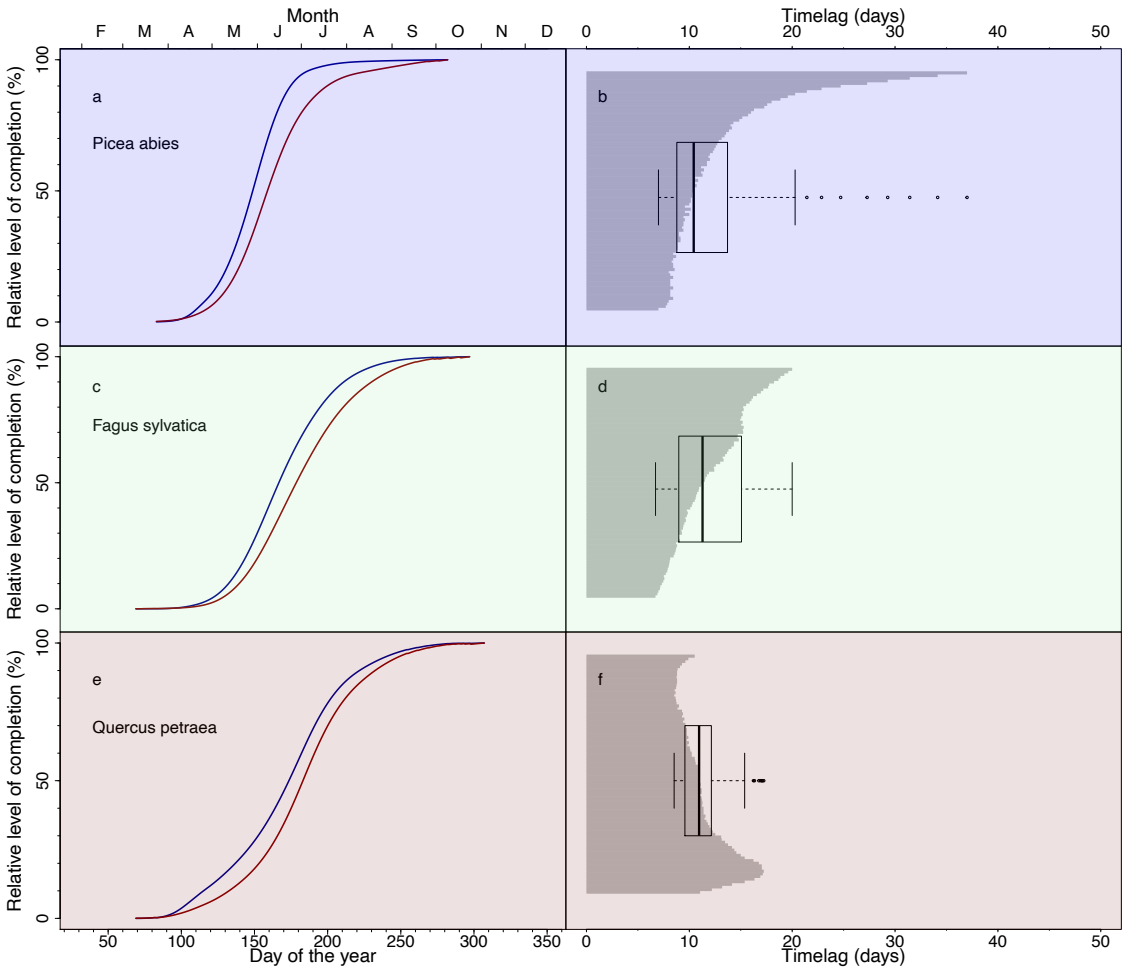
Gross primary productivity (GPP), non-structural carbon (NSC) concentration, xylem radial increment (XRI), and woody biomass production (WBP) presented contrasted dynamics along the monitored vegetation period for beech in Hesse forest [Figure 8.1]. The vegetation period was launched, at the beginning of April, by the increase of the GPP, to finish, at the end of November, with the stopping of it [Figure 8.1a]. GPP culminated at the summer solstice. XRI and SGV started after GPP, also in April, culminated before GPP, in June, and finished in early October, well before of all the other processes [Figure 8.1b,c]. WBP followed XRI with a lag of about two weeks in average, starting early in May, culminating in July, and finishing in November [Figure 8.1d]. NSC concentration presented very few variations along the seasonal cycle.

GPP, NSC, XRI, and WBP dynamics were not influenced by the same environmental factors: solar radiation was the main environmental factor influencing GPP, while day length was the main environmental factor influencing XRI, and temperature WBP. NSC didn't respond to any environmental factors.



73 — Figure 8.1 | Intra-annual dynamics of gross primary production, stem girth variation, xylem girth increment and woody biomass production in a mature beech forest.
Daily variation of GPP (**a**) with raw data, and GAM fitting (predicted values ± standard deviation), SGV (**b**), XGI (**c**), and WBP (**d**) among seven beech trees (mean values of predicted values from first derivative of GAMs fitted curves ± standard deviation). Figure from Andrianantenaina et al. {in preparation a}.

The coordination between GPP, NSC, XRI, and WBP seasonal dynamics were related to the tree-ring structure and wood density [Figure 8.2], but not to the site fertility or tree vitality. The time lag between GPP, XRI, and WBP was smaller for the studied beeches than for any observed conifer species. For beech, the time lag between GPP, XRI, and WBP was all the greater when trees presented denser wood.



74 — Figure 8.2 | Temporal coordination between intra-annual dynamics of stem growth in size and in biomass for spruce, beech and sessile oak.
In **a**, **c** and **e**, the blue curves correspond to the xylem radial increment (XRI) and the red curves to the aboveground woody biomass production (AWP), expressed in relative level of completion ranging from 0 to 100% along the growing season. In **b**, **d** and **f**, the grey horizontal bars represent the timelag (in days) between the two processes, while the box-plots represent the distribution of time-lag values (in days) along the growing season. Figure from Andrianantenaina et al. {in preparation b}.

8.2.5 | Conclusions and perspectives

These encouraging results underline the importance to better consider the variability of existing tree-ring structures, and to monitor more accurately NSC dynamics. These points will be tackled in our future works, by including oaks and spruce, and increasing sampling frequency for NSC monitoring.
We showed in this work that GPP, NSC, XRI, and WBP presented contrasted dynamics all along the growing season, and were influenced by different environmental factors and internal drivers. Our results suggest that in the complex pathway of the carbon in the tree, from the leaves to the wood, NSC dynamics, although poorly known, occupy a “strategic junction”, orchestrating up- and down-stream processes.

8.3 | Intra-annual dynamics of water transport in trees — The WatFlux project

8.3.1 | Context

Plants adjust leaf water potential and hydraulic conductance when confronted with drought, reducing sap-flow and optimizing water loss relative to carbon gain, in turn protecting their tissues from important water loss {Granier 1987; Granier et al. 1999}. Xylem hydraulic conductivity highly depends on the size and number of conduits per unit area {Tyree & Zimmermann 2002}. Therefore, even though the rate of water flux through and from the tree is largely determined by transpiration rates, it is also constrained by the xylem's anatomical features {Reichstein et al. 2005}.

Leaf and xylem water potentials directly influence cell turgor pressure, which in turn affect xylem cell division, expansion and maturation, and thus radial growth and wood properties {Kozlowski T.T., Pallardy 1997}. Xylem formation may therefore have long-lasting consequences for whole canopy water and carbon fluxes. However, much is still unknown about how trees adjust xylem structure to meet transpiration needs.

Xylem formation results from a sequence of cambium division and cell differentiation processes common to both angiosperms and gymnosperms, but that derives in different xylem structures with distinct hydraulic properties {Rathgeber et al. 2016}. Environmental conditions influence the timing, duration and rates of xylogenesis processes, which in turn affect vessel and tracheid size.

8.3.2 | Objectives

The aim of WatFlux project is to better understand the intra-annual dynamics of water fluxes between the canopy and the atmosphere in relation with xylem development and climatic conditions.

8.3.3 | Approaches

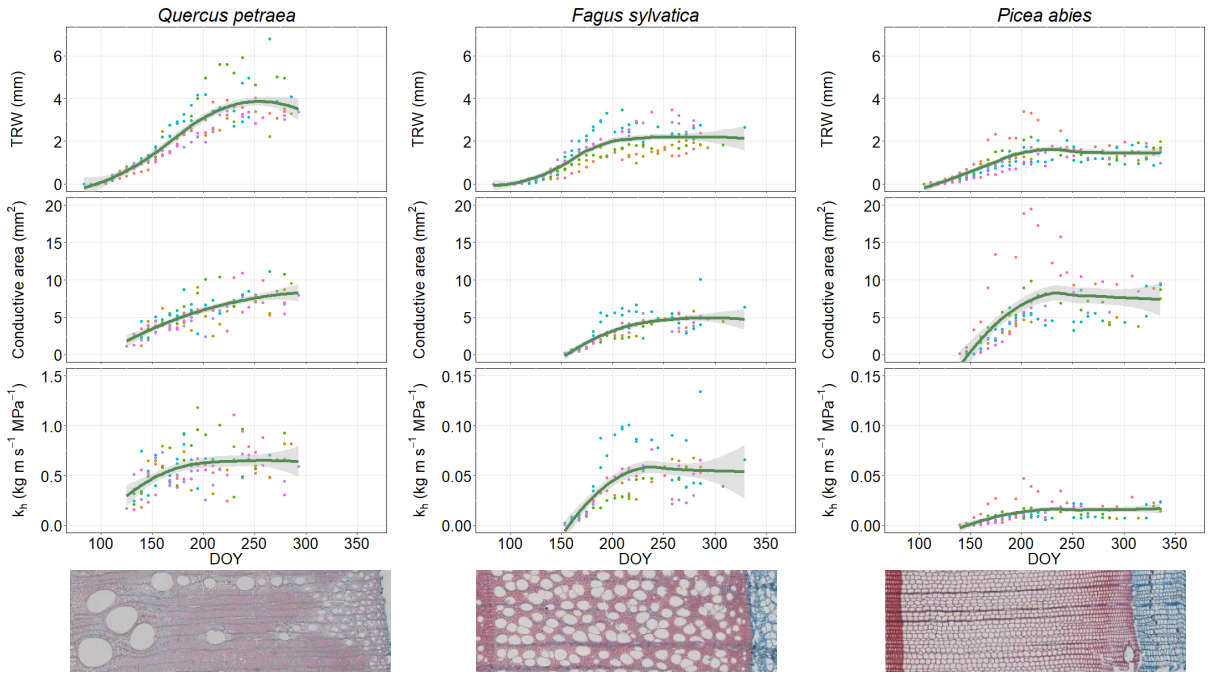
The high-resolution sap flow measurements will be compared to the xylem conductivity estimates calculated from the wood-formation-monitoring data. In addition, we will perform high resolution anatomical measurements of pit and perforation plate formation using scanning electron microscopy (SEM). The collected anatomical data will be used to obtain a better estimate of the changes in intra and inter-annual conductivity and resistance of the xylem to the transport of water.

This new project will add two years (2018, 2019) of high-quality data to our current dataset (2015, 2016, 2017) on Hesse forest. This unique dataset will allow us to: (1) explore the relationships between xylem anatomy, sap flow density, and eddy covariance flux measurements; (2) understand the adaptation of xylem conductivity to variations of climatic factors; and (3) model the water flux through the xylem in relation to the development of the tree and its physiological state.

8.3.4 | Preliminary results and discussion

Xylogenesis started in late-March to early-April in oak and in early-April to early-May in beech and spruce, depending on the year. Current-ring conduits started to be potentially hydraulically active earlier in oak (mid- to late-April) than in beech and spruce (mid-May).

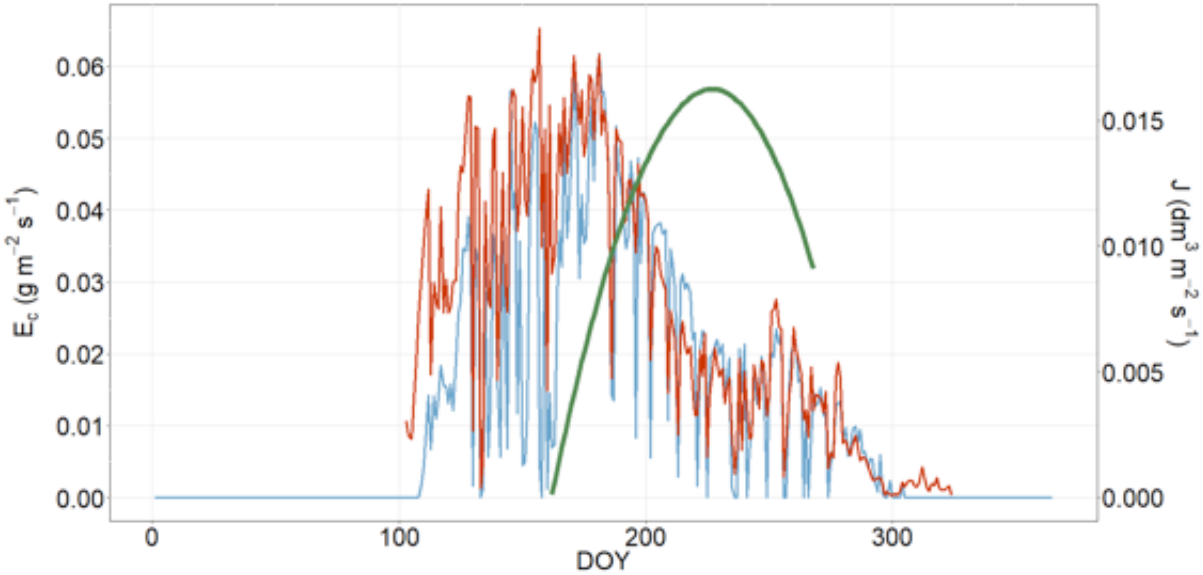
Both beech and spruce experienced an increase in the theoretical xylem hydraulic conductivity (kh) along the year as new conduits were formed and remained potentially hydraulically active all along the growing season [Figure 8.3] (i.e. no significant loss of conductivity occurred in the current ring at the end of the growing season). Maximum kh was then reached in beech and spruce in late-August. Conversely, oak reached its maximum kh in mid-July before experiencing a significant decrease in conductivity at the end of the growing season, due to the blockage of earlywood vessels by thyloses. These results show that wood formation monitoring methods can be an effective method to account for intra-annual changes in theoretical kh in species with different anatomical structures. Applying this method may be particularly interesting to study ring-porous species, such as sessile oak, which mostly rely on current-year vessels for water transport.



75 — Figure 8.3 | Intra-annual dynamics of xylem hydraulic conductivity for sessile oak, beech and spruce at Hesse forest in 2015.
In **a**, **e** and **i**, Tree-ring width (TRW) measurements. In **b**, **f** and **j**, Conductive area. In **c**, **g** and **k**, Theoretical xylem hydraulic conductivity (Kh). In **d**, **h** and **l**, Microscopic images of the contrasting tree-ring structures of the different species. Figure from Fernandez de Una et al. {in preparation a}.

Beech canopy transpiration (Ec) and sap flow started in mid-April, thus relying on previous-year vessels for water transport, and peaked in June, before maximum current-year ring kh was achieved [Figure 8.4]. Maximum current-year kh was reached in late-August-September, two months after transpiration rates peaked and when canopy transpiration needs were low. The inter-annual variation in

maximum and mean kh and Ec were also uncorrelated. This uncoupling between vessel formation and transpiration dynamics may therefore indicate that xylogenesis processes do not respond to current-year canopy transpiration needs. Moreover, current-year vessel contribution to transpiration of the year when they are formed may be negligible compared to total sapwood; however, it could have long-lasting legacy effects that may affect canopy processes for several years.



76 — Figure 8.4 | Intra-annual dynamics of canopy transpiration, sap flux density and xylem hydraulic conductivity.
The canopy transpiration (Ec) is in blue, the sap flux density (J) is in red, and the xylem hydraulic conductivity (Kh) is in green. Figure from Fernandez de Una et al. {in preparation b}.

8.3.5 | Conclusion and perspectives

The results of this project will provide new insights into the biological processes governing the carbon and water cycle of forest ecosystems, improving our understanding of its responses to climatic variability at short (growth and transpiration dynamics), medium (legacy effects), and long term (forest functioning).

8.4 | Understanding the creation of carbon and oxygen isotopic signals in tree-ring archives —The WoodIsotop project

8.4.1 | Context

Multiproxy tree-ring studies have played a major role in determining climate impacts on forests worldwide. Such records are paramount for past climate reconstructions as well as for the assessment of tree responses to future climate scenarios. Those studies rely on the fact that tree rings produced in climates with enough seasonal contrast can be univocally assigned to discrete time intervals (years) thanks to the fact that growing seasons interspersed with dormancy periods provokes anatomically distinguishable boundaries in wood.

In the last decades, there has been a proliferation of tree-ring studies dealing with intra-ring characteristics at the sub-annual scale by using radial increment, wood density, wood cell morphology, or stable isotope data. Sub-annual information archived in already mature wood is dated through a space-for-time conversion based on the cell row position, or instead on contingent/arbitrary anatomical features. Massive correlative analyses between intra-ring parameters and monthly climate data may ultimately serve to set a timescale to the tree ring by inferring a feasible time interval at which cell growth events occurred.

That approach requires splitting the ring into wood sections that are assumed to be formed in successive discrete time intervals (as it is done for tree rings). However, the fact that differentiation in a cell row overlaps that in neighboring ones hinders assignation of time intervals to intra-ring sections. Moreover, the number of forming cells at a given day varies according to changes in cell division rates and duration of cell differentiation (i.e. from 3 days up to 4 months in Pinus sylvestris). Hence, the length of the time window at which environmental conditions can affect individual cell development, as well as the amount of tissue affected by a given event, greatly changes along the growing season. In addition, possible differential environmental effects on simultaneously forming cells according to their respective developmental stage or position are largely unknown. Such a complex correspondence between cell age/lifespan and position blur the signal recorded in each isolated ring section, which is concurrent with other physiological constraints lagging tree growth responses to the environment. This somewhat jeopardizes reliability of tree-ring archives when used to infer processes at the sub-annual scale. Deciphering high-resolution information stored in tree rings therefore requires consideration of environmentally driven changes in cell differentiation kinetics, which cannot be assessed once the ring is completely formed.

Here, we advocate for a more systematic characterization of wood cell differentiation kinetics covering more species and longer periods and for implementing this knowledge on tree-ring analyses dealing with the sub-annual scale. This would contribute to find a more accurate correspondence between either cell position or recognizable anatomical features and time across species and environmental conditions.

8.4.2 | Objectives

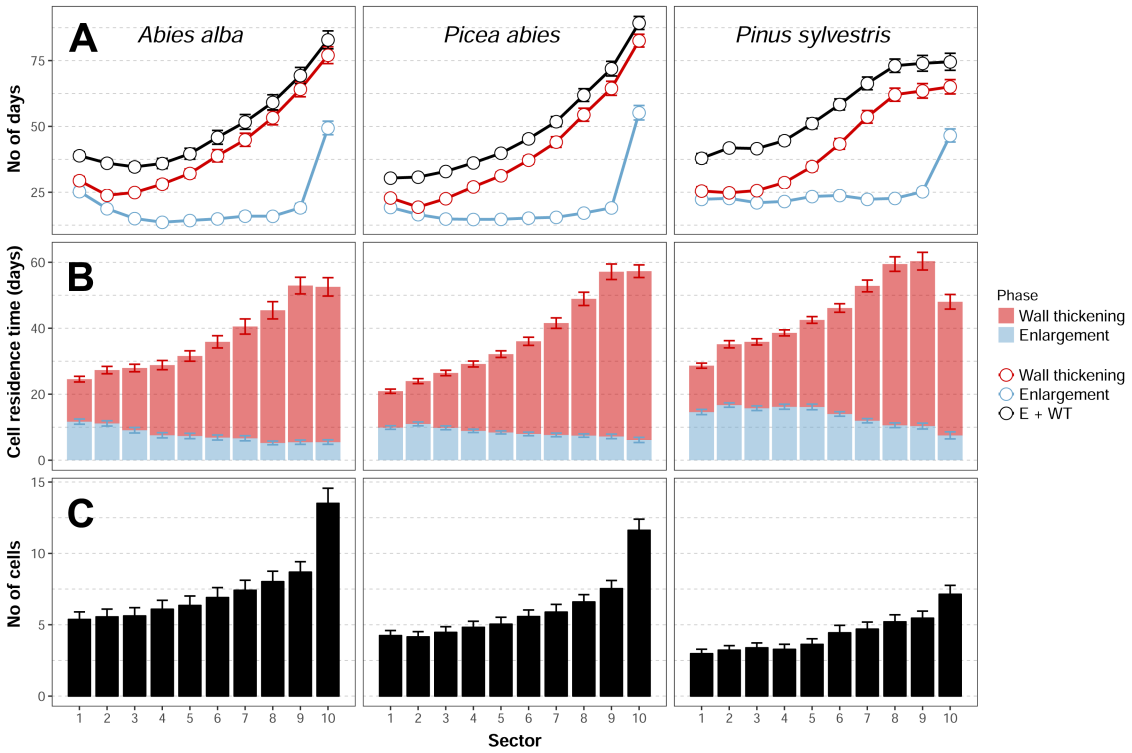
The WoodIsotop project aims to advance our knowledge of the interactions between environmental factors, developmental constraints, and physiological processes involved in wood formation. Our investigations focus on a better understanding of the mechanisms by which seasonal climate variations, and extreme events, leave permanent marks in tree rings. In particular, we focus on identifying (i) environmental factors influencing the intra-annual dynamics of wood formation and the characteristics of the rings formed (i.e. micro-densitometric profile, anatomical characteristics of the wood, and intra-ring variations in the carbon isotopes composition); (ii) understanding the mechanisms involved; and (iii) their time coordination.

8.4.3 | Approaches

To carry out this project, we rely on two monitoring networks of mixed coniferous forests: the Donon network (composed of 3 stands, containing 3 species, monitored over 3-4 years, and installed along an altitudinal gradient in the Vosges Mountains, France); and the Lötschental network (composed of 8 stands, containing 1-2 species, monitored over 9 years, and installed along two altitudinal gradients in the Valais, Switzerland). Both datasets have been completed to contain the same information on wood formation dynamics, quantitative anatomy and isotopes composition. We have also improved our model of wood formation dynamics to accurately relate cell differentiation kinetics, tracheid morphology, and wood isotopes composition to concomitant environmental conditions.

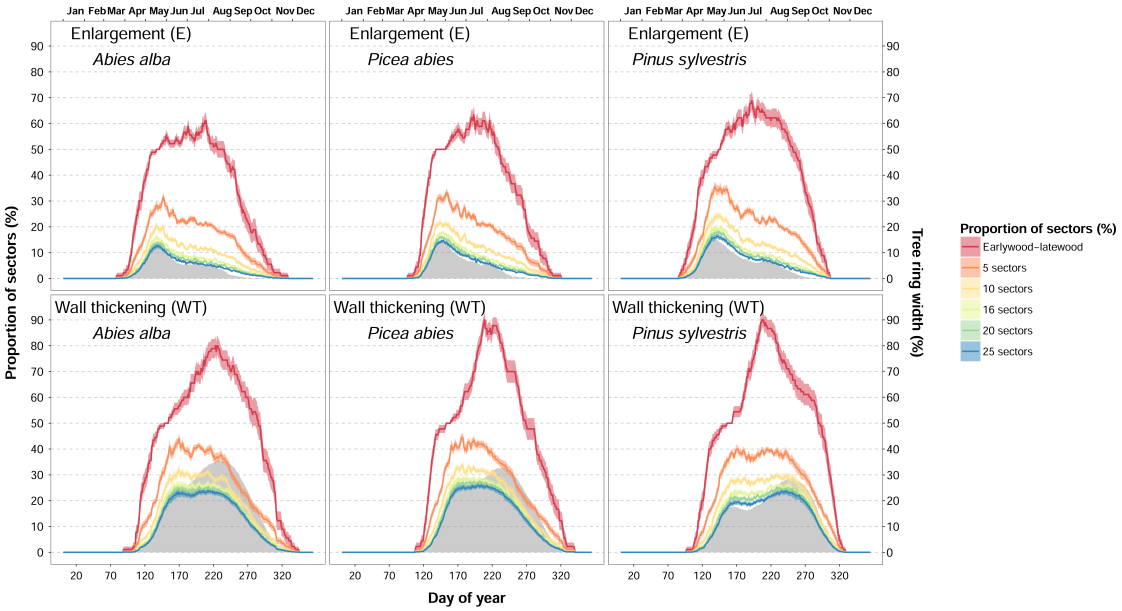
8.4.4 | Preliminary results

By connecting causes (environmental and developmental conditions) and consequences (growth ring characteristics and isotopic signature of wood) through a mechanistic understanding of the physiological processes involved in wood formation, the WoodIsotop project will not only enhance the reliability of dendrochronological variables used for climate reconstruction, but will also reduce uncertainties in future assessments of the impact of climate change on tree growth, forest ecosystem functioning, and the quantification of fluxes exchanged between the terrestrial biomass and the atmosphere. At present, the results obtained concern: (1) the allocation of a time window to the carbon isotopes composition in the tree ring [Fig. 8.5]; (2) the highlighting of the temporal "overlap" between the ring sections [Fig. 8.6]; (3) the evaluation of within-stand synchronicity of xylogenesis processes [Fig. 8.7].



77 — Figure 8.5 | Evolution of the duration of enlargement (blue) and wall thickening (red) across 10 ring sub-divisions (sectors), along with variation in the number of cells.

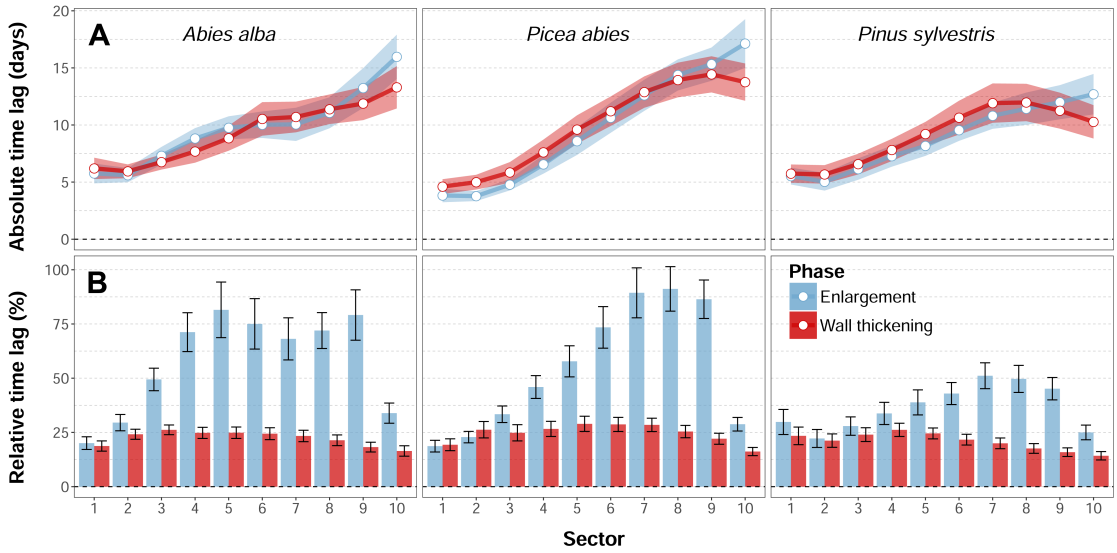
Total duration, in days, of sector formation (A); mean duration, in days, of individual cell formation for each sector (B); and number of cells per sector. Mean ± SE values are shown for each species. Figure from Perez de Lis et al. {in preparation}.



78 — Figure 8.6 | Simulated seasonal variation in the proportion of sectors containing enlarging cells (upper panel) and wall-thickening cells (lower panel).

Lines of different colours represent simulations for 5 to 25 regular sectors, as well as for earlywood-latewood divisions. The mean ± SE is shown for each species. Grey shadow area represents the mean percentage of the tree-ring width under enlargement (upper

panel) and wall thickening (lower panel) for each study species. Figure from Perez de Lis et al. {in preparation}.



79 — Figure 8.7 | Absolute (A) and relative (B) time lag among different individuals for each sector and species, considering enlargement (blue) and wall thickening (red) processes.
Lines in the upper panel show the mean ± SE time lag in number of days between each tree and the average period for a given species, site and year. Bars in the lower panel show the mean ± SE time lag between each tree and the average period for a given species, site and year expressed as a percentage of the total duration of the average period. Figure from Perez de Lis et al. {in preparation}.

8.4.5 | Conclusion and perspectives

At present, the results obtained for fir in the Vosges Mountains using the intra-annual variations of δ13C and micro-densitometric profiles are quite disappointing from a dendroecological point of view, but we are waiting for the final results to conclude. We believe this is due to the low variability observed between the study sites and the monitored years, as well as the physiological behaviour of the species, which changes little during the growing season. The second part of the project, which will focus on the variations of δ18O in larch rings along the altitudinal gradient of the Lötschental, is likely to provide more salient results.
Despite many methodological difficulties inherent to the wood formation monitoring technique, we were able to improve our statistical model of xylogenesis dynamics sufficiently to assign a precise time window to each section of wood cut within the ring. This allowed us to show that the sections of wood thus delimited, although completely spatially separated, actually "overlapped" largely from a temporal point of view. Therefore, these sections cannot provide independent climatic information, unlike what is regularly proposed in related scientific publications.

8.5 | For a broad, integrated approach to tree-ring sciences and wood quality

8.5.1 | Introduction

Wood density (i.e. the ratio of the dry weight of wood to its volume) is the expression of how much organic matter is present in a given piece of wood {Zobel & Van Buijtenen, 1989}. Density is generally considered to be the main feature used to assess wood quality, because it has a major effect on both the yield and the quality of fibrous and solid wood products, and because it can be manipulated by silvicultural practices and genetic selection {Barnett & Jeronimidis, 2003}.

Wood density represents an important characteristic of tree radial growth, which is necessary to accurately describe biomass production and carbon sequestration in forest ecosystems {Bouriaud et al., 2015}. It is also regarded as a key functional trait by ecologists {Chave et al., 2009}. However, wood density is a complex property resulting from the combination of several tree-ring structural features (e.g. the ratio of earlywood to latewood), and xylem anatomical and chemical characteristics (e.g. the amounts, dimensions and composition of the different types of constitutive cells: tracheids, ray cells, tracheary elements).

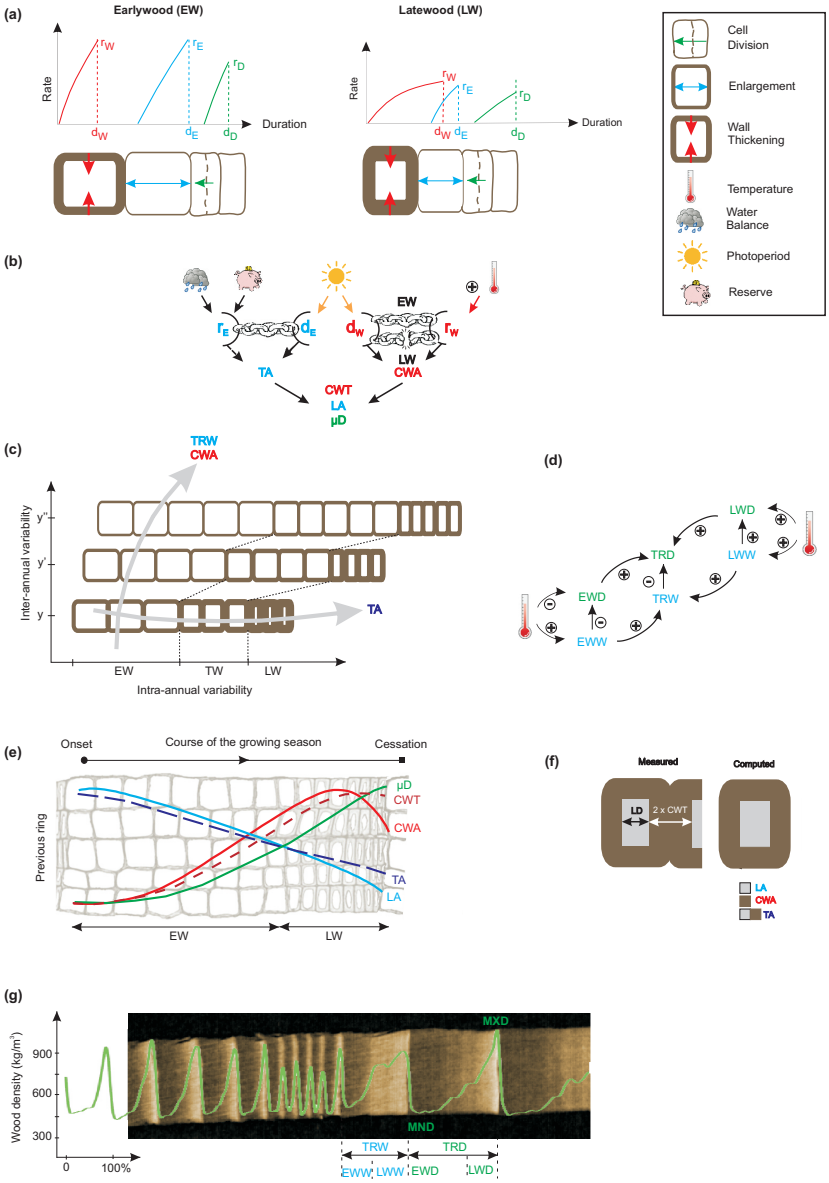
Wood density varies between tree species, but also between individuals from the same species, and between organs from the same individuals – branches tend to be denser than stems, which are denser than roots {Pratt et al., 2007}. Inside stems, wood density varies from the bottom to the top – density tends to increase with height {Swenson & Enquist, 2008}; and from the bark to the pit – heartwood is often significantly denser than sapwood {Woodcock & Shier, 2002}.

As well as numerous 'ontogenic' sources of variability, wood density is also known to be sensitive to environmental conditions. Factors such as site quality, stand characteristics, forest management and biological interactions, all have significant influences on it. However, climatic conditions are the most studied source of variability. Tree-ring density and width parameters have been extensively used to reconstruct past climatic conditions (summer temperatures especially) over the last 2000 years {St George, 2014; Wilson et al., 2016; Anchukaitis et al., 2017}.

Björklund et al. {2017} developed a novel and promising approach combining an 'extensive' dataset of widespread and well-replicated annually resolved tree-ring parameters, with an 'intensive' but more spatially limited dataset containing wood cell anatomical measurements. An exploration of the global tree-ring dataset allows for the detection of large structural, biogeographical and environmental patterns, while a complementary use of the detailed anatomical data facilitates interpretations.

8.5.2 | Structural relationships between tree-ring width, wood density and xylem anatomy

In forest science, growth rate is seen as the factor that can most affect wood density. It is generally accepted that, in conifers, when tree-ring width increases tree-ring density decreases in return {Zobel & Van Buijtenen, 1989}. With the power of a global dataset, Björklund et al. show that correlations between ring width and density generally shift from negative in earlywood – larger earlywood widths correspond to lower earlywood densities – to positive in latewood – larger latewood widths correspond to higher latewood densities [Fig. 8.8d]. Such results show that width/density structural relationships must be explored at the intra-ring level to reach sound conclusions, and advocate for further investigations at the anatomical level. Moreover, Björklund et al. confirm, at global scale and for a large number of conifer species, that tracheid size is the main driver of the inter-annual variability of earlywood density, while cell wall amount is more influential for latewood density [Fig. 8.8c]. The progressive shift, from early- to late-wood, in tracheid allometry is the key to understand the structural relationships between ring width and wood density: in earlywood, cell-wall area is quite independent of tracheid area; while in latewood, cell-wall area is positively related to tracheid area. Xylogenesis processes at the origin of this allometric shift have recently been unveiled {see Rathgeber et al., 2016 for more details}. During the first part of the growing season cell division is intense, while cell enlargement duration is long and wall thickening is relatively short, producing a high number of large, thin-walled cells of light earlywood; during the second part of the growing season, cell division slows down, while enlargement duration shortens and thickening duration extends, producing a relatively small number of narrow, thick-walled cells of dense latewood {Cuny et al., 2014}.



80 — Figure 8.8 | Origin and description of ring width, wood density, and xylem anatomy variability in conifers.
(**a**) Processes of tracheid differentiation in early- and late-wood. Duration of: dD, cell division; dE, cell enlargement; dW, cell-wall thickening. Rate of: rD, cell division; rE, cell enlargement; rW, wall thickening. (**b**) Cascade of influences of external and internal factors on the processes of cell differentiation and resulting tracheid characteristics and tree-ring structure. CWA, cell-wall area; CWT, cell-wall thickness; LA, lumen area; TA, tracheid area; lD, microdensity. (**c**) Unravelling tree-ring structure intra- and inter-annual variability components. EW, TW, LW, early-, transition-, and late-wood, respectively; TRW, tree-ring width. (**d**) Network of relationships between ring-width and wood- density parameters, and climatic factors. EWD, earlywood density; EWW, earlywood width; TRD, tree-ring density; LWD, latewood density; LWW, latewood width. (**e**) Intra-ring profile of the main variables describing tree-ring structure. (f) Presentation of the main tracheid features used in quantitative wood anatomy. LD, lumen diameter. (g) Wood micro-density profile along with the associated X-ray image and measured parameters. MND, minimal tree-ring density; MXD, maximal tree-ring density. Figure from Rathgeber {2017}.

8.5.3 | Functional significance of tree-ring width, wood density and xylem anatomy

Tree-ring width and density parameters are widely used as ecological and environmental proxies; however, understanding of their formation process, structural determinism and functional significance remains neglected {Vaganov et al., 2007}. In coniferous trees, it is commonly stated that the wide band of large-lumen earlywood tracheids [Fig.8.8g] is optimized for the efficient transport of raw sap from the roots to the needles, supplying the bulk of the crown water demand; while the narrow band of thick-walled, small-diameter latewood tracheids are optimized to provide mechanical support to the tree structure, but have limited water transport capacity {Tyree & Zimmermann, 2013}. Björklund et al. {2017} show that earlywood density is negatively related to cell number and lumen area [Fig. 8.8e]. Thus, because water-conduction efficiency increases linearly with the number of tracheids, and exponentially with the diameter of their lumens, a small decrease in earlywood density must result in a dramatic increase in water-conduction efficiency. On the other hand, interpreting latewood density directly in terms of mechanical support seems perilous because the specific contribution of the latewood tracheids to this function is still difficult to assess. However, the mechanical properties of the tracheids are related to the thickness-to-span ratio, which increases linearly with cell-wall thickness and decreases with lumen diameter {Sperry et al., 2006}. Latewood is then considered to be the compartment most resistant to cell implosion risk under water tension {Hacke & Sperry, 2001}. Björklund et al. {2017} show that, in latewood, density is strongly positively related with cell number and cell-wall thickness. Thus an increase in latewood density must result in a proportional increase in water-conduction security. The work of Björklund et al. {2017} opens a very promising research avenue to the use of ring density parameters as proxies for tree functional responses to changing environments. Indeed, thanks to the strong and stable relationship between earlywood density and water conduction-efficiency, and between latewood density and water conduction-security, tree-ring networks exhibiting high individual replication, wide geographical coverage and deep temporal representation, could be used to investigate water transport variability both spatially and over time {Camarero et al., 2014, 2017}. Future developments could explore the evolution of the trade-off between efficiency and security, including stable isotope data {Frank et al., 2015}.

8.5.4. Influence of environmental factors on tree-ring width, wood density and xylem anatomy

In their study, Björklund et al. {2017} show that the correlations between ring parameters and temperatures are complex, varying during the growing season and across biogeographic zones. The temperature response of ring-width parameters progressively changes from a strong mid-summer positive correlation at higher latitudes (above 55°N), to a negative previous late-summer correlation at an intermediate latitude (40–55°N), and finally a negative late-spring correlation at lower latitudes (under 40°N). The ring-density parameters exhibit even stronger correlations with temperature, with earlywood parameters correlating positively

with spring temperatures at lower latitude, while latewood parameters (maximal density in particular) correlate strongly with whole summer temperatures at higher latitudes.

The fact that earlywood density and minimal density show consistently positive responses to the previous year temperatures, while latewood density and maximal density exhibit pronounced responses to early spring, suggests that a significant amount of the carbon used for cell-wall formation has been captured earlier in the growing season, or even during the previous year. This interpretation is supported by several recent experimental studies reporting that earlywood formation strongly relies on photosynthates from the previous year, and that secondary growth may even mobilize carbon reserves that have been stored for several years if necessary {Kagawa et al., 2006; Gessler et al., 2009; Kuptz et al., 2011}. These results may also reflect an evolution of the source–sink relationships throughout the growing season: latewood would record the direct influences of the current climatic conditions on cambial activity (sink limitation), whereas earlywood would bear witness to the indirect influences of previous climatic conditions on photosynthetic activity (source limitation).

Björklund et al. {2017} show that during earlywood formation, spring temperatures mainly influence tracheid size, whereas during latewood formation, summer temperatures mainly influence cell-wall dimensions, providing a sound anatomical basis to the well documented, and widely used, relationship between summer temperature and tree-ring maximal density. These results echo recent works showing that temperature drives the rate of deposition of wall material [Fig. 1b], reaching its maximal influence during latewood formation when the coupling between wall thickening rate and duration is broken {Balducci et al., 2016; Cuny & Rathgeber, 2016}. Among the cellular processes involved in cell-wall deposition, lignification is known to be the slowest and the most constrained by temperatures {Gindl et al., 2000}. Interestingly, Björklund et al. {2017} also show that in response to the same favourable climatic conditions – independently of phylogeny or biogeography – conifers may increase earlywood formation (i.e. producing more lighter earlywood cells and enhancing water-conduction efficiency), and/or increase latewood formation (i.e. producing denser latewood cells and enhancing water conduction security) depending on the time of the year. This suggests that, in general, the earlywood–latewood transition is relatively independent of environmental factors, but it can be triggered by particular climatic conditions such as drought or cold spells {e.g. Begum et al., 2016}. The earlywood–latewood transition must, therefore, be under strong developmental and genetic control, recording tree physiological state and growing season progression, and modifying the coordination between the xylogenesis processes accordingly.

8.5.5 | Conclusions and perspectives

Tree radial growth, wood density and xylem anatomy reflect tree strategies to best allocate – in the time course of the growing season, and year after year – the available resources under changing environmental conditions. Therefore, tree-ring structure radial variability provides crucial information, first on tree responses to growing conditions, and second on the environment itself. Björklund et al. {2017} combine these three sources of information at large spatial and temporal scales, and reconciled quantitative wood anatomy and dendrochronology, opening a new

research area for tree-ring science. The next challenge I foresee will be to incorporate the knowledge recently gained by wood-formation-monitoring studies into this framework; this will really open the door for a mechanistic approach in tree-ring science, and will allow the combination of tree short-, medium- and long-term responses to the environment, which is pivotal for reconstructing past environmental conditions, as well as for forecasting the future impact of climate changes.

8.6 | References

This list of references is not formatted!

Babst, F., Poulter, B., Bodesheim, P., Mahecha, M.D. & Frank, D.C. (2017). Improved tree-ring archives will support earth-system science. Nat. Ecol. Evol. Publ. online 24 January 2017; | doi10.1038/s41559-016-0008, 1, 0008.

Belmecheri, S., Wright, W.E., Szejner, P., Morino, K.A. & Monson, R.K. (2018). Carbon and oxygen isotope fractionations in tree rings reveal interactions between cambial phenology and seasonal climate. Plant Cell Environ., 41, 2758–2772.

Belokopytova, L. V., Babushkina, E.A., Zhirnova, D.F., Panyushkina, I.P. & Vaganov, E.A. (2018). Pine and larch tracheids capture seasonal variations of climatic signal at moisture-limited sites. Trees - Struct. Funct., 0, 0.

Björklund, J., Seftigen, K., Schweingruber, F., Fonti, P., von Arx, G., Bryukhanova, M. V., et al. (2017). Cell size and wall dimensions drive distinct variability of earlywood and latewood density in Northern Hemisphere conifers. New Phytol.

Carrer, M., Castagneri, D., Prendin, A.L., Petit, G. & von Arx, G. (2017). Retrospective Analysis of Wood Anatomical Traits Reveals a Recent Extension in Tree Cambial Activity in Two High-Elevation Conifers. Front. Plant Sci., 8, 1–13.

Castagneri, D., Fonti, P., Von Arx, G. & Carrer, M. (2017). How does climate influence xylem morphogenesis over the growing season? Insights from long-Term intra-ring anatomy in Picea abies. Ann. Bot., 119, 1011–1020.

Cuny, H.E. & Rathgeber, C.B.K. (2016). Xylogenesis: Coniferous Trees of Temperate Forests Are Listening to the Climate Tale during the Growing Season But Only Remember the Last Words! Plant Physiol., 171, 306–17.

Cuny, H.E., Rathgeber, C.B.K., Frank, D., Fonti, P. & Fournier, M. (2014). Kinetics of tracheid development explain conifer tree-ring structure. New Phytol., 203, 1231–41.

Cuny, H.E., Rathgeber, C.B.K., Frank, D., Fonti, P., Mäkinen, H., Prislan, P., et al. (2015). Woody biomass production lags stem-girth increase by over one month in coniferous forests. Nat. Plants, 1, 15160.

Granier A. (1987) Evaluation of transpiration in a Douglas-fir stand by means of sap flow measurements. Tree Physiology, 3, 309–320.

Granier A., Bréda N., Biron P., Villette S. (1999) A lumped water balance model to evaluate duration and intensity of drought constraints in forest stands. Ecological Modelling, 116, 269–283.

Kozlowski T.T., Pallardy S.G. (1997) Physiology of woody plants (Academic Press, San Diego, USA).

McCarroll, D., Whitney, M., Young, G.H.F., Loader, N.J. & Gagen, M.H. (2017). A simple stable carbon isotope method for investigating changes in the use of recent versus old carbon in oak. Tree Physiol., 37, 1021–1027.

Pacheco, A., Camarero, J.J. & Carrer, M. (2018a). Shifts of irrigation in Aleppo pine under semi-arid conditions reveal uncoupled growth and carbon storage and legacy effects on wood anatomy. Agric. For. Meteorol., 253–254, 225–232.

Pacheco, A., Camarero, J.J., Ribas, M., Gazol, A., Gutierrez, E. & Carrer, M. (2018b). Disentangling the climate-driven bimodal growth pattern in coastal and continental Mediterranean pine stands. Sci. Total Environ., 615, 1518–1526.

Rathgeber C.B.K., Cuny H.E., Fonti P. (2016) Biological Basis of Tree-Ring Formation: A Crash Course. Frontiers in Plant Science 7(734):1–7.

Reichstein M., Falge E., Baldocchi D., Papale D., Aubinet M., Berbigier P., Bernhofer C., Buchmann N., Gilmanov T., Granier A., Grünwald T. , Havránková K. , Ilvesniemi H., Janous D., Knohl A., Laurila T., Lohila A., Loustau D., Matteucci G., Meyers T., Miglietta F., Ourcival J., Pumpanen J., Rambal S., Rotenberg E., Sanz M., Tenhunen J., Seufert G., Vaccari F., Vesala T., Yakir D., Valentini R. (2005) On the separation of net ecosystem exchange into assimilation and ecosystem respiration: review and improved algorithm. Global Change Biology, 11: 1424-1439

Szejner, P., Wright, W.E., Babst, F., Belmecheri, S., Trouet, V., Leavitt, S.W., et al. (2016). Latitudinal gradients in tree ring stable carbon and oxygen isotopes reveal differential climate influences of the North American Monsoon System.

Szejner, P., Wright, W.E., Belmecheri, S., Meko, D., Leavitt, S.W., Ehleringer, J.R., et al. (2018). Disentangling seasonal and interannual legacies from inferred patterns of forest water and carbon cycling using tree-ring stable isotopes. Glob. Chang. Biol., 24, 5332–5347.

Tyree M.T., Zimmermann M.H. (2002) Xylem Structure and the Ascent of Sap, 2nd edition. Springer, Berlin, Germany.

CHAPTER 9 | GENERAL SYNTHESIS AND PERSPECTIVES

9.1 | Introduction

Xylogenesis has led to a major transformation of our planet by forming a tissue that provides both the mechanical support necessary for stability in the unbearable air environment, as well as the ability to transport water upwards and over long distances. Xylogenesis has given trees a competitive advantage that has allowed them to colonize the Earth's emerged lands, developing sizes that can be huge.

In trees, wood performs four essential functions: it supports and spatially distributes the photosynthetic tissues above ground; it conducts the raw sap (i.e. water and nutrients) from the roots up to the leaves; it stores carbohydrates, water, and other compounds; and finally, it protects the tree from pathogens, by storing and distributing defensive compounds.

Humankind's dependence on wood is so strong that, throughout history, the availability of wood has been closely linked to the success or decline of civilizations. Today, wood still plays a crucial role in our societies by being a preferred material in many fields such as building construction, furniture manufacturing or energy.

In this Chapter, we make a brief synthesis of all the aspect of intra-annual dynamics of wood formation, which are developed in the height previous chapters, and propose some perspectives for future researches.

9.2 | Biological basis of wood formation

9.2.1 | Synthesis

In the 'Biological basis of wood formation' chapter, we presented the fundamental processes involved in intra-annual dynamics of wood formation, along with their relationship with the developmental, physiological and environmental factors.

Xylogenesis produces wood cells that are adapted to perform vital functions in woody plants. It involves several processes and represents an extremely complex example of cellular specialization.

During wood formation, new xylem cells produced by the cambium are undergoing profound transformations, passing through successive differentiation stages, which will enable them to perform their future functions in trees. Xylem cell differentiation can be divided in five major phases: (1) the division of a cambial mother cell that creates a new cell; (2) the enlargement of this newly formed cell; (3) the deposition of its secondary wall; (4) the lignification of its cell wall; and finally, (5) its programmed cell death.

Later on, heartwood formation complete the process providing natural durability and ornamental values to wood products as well as antimicrobial properties to protect living stems from decay.

9.2.2 | Perspectives and conclusion

This knowledge at the cellular level still needs to be further developed and integrated with recent advances in genetics and genomics, as well as a more global understanding of xylogenesis in relation with tree development, physiology and environment.

This should ultimately lead to a more complete knowledge of the functioning and regulation of the xylogenesis process, which is particularly important in view of the need to protect forest biodiversity and optimise wood supply in a context of global change.

I claim that without a clear understanding of the xylem formation process, it is not possible to comprehend how tree-rings and typical wood structures are formed, recording normal seasonal variations of their environments as well as extreme climatic events.

9.3 | Methods for monitoring wood formation

9.3.1 | Synthesis

In the last decade, the pervasive question of climate change impacts on forests has revived investigations on intra-annual dynamics of wood formation, involving disciplines such as plant ecology, tree physiology, and dendrochronology. This resulted in the creation of many research groups working on this topic worldwide and a rapid increase in the number of studies and publications.

Wood-formation-monitoring studies are generally based on a common conceptual model describing xylem cell formation as the succession of four differentiation phases (cell division, cell enlargement, cell wall thickening, and mature cells). They generally use the same sampling techniques, sample preparation methods, and anatomical criteria to separate between differentiation zones and discriminate and count forming xylem cells, resulting in very similar raw data. However, the way these raw data are then processed, producing the elaborated data on which statistical analyses are performed, still remains quite specific to each individual study. Thereby, despite very similar raw data, wood-formation-monitoring studies yield results, which are still quite difficult to compare.

In the 'Methods for monitoring wood formation' chapter, we presented CAVIAR, an R package specifically dedicated to checking, displaying and processing wood-formation-monitoring data. We showed how to import formatted wood-formation-monitoring data into R, and we proposed a visual 5-step procedure designed to meticulously check raw data.

We also demonstrated how to manipulate and standardize raw data in order to maximize the growth signal and reduce the noise in the dataset before producing elaborated data. CAVIAR provides robust and efficient functions to compute (based on logistic regressions), display and analyse (based on bootstrap tests) wood formation phenology critical dates and durations.

CAVIAR provides also robust and efficient algorithms to model (based on Gompertz functions), display and analyse (based on bootstrap tests) intra-annual dynamics of xylogenesis. For temperate and cold forest trees, logistic regressions provide the best estimates of tree-ring formation critical dates, while Gompertz

models very well capture wood formation dynamics during the main part of the growing season.
We believe that the current version of CAVIAR represents a 'classic standard' in wood-formation-monitoring data processing. Indeed, for the first time, robust and efficient algorithms allowing performing the main classical data treatments (e.g., raw data verification, data standardization, wood formation critical dates and durations computation, and wood formation dynamics modelling) are gathered together in the same coherent package.
In the 'Methods for monitoring wood formation' chapter, we also presented the GloboXylo initiative, which aims to collect, store and use global wood-formation-monitoring data to identify large-scale biogeographic and ecological trends in xylogenesis, and can certainly contribute to the development of this new discipline.

9.3.2 | Perspectives and conclusion

Data collected in the GloboXylo database are currently available upon request. We aim to improve the visibility of GloboXylo and develop it further by building an online relational database.
In the third version of CAVIAR we plan to implement new algorithms based on generalized additive models to upgrade wood formation phenology and dynamics functions and develop new functions devoted to cell differentiation kinetics. This should improve the robustness and accuracy of CAVIAR in case of missing or scarce data (e.g., bi-weekly or monthly sampling), and extend its scope further to new environments (e.g., Mediterranean and tropical areas) and new species (e.g., angiosperms exhibiting ring- or diffuse-porous wood).
Moreover, a smooth connexion between CAVIAR and GloboXylo would be without any doubt a wonderful tool to make new discoveries concerning wood formation dynamics.
Thanks to recent advances in wood sample collection, microcore preparation, and anatomical section observation; as well as in raw data manipulation, data visualisation and elaborated data analyses; wood-formation-monitoring studies produce now abundant and high-quality data, which can be used to better understand the process of tree-ring formation dynamics and its relationships with tree development stage, physiological state, and environmental conditions.
To conclude, we believe that the GloboXylo initiative and the CAVIAR package will both help to strengthen tree-ring formation science, contributing to standardize its methodologies, improve further the quality of its data, and reinforce the significance of its results.

9.4 | Phenology of cambial activity and tree-ring formation

9.4.1 | Synthesis

In the 'Phenology of cambial activity and tree-ring formation' chapter, we presented our approach to describe and quantify accurately tree-ring formation phenology using the CAVIAR R package.
We proposed a synthesis of the most recent knowledge on the influence of environmental cues on the phenology of tree leaves and reproductive structures,

fine roots, and wood, and we also consider carbon and nitrogen reserves as well as the relationships between organs, which are all essential to understand the functioning of trees.
We explored the variability of tree-ring formation phenology according to tree age, size and social status, tree species, and site conditions. We have shown that tree characteristics have as much influence as site and climatic conditions in determining wood formation calendar.
We have shown that temperatures are the main driver of the onsets of cambial activity and wood formation, while photoperiod determine their cessations, which could be occasionally hasten by water shortage or cold spell.
We also have shown through a functional approach based on a meta-analysis of wood and leaf phenology data, that tree-ring structure and leaf traits determine the coordination of tree organ 'phenologies' according to plant functional type life strategies.
Finally, we developed a new process-based model approach, and applied it to data from four conifer species covering a wide range of temperature and photoperiod conditions in the Northern Hemisphere to show that forcing and chilling temperatures (influenced by photoperiod) determine the onsets of xylem formation.

9.4.2 | Perspectives and conclusion

the 'Phenology of cambial activity and tree-ring formation' chapter has highlighted several knowledge gaps, thus we would like to propose some research avenues that could fill these gaps in the future.
The first promising research avenue is the development of the GloboXylo database in order to include more conifer species, but also angiosperm species and more climatic conditions for the different tree species. Collecting large dataset will allow us to explore the space-time variability in order to find global ecological trends. Moreover, this will allow us to develop, parametrize and test process-based models of wood formation phenology. And we showed that this is the best way to understand the influence of the environmental factors on wood formation phenology. We have submitted a new PhD thesis project on this subject in 2019 together with Nicolas Delpierre (Université Paris-Sud).
All studies on wood formation encounter the difficulty of correctly defining the beginning and the end of cambial activity. Indeed, if the beginning of xylem formation is clearly defined by the appearance of the first thickening xylem cells, the beginning of cambial activity is often determined using the number of cambial cells only, and we have shown that this criterion is poor. However, the resumption of cambial activity is the first event in the wood formation phenological chain, and therefore probably the most influential. It is therefore important to find reliable anatomical criteria to define when the cambium is active, or when the cambial divisions resume, and to document these new critical dates for the greatest number of species and situations.
This work on better defining cambial activity resumption would benefit from a work on the dynamics of phloem formation. Phloem formation represent the other side of the coin, and is even more important for the functioning of a tree, than xylem formation. However, it is still often discarded because observations are still challenging. However, Prislan et al. {2019}, showed that the formation of the phloem can be describe very accurately for beech using a combination of light

microscopy as well as scanning and transition electron microscopy. It would be very interesting to develop such a work on Hesse site, for example. This could be the subject of another PhD thesis project, in collaboration with Daniel Epron (Lorraine University).

9.5 | Seasonal dynamics of tree-ring formation

9.5.1 | Synthesis

In the 'Seasonal dynamics of tree-ring formation' chapter, we presented our approach to describe and quantify accurately tree-ring formation dynamics using the CAVIAR R package.

Then, we explored the variability of tree-ring formation dynamics according to tree age, size and social status, tree species, and site conditions. We have discovered two distinct strategies concerning the dynamics of wood formation: the 'intensive' strategy, in which trees grow quickly but not long; and the 'extensive' strategy, in which trees grow long but slowly. We have also shown that trees adapt to high elevation by increasing their number of cambial cells to compensate for the increase in cell cycle length caused by lower temperatures.

We also used the large GloboXylo data base to show that the rate of xylem cell production by the cambium is the main contributor to tree-ring width and wood production, while the duration of the growing season only played a secondary role. We have confirmed the major influence of temperatures on the duration of wood production, while the rate is rather influenced by microsite conditions, stand dynamics and tree characteristics.

We have confirmed that recent global warming could result in extended period of growth explaining enhanced forest growth. However, we have also showed that the rate of growth, along with species behaviour and site conditions must be considered into vegetation models to assess the impact of future climatic changes on forest productivity.

9.5.2 | Perspectives and conclusion

In the near future we would like to include more arid sites in the GloboXylo data base and complete our work on the 'Ecological trends and life strategies in tree-ring formation dynamics'.

In a second time we would like to integrate the generalized additive model approach in CAVIAR to describe wood formation dynamics. This will allow to work on more extreme sites or years and provide more accurate estimates of timings and rate of wood formation dynamics.

Working with more accurate rates will allow to launch a new analysis of the GloboXylo data base to check the synchronisation between the maximal rate of xylem cell production and the summer solstice, and look for the influence of other potentially important climatic factors (e.g., soil water content, temperature).

9.6 | Kinetics of tracheid differentiation in conifers

9.6.1 | Synthesis

In the 'Kinetics of tracheid differentiation in conifers' chapter, we presented our approach to describe and quantify accurately the kinetics of xylem cell

differentiation using a conceptual model of wood formation dynamics and generalized additive models.
We have provided novel quantifications of wood formation kinetics that explain the development of the typical, widely observed, conifer tree-ring structure. Importantly, we refute the long-lasting assumption that the increase in wall thickness and wood density along the ring is driven by the fixation of more biomass during the wall-thickening process. Instead, we demonstrated that the amount of wall material per cell is almost constant along a ring. We have also shown that the duration of cell expansion decreases along the ring (from about 3 to 1 weeks), while the duration of cell-wall deposition increase (from about 1 to 2 months), and the rate of cell-wall deposition decrease. Consequently, we have demonstrated that changes in cell-wall thickness and wood density are principally driven by changes in cell size. Moreover, we have estimated that the duration of cell enlargement contributes to 75 % of the changes in cell size, whereas the rate only contributes to the other 25 %.
We explored the relationship between tracheid differentiation kinetics and environmental factors to show that temperature and water stress have significant influences on the rate of cell expansion and cell wall deposition. However, we have shown that these influences only remain visible in the very last latewood cells of a tree-ring.
We have also shown how couplings between the rate and the duration of cell differentiation kinetics allow trees to adjust the xylogenesis process to the changing environmental conditions to produce typical conifer tree-ring structure. Moreover, we have also shown how compensatory mechanisms allow trees to adapt to cold temperature at high elevation, or to water stress in dry conditions.
Finally, in the last section of this chapter, we presented the XyDyS modeling framework, which was developed to simulate tracheid differentiation kinetics and resulting tree-ring structure. This approach allowed us to refute the classical theory of the morphogenetic gradient. At least two distinct biochemical signals (e.g., auxin and cytokine or TDIF) must provide concentration gradients to organise cell division in the cambium and cell differentiation in the developing xylem, and thus form realistic conifer tree-ring structure.
The kinetic model presented here has been established on four major European conifer species, whose typical tree-ring structure is representative of most conifers. We believe that the conceptual framework provided by this model can be applied to many species and other environments. However, further investigations are needed to understand how the relationships between cell development kinetics and wood anatomy are modified for conifer species with narrow latewood (e.g., Pinus cembra, Juniperus species) or rings formed under stressful conditions (e.g. xeric site, drought year, insect attacks, late frosts) and typically associated with wood anatomical anomalies (e.g. light latewood, false rings).

9.6.2 | Perspectives and conclusion

We started to develop our conceptual model and the associated GAM approach since 2012, and published the methods and the first results in 2013. Since this time we developed the method further and applied it to other species (e.g. *Picea Marianna*, *Larix decidua*). This method starts to be applied now by other teams,

but not always correctly. Thus, integrating the computation of tracheid differentiation kinetics into CAVIAR may help the diffusion of this approach.
Integrating the kinetic approach into CAVIAR will also allow to explore large data base (GloboXylo), looking for global patterns and ecological trends in xylem cell differentiation.
Up to now the kinetic approach has been restricted to conifer tracheids, a grand challenge of the coming years is to apply it to angiosperm tracheids, vessels and parenchyma cells, from the less problematic diffuse porous species to the more difficult ring porous species. Also, this approach may be applied to the differentiation of phloem cells, for conifer species first, and then for angiosperm species.
Another research line we started to develop in collaboration with colleagues from SCION (New Zealand) is a better description of the kinetics of the lignification process using confocal microscopic analysis.

9.7 | Intra-annual dynamics of woody carbon sequestration

9.7.1 | Synthesis

Wood is the main terrestrial biotic reservoir for long-term carbon sequestration, and its formation in trees consumes around 15% of anthropogenic carbon dioxide emissions each year. However, the seasonal dynamics of woody biomass production cannot be quantified from eddy covariance or satellite observations. As such, our understanding of this key carbon cycle component, and its sensitivity to climate, remains limited.
In the 'Intra-annual dynamics of woody carbon sequestration' chapter, we presented our approach to describe and quantify accurately the intra-annual dynamics of carbon sequestration into tree stem.
We have shown that stem woody biomass production lags behind stem-girth increase by over 1 month. We also analysed more general phenological observations of xylem tissue formation in Northern Hemisphere forests and find similar time lags in boreal, temperate, subalpine and Mediterranean forests. These time lags question the extension of the equivalence between stem size increase and woody biomass production to intra-annual time scales. They also suggest that these two growth processes exhibit differential sensitivities to local environmental conditions. Indeed, in the well-watered French sites the seasonal dynamics of stem-girth increase matched the photoperiod cycle, whereas those of woody biomass production closely followed the seasonal course of temperature. We discussed that forecasted changes in the annual cycle of climatic factors may shift the phase timing of stem size increase and woody biomass production in the future.
Finally, this work has help to better understand the seasonal balance of terrestrial carbon, providing insights and methods to link forest–atmosphere exchanges and woody carbon sequestration at intra-seasonal time scales. Modelling the dynamics of the processes that drive carbon fluxes in different ecosystem components is a key issue, especially for assessing how climate change impacts these ecosystems. Our detailed mechanistic representation of when and how carbon is sequestered into the wood during the growing season provides crucial information on a major

carbon flux and storage component of forest ecosystems. Such information is essential for further developing the process-based biosphere models to better constrain modern carbon budgets and to predict future carbon–climate interactions.

9.7.2 | Perspectives

This work opened a new research avenue, which Anjy Andriantenaina's PhD thesis have followed (see following section for more information).

9.8 | Frontiers in tree-ring formation dynamics

9.8.1 | Intra-annual dynamics of carbon fluxes in trees — The WoodCap project

Synthesis

Plant sequester nearly half of the 120 Pg of carbon captured annually by photosynthesis, contributing to uptake 15 % of anthropogenic CO2 emissions. Most of the carbon is sequestered into the wood, which represents about 80 % of the living terrestrial biomass. The biomass accumulation, in northern hemisphere woody plants, closely matches the seasonal cycles, with modifications that can strongly impact on the global carbon cycle. However, the seasonal dynamics of woody carbon sequestration remains poorly quantified, limiting our understanding of the terrestrial carbon cycle, and its sensitivity to on-going and future climate changes.

In order to better understand the process of carbon sequestration into stem wood, along with its environmental drivers, we propose to quantify the dynamics of the carbon flux, from its initial capture by the leaves, to its final sequestration into the wood. The aim of WoodCap project is to accurately assess the seasonal dynamics of gross primary productivity, non-structural carbohydrates, tree radial growth, and woody carbon sequestration. In addition, this work will allow us to explore the complex relationships between the seasonal dynamics of carbon pools and fluxes, and the course of the environmental factors.

Gross primary productivity (GPP), non-structural carbon (NSC) concentration, xylem radial increment (XRI), and woody biomass production (WBP) presented contrasted dynamics along the monitored vegetation period for beech in Hesse forest. Moreover, GPP, NSC, XRI, and WBP dynamics were not influenced by the same environmental factors: solar radiation was the main environmental factor influencing GPP, while day length was the main environmental factor influencing XRI, and temperature WBP. NSC didn't respond to any environmental factors. The coordination between GPP, NSC, XRI, and WBP seasonal dynamics were related to the tree-ring structure and wood density, but not to the site fertility or tree vitality.

Perspectives and conclusion

These encouraging results underline the importance to better take into account the variability of existing tree-ring structures, and to monitor more accurately NSC

dynamics. These points will be tackled in our future works, by including oaks and spruce, and increasing sampling frequency for NSC monitoring.
We showed in this work that GPP, NSC, XRI, and WBP presented contrasted dynamics all along the growing season, and were influenced by different environmental factors and internal drivers. Our results suggest that in the complex pathway of the carbon in the tree, from the leaves to the wood, NSC dynamics, although poorly known, occupy a "strategic junction", orchestrating up- and down-stream processes.

9.8.2 | Intra-annual dynamics of water conduction in stem wood — The WatFlux project

Plants adjust leaf water potential and hydraulic conductance when confronted with drought, reducing sap-flow and optimizing water loss relative to carbon gain, in turn protecting their tissues from important water loss. Xylem hydraulic conductivity highly depends on the size and number of conduits per unit area. Therefore, even though the rate of water flux through and from the tree is largely determined by transpiration rates, it is also constrained by the xylem's anatomical features.
Xylem formation results from a sequence of cambium division and cell differentiation processes common to both angiosperms and gymnosperms, but that derives in different xylem structures with distinct hydraulic properties. Environmental conditions influence the timing, duration and rates of xylogenesis processes, which in turn affect vessel and tracheid size.
The aim of WatFlux project is to better understand the intra-annual dynamics of water fluxes between the canopy and the atmosphere in relation with xylem development and climatic conditions.
Preliminary results have shown that both beech and spruce experienced an increase in the theoretical xylem hydraulic conductivity (kh) along the year as new conduits were formed and remained potentially hydraulically active all along the growing season. Conversely, oak reached its maximum kh in mid-July before experiencing a significant decrease in conductivity at the end of the growing season, due to the blockage of earlywood vessels by thyloses. Moreover, beech canopy transpiration (Ec) and sap flow started in mid-April, thus relying on previous-year vessels for water transport, and peaked in June, before maximum current-year ring kh was achieved. Maximum current-year kh was reached in late-August-September, two months after transpiration rates peaked and when canopy transpiration needs were low. The inter-annual variation in maximum and mean kh and Ec were also uncorrelated. This uncoupling between vessel formation and transpiration dynamics may therefore indicate that xylogenesis processes do not respond to current-year canopy transpiration needs.
The results of this project will provide new insights into the biological processes governing the carbon and water cycle of forest ecosystems, improving our understanding of its responses to climatic variability at short (growth and transpiration dynamics), medium (legacy effects), and long term (forest functioning).

9.8.3 | Understanding the creation of carbon and oxygen isotopic signals in tree-ring archives — The WoodIsotop project

Synthesis

Multiproxy tree-ring studies have played a major role in determining climate impacts on forests worldwide. Such records are paramount for past climate reconstructions as well as for the assessment of tree responses to future climate scenarios. Those studies rely on the fact that tree rings produced in climates with enough seasonal contrast can be univocally assigned to discrete time intervals (years) thanks to the fact that growing seasons interspersed with dormancy periods provokes anatomically distinguishable boundaries in wood.

In the last decades, there has been a proliferation of tree-ring studies dealing with intra-ring characteristics at the sub-annual scale by using radial increment, wood density, wood cell morphology, or stable isotope data. Sub-annual information archived in already mature wood is dated through a space-for-time conversion based on the cell row position, or instead on contingent/arbitrary anatomical features. Massive correlative analyses between intra-ring parameters and monthly climate data may ultimately serve to set a timescale to the tree ring by inferring a feasible time interval at which cell growth events occurred.

That approach requires splitting the ring into wood sections that are assumed to be formed in successive discrete time intervals (as it is done for tree rings). However, the fact that differentiation in a cell row overlaps that in neighboring ones hinders assignation of time intervals to intra-ring sections. Moreover, the number of forming cells at a given day varies according to changes in cell division rates and duration of cell differentiation. Hence, the length of the time window at which environmental conditions can affect individual cell development, as well as the amount of tissue affected by a given event, greatly changes along the growing season. In addition, possible differential environmental effects on simultaneously forming cells according to their respective developmental stage or position are largely unknown.

Such a complex correspondence between cell age/lifespan and position blur the signal recorded in each isolated ring section, which is concurrent with other physiological constraints lagging tree growth responses to the environment. This somewhat jeopardizes reliability of tree-ring archives when used to infer processes at the sub-annual scale. Deciphering high-resolution information stored in tree rings therefore requires consideration of environmentally driven changes in cell differentiation kinetics, which cannot be assessed once the ring is completely formed.

We advocate for a more systematic characterization of wood cell differentiation kinetics covering more species and longer periods and for implementing this knowledge on tree-ring analyses dealing with the sub-annual scale. This would contribute to find a more accurate correspondence between either cell position or recognizable anatomical features and time across species and environmental conditions.

The WoodIsotop project aims to advance our knowledge of the interactions between environmental factors, developmental constraints, and physiological processes

involved in wood formation. Our investigations focus on a better understanding of the mechanisms by which seasonal climate variations, and extreme events, leave permanent marks in tree rings. In particular, we focus on identifying (i) environmental factors influencing the intra-annual dynamics of wood formation and the characteristics of the rings formed (i.e. micro-densitometric profile, anatomical characteristics of the wood, and intra-ring variations in the carbon isotopes composition); (ii) understanding the mechanisms involved; and (iii) their time coordination.
By connecting causes (environmental and developmental conditions) and consequences (growth ring characteristics and isotopic signature of wood) through a mechanistic understanding of the physiological processes involved in wood formation, the WoodIsotop project will not only enhance the reliability of dendrochronological variables used for climate reconstruction, but will also reduce uncertainties in future assessments of the impact of climate change on tree growth, forest ecosystem functioning, and the quantification of fluxes exchanged between the terrestrial biomass and the atmosphere. At present, the preliminary results obtained concern: (1) the allocation of a time window to the carbon isotopes composition in the tree ring; (2) the highlighting of the temporal "overlap" between the ring sections; (3) the evaluation of within-stand synchronicity of xylogenesis processes.

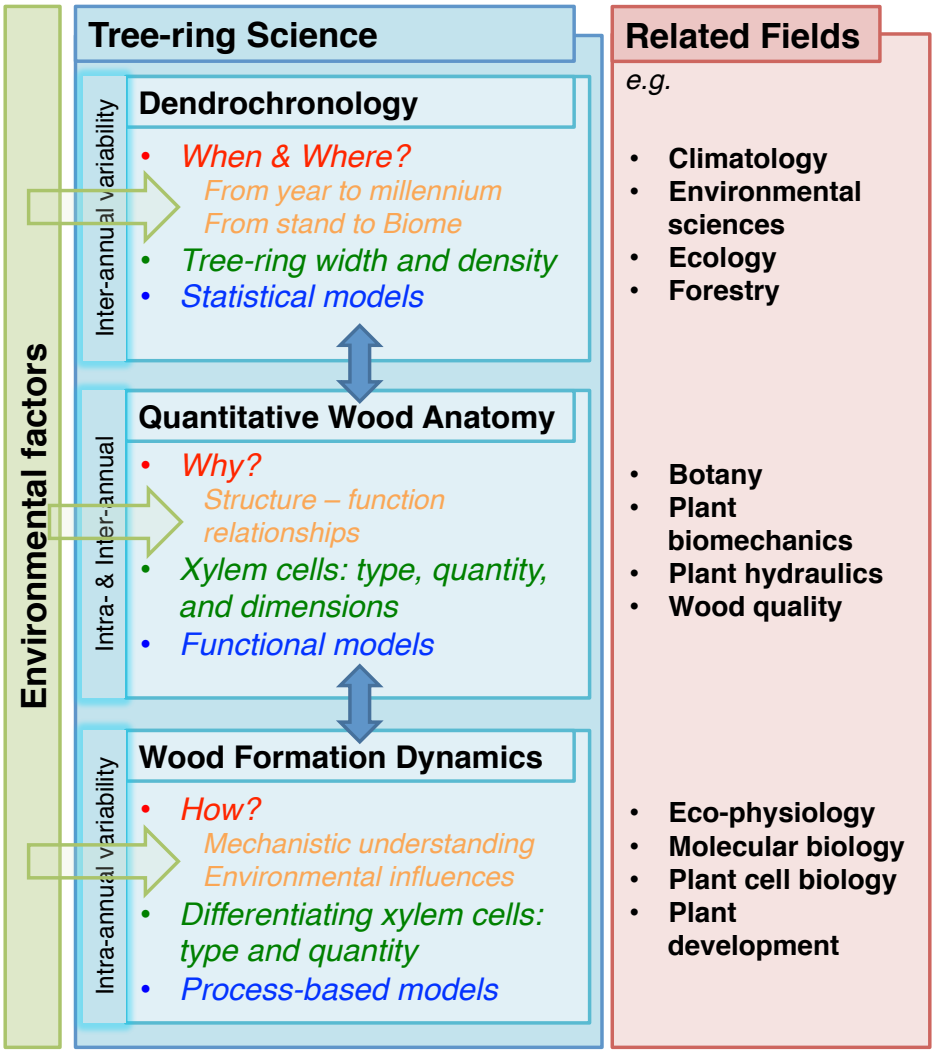
Perspectives and conclusion

Despite many methodological difficulties inherent to the wood formation monitoring technique, we were able to improve our statistical model of xylogenesis dynamics sufficiently to assign a precise time window to each section of wood cut within the ring. This allowed us to show that the sections of wood thus delimited, although completely spatially separated, actually "overlapped" largely from a temporal point of view. Therefore, these sections cannot provide independent climatic information, unlike what is regularly proposed in related scientific publications.
At present, the results obtained for fir in the Vosges Mountains using the intra-annual variations of δ13C and micro-densitometric profiles are quite disappointing from a dendroecological point of view, but we are waiting for the final results to conclude. The second part of the project, which will focus on the variations of δ18O in larch rings along the altitudinal gradient of the Lötschental, is likely to provide more salient results.

9.9 | Towards and integrated approach to tree-ring sciences and wood quality

Tree radial growth, wood density and xylem anatomy reflect tree strategies to best allocate — in the time course of the growing season, and year after year — the available resources under changing environmental conditions. Therefore, tree-ring structure radial variability provides crucial information, first on tree responses to growing conditions, and second on the environment itself. I believe that combining these three sources of information at large spatial and temporal scales, and

reconciling quantitative wood anatomy and dendrochronology, will open new research areas for tree-ring sciences.
The next grand challenge I foresee will be to incorporate the knowledge recently gained by wood-formation-monitoring studies into this framework [Fig. 9.1]; this will really open the door for a mechanistic approach in tree-ring sciences, and will allow the combination of tree short-, medium- and long-term responses to the environment, which is pivotal for reconstructing past environmental conditions, as well as for forecasting the future impact of climate changes.



81 — Figure 9.1 | A novel extended framework for tree-ring science.
Figure from Rathgeber {2017}.

9.10 | Conclusion

Wood, the most abundant biological compound on earth, is of crucial importance for Humankind and Biosphere. Although tree rings and the process of xylogenesis that creates them have long been under the microscope of biologists, it is only recently that the invention of new techniques has made it possible to monitor the intra-annual dynamics of xylem formation by the cambium, along with the environmental conditions that influence it.
In most regions of the world cambial activity follows a seasonal cycle. At the beginning of the growing season, when temperatures increase, the cambium

resumes activity. During the growing season, new xylem cells produced by the cambium are undergoing profound transformations, passing through successive differentiation stages, which will enable them to perform their future functions in trees. Finally, at the end of the growing season, when climatic conditions deteriorate, cambial activity stops, soon followed by the cessation of all the differentiation processes.

In this manuscript, after reviewing the biological basis of wood formation and describing the methods that allow its monitoring, we investigate and model the relationships between cambium phenology, tree-ring formation dynamics, tracheid differentiation kinetics, and the climatic factors. The results obtained are used to describe the coordination between stem growth in size and in biomass, as well as the relationships between the source (the canopy) and the main sink (the cambium) of carbon in trees.

Finally, three promising research avenues are presented, exploring the relationships between: (1) the intra-annual dynamics of woody biomass production and gross primary productivity; (2) the intra-annual dynamics of water conduction in xylem and stem sap flow and stand evapotranspiration; (3) the kinetics of tracheid differentiation and the creation of the isotopic signals in tree-ring archives.

In the context of accelerating global changes, it is crucial to investigate what is ruling tree-ring formation and wood production, in order to better evaluate how the ongoing modifications of the environmental factors are impacting trees, biogeochemical cycles, human societies, and ultimately the climate itself.