Dissertationes Forestales 229

Dynamic variations in bark hydraulics – understanding whole tree processes and its linkage to bark hydraulic function and structure

Tommy Chan

Department of Forest Sciences
Faculty of Agriculture and Forestry
University of Helsinki
Helsinki, Finland

Academic Dissertation

To be presented, with the permission of the Faculty of Agriculture and Forestry of the University of Helsinki, for public criticism in auditorium 1 of Infokeskus Korona (Viikinkaari 11), on November 4th, 2016 at 12 o'clock noon.

Title of dissertation: Dynamic variations in bark hydraulics – understanding whole tree processes and its linkage to bark hydraulic function and structure

Author: Tommy Chan

Dissertationes Forestales 229

http://dx.doi.org/10.14214/df.229

Thesis supervisors:
Professor Eero Nikinmaa
Department of Forest Sciences, University of Helsinki

Assistant Professor Teemu Hölttä
Department of Forest Sciences, University of Helsinki

University Lecturer Frank Berninger
Department of Forest Sciences, University of Helsinki

Pre-examiners:
Senior Lecturer Remko Duursma
Hawkesbury Institute for the Environment, University of Western Sydney

Docent Ari Pekka Mähönen
Department of Biosciences, University of Helsinki

Opponent:
Professor Roderick Dewar
Research School of Biology, The Australian National University

ISSN 1795-7389 (online)
ISBN 978-951-651-548-2 (pdf)

ISSN 2323-9220 (print)
ISBN 978-951-651-549-9 (paperback)

Publishers:
Finnish Society of Forest Science
Natural Resources Institute Finland
Faculty of Agriculture and Forestry of the University of Helsinki
School of Forest Sciences of the University of Eastern Finland

Editorial Office:
Finnish Society of Forest Science
P.O. Box 18 FI-01301 Vantaa, Finland
http://www.metla.fi/dissertationes

Chan T. (2016). Dynamic variations in bark hydraulics – understanding whole tree processes and its linkage to bark hydraulic function and structure. Dissertationes Forestales 229. 39 p. Available at http://dx.doi.org/10.14214/df.229

ABSTRACT

A mature tree stem generally consists of a column of wood that is composed of a series of annual incremental layers and enclosed in a covering of bark. The dynamic variations of the bark are complex due to its structure and function: the thick outer-bark acts as a protective barrier against the abiotic and biotic environment; while the phloem is where sugar transport occurs. Much of the bark variation is due to the transport of sugars and its related processes. The driving force for sugar transport in the phloem is generated by the accumulation of sugars at source sites (e.g. leaves), which creates differences in gradients in turgor pressure along the stem. As a result, mass flow occurs – transporting sugars to sink regions that require it (e.g. stem and roots) for active growth, respiration and storage. The xylem pathway, which transports water in the opposite direction, is connected to the phloem in parallel along the entire length of the stem. The immediate connection between these two transport pathways suggests a functional linkage, as the phloem draws water from the xylem in order for mass flow to occur. The dynamic interactions between the xylem and phloem, and the processes occurring within the bark have great implications for whole tree physiology.

The purpose of this thesis is to study the dynamic processes that occur within the bark and its interaction with other internal tree processes and the external environment. This is accomplished by first understanding the bark hydraulic architecture and its linkage to the environment, followed by its linkage to various tree processes. These linkages have not been thoroughly quantified, especially on an intra-annual (e.g. daily) scale. The study of bark hydraulic dynamics is of great interest because it is a relatively new topic with great potential. The changes of the bark in response to the environment may play a large part as a regulator to other tree processes.

The thesis consists of four papers, of which one is a modelling paper and three are experimental (field and laboratory) studies. The model estimates growth by using dendrometer measurements as inputs for the model. Growth is estimated by separating the water-related influences from measured inner-bark, revealing a growth signal – proxy for cambial stem growth. Using this growth signal, a correlation study to microclimate variables is examined in one paper; and to assumed growth respiration in a second paper. The remaining two papers explore the seasonality of photosynthesis and respiration, and bark stem dynamics during the winter recovery period in the spring.

As a conclusion of this thesis, these four papers show how inextricably linked, the individual tree processes are to the changes within the bark, due to the tight coupling with sap flow-related changes of the xylem. The culmination of this thesis opens new opportunities to further understand the dynamics of bark hydraulics and ecophysiological processes by implementing field measurements and state-of-the-art modelling.

Keywords: xylem, phloem, growth, respiration, photosynthesis, spring recovery

Acknowledgements

My doctoral studies began shortly after obtaining my MSc. with the University of Helsinki. Initially I was employed as a researcher led by Eija Juurola, and soon thereafter, attached to the DECADE project working under Eero Nikinmaa – and with it, a possibility to continue with research as a doctoral student. The transition from environmental science during my BA studies, to tropical silviculture during my MSc. studies and finally to tree ecophysiology for my PhD studies has been challenging and took an adjustment period to grasp the different scientific concepts specific to each field. However, the journey has been unforgettable and rewarding, and I am deeply indebted to our research group to have adopted me with open arms since day one.

I would like to thank foremost, Eero Nikinmaa for taking me under his wing and continually instilling faith in me as a researcher. His mentoring has given me inspiration and the drive for quality research. I am grateful to Jaana Bäck and Eija Juurola for supporting me not only on my doctoral studies, but as a future step towards a career in forest sciences. I thank Teemu Hölttä for his patience and supervision. His jovial demeanor and effervescent enthusiasm has given me confidence as a researcher. I also thank Frank Berninger and Pasi Kolari for the guidance and constantly challenging me to think outside the box. To all my peers and colleagues in our group, I also thank you all for sharing your research and personal anecdotes about life and science. It has been absolutely delightful to work with passionate and hardworking people towards a greater good. Going through the motions during these times – that is, experiencing the highs and lows as a doctoral student is a great challenge in and of itself; and the support, motivation and understanding that we all shared has certainly made my journey more reposed.

To my co-authors, I also thank you for your insightful comments and making me a better scientific writer. I extend my thanks to Raisa Mäkipää and Takayoshi Koike for allowing me to research at Hokkaido University, Japan. The opportunity to work with another group and the exchange of knowledge has been extremely rewarding. Notwithstanding, I thank the Academy of Finland and Centre of Excellence for the financial support.

Finally, I would like to thank my family and friends for the unconditional support and understanding over the years. To Aili, my wife, who has been with me since the very beginning: you have been the indelible calm when tumultuous waters roar and heave – a kindred spirit stronger than the sea's embrace. Over the years, you have made me a better person – and with our newborn daughter Elisabet, a better father.

Helsinki, October 2016

Tommy Chan

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, which are referred to in the text by the Roman numerals I–IV. The publications are reprinted here with the kind permission of the publishers.

I Chan T., Hölttä T., Berninger F., Mäkinen H., Nöjd P., Mencuccini M. and Nikinmaa E., (2016). Separating water-potential induced swelling and shrinking from measured radial stem variations reveals a cambial growth and osmotic concentration signal. Plant, cell & environment, 39(2), pp.233-244. http://dx.doi.org/10.1111/pce.12541

II Chan T., Berninger F., Kolari P., Nikinmaa E. and Hölttä T., (2016). Linking stem growth respiration to the seasonal course of stem growth and GPP of Scots pine. Manuscript.

III Kolari P., Chan T., Porcar-Castell A., Bäck J., Nikinmaa E. and Juurola E., (2014). Field and controlled environment measurements show strong seasonal acclimation in photosynthesis and respiration potential in boreal Scots pine. Frontiers in plant science, 5. http://dx.doi.org/10.3389/fpls.2014.00717

IV Vanhatalo A., Chan T., Aalto J., Korhonen J.F., Kolari P., Hölttä T., Nikinmaa E. and Bäck J., (2015). Tree water relations can trigger monoterpene emissions from Scots pine stems during spring recovery. Biogeosciences, 12(18), pp.5353-5363. http://dx.doi.org/10.5194/bg-12-5353-2015

Author's contribution:

In **Study I**, the author contributed in the design of the study. He performed the modelling work, analysed and interpreted the results. He wrote the article together with the co-authors. In **Study II**, the author contributed in the design of the study. He performed the modelling work and analysed and interpreted the results. He wrote the article together with the co-authors. In **Study III**, the author contributed in the design of the study. He performed the on-site field work and participated with the co-authors to analyse and interpret the results. He wrote the article together with the co-authors. In **Study IV**, the author contributed in the design of the study. He helped analyse the data and interpreted results. He wrote the article together with the co-authors.

TABLE OF CONTENTS

1. INTRODUCTION

The stem of trees serve many purposes: to support the crown; to store water, carbohydrates and minerals; transport sugars and hormones from points of synthesis to areas that require them for growth or storage; and to conduct water and minerals upwards from the roots (Pallardy 2010). The bark, located to the outside of the stem, does not only protect the tree from external agents; but is an essential part of the vascular system, responsible for translocating organic nutrients and defensive and signaling compounds (van Bel 2003a; De Schepper et al. 2013b). These organic nutrients are soluble organic material (i.e. sugars) made during photosynthesis and transported away from assimilatory regions (source locations) to regions that require these sugars for metabolism and growth (sink locations). The bark provides the pathway for assimilate transport from source to sink. It is essential that this pathway is maintained and continuous because without sufficient transport capacity, the assimilate storage capacity at the source will eventually become saturated (Hölttä et al. 2014). A saturation of sugar and starch at these sources can lead to whole-tree stress and adversely alter its physiological state, which can become permanent if the cause is not alleviated. When stressed, down-regulation of photosynthesis, carbon starvation, decrease of cell turgor and reduction of osmotic refilling of embolized vessels may occur. These have been observed in response to drought, phloem girdling and under elevated CO_2 conditions (Bel and Gamalei 1992; Franck et al. 2006; Nardini et al. 2011). Although the term “bark hydraulics” is frequently associated solely within the context of translocation of assimilate (i.e. sugars) from source to sink locations, there is an emergent acceptance that the bark also regulates an array of important whole-tree processes (Woodruff 2014).

Considering bark as a single entity can obscure important aspects of its function and structure. Structurally, the bark is made up of complex tissues located outwards from the vascular cambium, and it includes both living and dead cells produced from the phellogen and vascular cambium (Figure 1). The vascular cambium develops inwardly xylem tissue (towards the pith) and outwardly, phloem tissue (or inner-bark). This development is responsible for circumferential stem increment. Meanwhile, the phellogen is responsible for the development of the periderm, i.e. outer-bark. Cells that grow inwards from the phellogen make up the phelloderm, and cells that develop outwards are called phellem. The phelloderm separates the inner-bark from the outer-bark, while the phellem protects the tree from external agents (e.g. fire, disease, environmental extremes and foraging).

For gymnosperms (e.g. *Pinus Sylvestris* L., *Picea abies*), it is within the phloem that translocation of sugars from source tissues (photosynthetic leaf cells) to sink tissues (stems and roots) occur. The phloem is comprised of living cells, called sieve elements (Figure 2) and are the conduits for transport of phloem sap (Fisher 1978; Blechschmidt-Schneider 1990; Franceschi and Tarlyn 2002). Sieve elements are comprised of elongated sieve tube pathways and adjoining sieve cells, which function together to translocate sugars to different regions of the tree. Sieve pathways have pores located at each end and are interconnected end-to-end to form a longitudinal network from the leaves to roots. Since sieve pathways lack the capacity for protein synthesis, physiological functions are maintained by the sieve cells. These cells aid sieve pathways by providing energy as ATP, and can even perform the metabolic functions that are surrendered during differentiation of sieve elements (Bostwick et al. 1992; Lucas et al. 2013). It is important to note that the bark anatomy of angiosperms (e.g. *Betula pendula*) differs from gymnosperms. For example, companion cells are present within the phloem of angiosperms, where these cells maintain sieve elements.

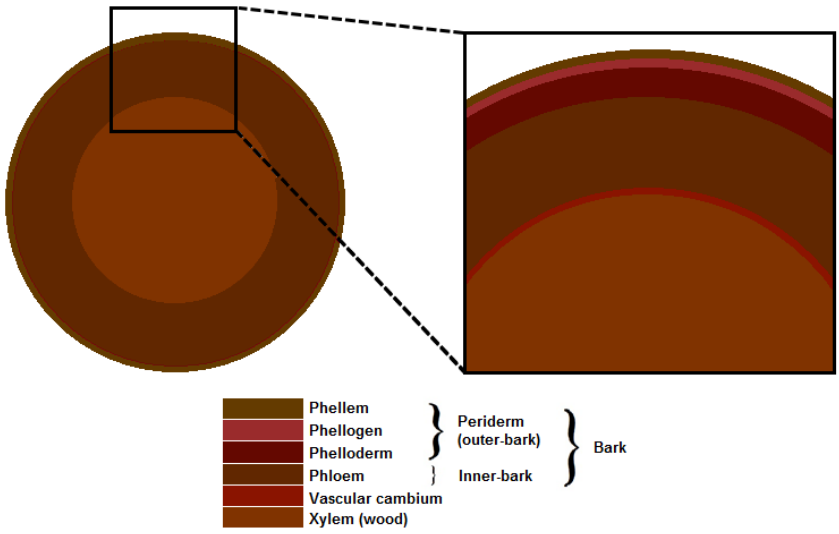

Figure 1. Cross-section of a stem showing the various tissues.

1.1 Pressure-Flow Hypothesis

The pressure-flow hypothesis (or Münch theory) explains how products of photosynthesis from leaves (e.g. sugars and starch) are translocated to regions (e.g. stem and roots) that require it for maintenance and growth. This theory suggests that mass flow of sugars through sieve elements is driven by differences in turgor pressure gradients between source and sink locations, with sap flowing from high to low pressure (Münch 1930; Windt et al. 2006; Knoblauch et al. 2016). At source regions, sugars produced in leaf mesophyll cells are loaded into sieve pathways from sieve cells. This transport process requires energy (molecular transporters that pump sugars from cell-to-cell). As sugars are loaded, the solute concentration increases, thereby drawing water (from the xylem) into the phloem. As water enters, the turgor pressure increases and water-sugar solution flows along the phloem tract, unloading at sink regions that require sugar. At these regions, the unloading of sugars from sieve elements reverses water potentials – water flows into the xylem and the turgor pressure decreases relative to that of sieve elements in source regions. The translocation of sugars from source to sink is a passive, symplastic process; thus no energy input is required. In terms of whole-tree allocation, sugars would tend towards highly metabolized active sink organs, as the local consumption of sugars should cause the turgor pressure within the unloading phloem to decrease, thereby increasing the pressure differential between the source region and sink (Ham and Lucas 2013). Because of this, phloem sap has been observed to have unidirectional flow (Fetene et al. 1997).

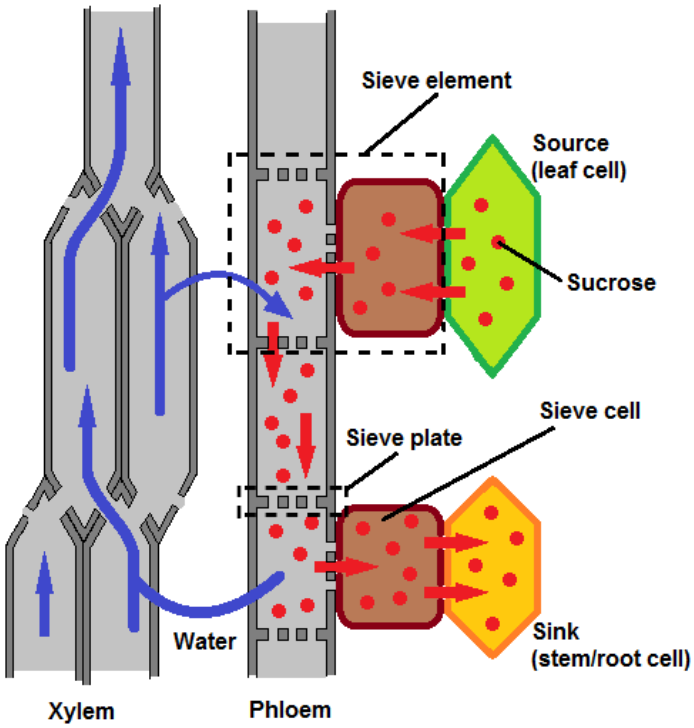

Figure 2. A schematic drawing of the pressure-flow hypothesis of sugar transport in the phloem of gynmosperms.

1.2 Sink compartmentation and sink allocation

Sugars that have been unloaded into sinks are primarily invested in competing compartmentation activities, such as for cell growth and cell maintenance, or placed into storage pools (Patrick 1997). Trees may also reserve sugars for defense, such as increasing shade tolerance (Chapin et al. 1990; Imaji and Seiwa 2010). Sugar allocation to these compartments vary over the course of cell development and over shorter time scales depending on the tree's physiological state (Dickson 1989; Chapin et al. 1990). Sugars allocated for growth either become substrates for synthesis of new structural biomass or undergo catabolism in metabolic pathways that support growth respiration growth pools. These growth pools are only stored temporarily, and will be used locally for growth synthesis. Sugars allocated towards maintenance respire to provide the energy required to maintain cell function and structure. Sugars directed to storage sinks will ultimately be translocated to other regions (or temporarily stored) to support growth and storage processes. These sugars can stay in storage lasting from a few hours to months, or even years (Lewis et al. 2009; Sala et al. 2012). Long-term storage pools provide the substrate needed to ensure a constant and steady hydraulic transport capacity throughout the tree (Schnyder 1993). Mature trees may also require large storage pools as a precaution, particularly in the event of severe stress; for example, drought, freeze-thaw events and

increasing tree height can impose additional constraints on the bark hydraulic system (Sala et al. 2012). In the short-term, sugars in storage sinks can regulate turgor gradients by acting as a buffer to changes in sugar loading from source regions. In addition, sugar accumulation can play an important role in regulating the water balance (e.g. when under stress) by the process of osmoregulation (Yakushiji et al. 1996).

1.3 Quantifying bark hydraulics

Quantifying bark hydraulics and its processes (e.g. translocation, allocation, sugar loading/unloading) is difficult as it is comprised of complex living tissue that readily responds to external forces. The phloem itself is a pressurized system and any disturbance may result in rupturing this pressure, altering its structure and function (Ehlers et al. 2000; Turgeon and Wolf 2009). It has only been in the last fifty years that non-intrusive studies were conducted to understand bark hydraulics. Tyree et al. (1974) were the first to mechanistically quantify the driving force (i.e. turgor pressure) required to drive the pressure-flow hypothesis using physical parameters that govern phloem hydraulic flow. In simple terms, the model quantified gradients of concentration and pressure at a specific point, and iteratively calculated adjacent points by multiplying their known rate of change with distance by a small interval of distance. The fundamental equation used in Tyree et al. (1974) and in many subsequent phloem transport models is the Hagen-Poiseuille law – a physical law that estimates the pressure change in a fluid flowing through a long cylindrical pipe. Precisely, the law states that the pressure (P; Pa) difference between the source and sink is proportional to the flow rate (J; m s^{-1}), which can be estimated as the solute concentration (c; mol m^{-3}) difference between source and sink:

$$J = \frac{\pi r^4}{8L\mu}(P_{source} - P_{sink}) = \frac{\pi r^4}{8L\pi} RT(c_{source} - c_{sink}), \quad (1)$$

where R is the gas constant, T is temperature, μ is the fluid viscosity and r and L are related to the conduit size; radius and length, respectively. More recent modelling efforts have addressed the limitations of long-distance phloem transport that plagued previously published models (Phillips and Dungan 1993). For example, Tyree et al. (1974) revealed that sugar transport in trees greater than 50 m would take over 15 days; a contrary result to modern results of just days (Mencuccini and Hölttä 2010). Although these models are robust in maintaining the underlying theory behind the pressure-flow hypothesis, one large omission is the radial transfer of water between the xylem and phloem vascular systems. The force required for this radial transfer is due to the differences in water potential between the xylem and phloem (Pfautsch et al. 2015). Radial transfer has been observed in models from Ferrier and Christy (1975) and Daudet et al. (2002), but only extending to the effect of sinusoidal variation of xylem water potential on phloem translocation (Hölttä et al. 2006). The functional links between these two vascular systems need to be clearly understood and included in current models to better understand bark hydraulic processes in trees.

The phloem vascular system is hydraulically linked with the xylem vascular system – in which the xylem is responsible for carrying water from the roots to the crown of the tree (Hölttä et al. 2006; Hall and Minchin 2013). Water is pulled up the xylem conduits under tension that arises from the evaporation at the leaf surface (i.e. due to transpiration) or by capillary action (Denny 2012). Concomitantly, water is exchanged readily to and from the xylem into the phloem, as required for phloem translocation. As a water potential

equilibrium is the necessary end state between the two tissues for phloem transport (Thompson and Holbrook 2003), changes in water potential within the xylem can directly alter the efficacy of phloem sap delivery from source to sinks. It is also suggested that regulation of osmotic potentials in the phloem can impact water potential in the xylem (Hölttä et al. 2006; Hölttä et al. 2009). The interactions between the negative pressure (tension) in the xylem and osmotic strength in the phloem will ultimately affect its transport capacity, tissue growth and physiological processes (e.g. respiration and gas exchange). Hölttä et al. (2006) resolved the coupling between the xylem and phloem transport system by modelling the behaviour of these two systems simultaneously. The model incorporates by design, a simple representation of phloem transport by using measured variables such as transpiration, soil water tension and sugar loading and unloading rates. By including changes of water potential from the xylem, the results gave insight into the conditions under which radial water exchange, according to the pressure-flow hypothesis, works. Moreover, by altering input parameters to simulate explicit environmental conditions, the model tested the certainty of the pressure-flow hypothesis, and how it performed under variable environmental conditions (e.g. drought, elevated CO_2 conditions). The integration of xylem transport and water exchange into phloem transport models gave renewed interest in bark hydraulics (see studies from Lacointe and Minchin (2008), De Schepper and Steppe (2010) and Mencuccini et al. (2013) for successive xylem and phloem-coupled models).

For example, Mencuccini et al. (2013) introduced a simple approach to radial flow of water between the xylem and phloem. In general, the direction and rate of water movement (J, $m^3 s^{-1}$) between the xylem (P_x) and inner-bark (P_b) is:

$$J = LA(P_x - (P_b - \Pi)), \tag{2}$$

where L is the area-specific radial hydraulic conductance between xylem and inner-bark, A is the cross-sectional area through which water exchange occurs and Π is the osmotic pressure of the inner-bark. Radial water exchange only occurs when both the xylem and inner-bark are in water potential disequilibrium. By incorporating Eq. (2) with Hooke's Law, radial water exchange can observe the dimensional changes within the inner-bark (Perämäki et al. 2001). This novel modelling approach to understanding bark hydraulics in both vertical and horizontal dimensions has made it accessible to observe sugar transport, radial water exchange and radial growth dynamics.

1.4 Current research trends in Forest Sciences

The pressure-flow hypothesis has been the most widely accepted mechanism for phloem transport, and much of the current research is centred on this theory. While the core of the theory remains intact, there have been adaptations to explain the uncertainties that Münch did not originally realize. For example, the role that xylem plays in phloem transport has largely been omitted, and only recently has there been efforts to understand its importance in not only to maintain transport capacity but for whole-tree function. Further, Münch hypothesized that the transport pathway from source to sink is similar to an impermeable pipe – such that the regions of loading and unloading of sugars occur only at the sources and sinks, respectively. It has been demonstrated that sieve pathways are, in fact, permeable and loading and unloading may occur along the pathway (Thorpe et al. 2005). Hence, the phloem transport pathway functions not only as a sink endpoint, but also supports and receives carbohydrates at lateral sinks along the tree axis (van Bel 2003b; De Schepper et al. 2013a). There has been a lot of uncertainty surrounding the pressure-flow hypothesis in

respect to tall trees (due to large transport distances). Little is known, and much of the studies contradict one another about the variation in phloem conduit properties and flow conducting area with changing tree size and axial position (Hölttä et al. 2014). Studies have found that the phloem pressure gradient is constant (Windt et al. 2006; De Schepper et al. 2013a; Hölttä et al. 2013), but may also increase with tree height (Mencuccini and Hölttä 2010; Dannoura et al. 2011). The latter result contradicts Turgeon (2010), who suggested that phloem turgor pressure differences does not scale to tree size.

An alternative proposal to explain Münch's problem of long-distance travel is the relay hypothesis. It has been suggested that sieve pathways may be shorter than the whole-tree axial length, and the pathway is instead, comprised of a series of short, overlapping sieve pathways (Hölttä et al. 2009; Turgeon 2010; Hölttä et al. 2014). If such a case is plausible, the sieve pathways would not be symplastically connected along the whole transport length, but composed of specific apoplastic loading and unloading steps along the transport pathway, called relays (Lang 1979). The turgor gradient, speed and direction would then be controlled near the relays (Thompson and Holbrook 2003), and any changes of these properties would be in proportion to the number of relays (Hölttä et al. 2009; Hölttä et al. 2014). Although the original pressure-flow hypothesis supports the relay hypothesis, the problem lies in the phloem transport mechanisms. The relay hypothesis requires a complex set of transporters since the phloem sap is rich in organic molecules and ions (Turgeon 2010; De Schepper et al. 2013b). These transporters are specific only to apoplastic sap loading – a mechanism not recognized in the pressure-flow hypothesis. However, the relay hypothesis agrees with the permeable phloem pathway. There is currently no substantial evidence either for or against the existence of the relay hypothesis, and more studies are needed to understand how phloem transport works (Hölttä et al. 2014).

At the whole-tree level, changes in bark hydraulics due to external environmental conditions can impact the internal physiology of the tree. By studying the hydraulic response to certain conditions, it can lead to better predictive tools and understanding of growth and development cycles. Water, light availability and mineral nutrients, for example, affect the relative allocation of carbon to different regions of the tree. A decrease in water and nutrient inputs into the tree would have lower overall carbon found in shoots than in the roots, whereas low irradiance may induce an opposite response (Wilson 1988; Lacointe 2000). It is also suggested that phloem turgor collapse may occur in drought situations (Sevanto 2014). Recent studies have shown that changes within the hydraulic function of bark may also impact other internal processes. Nikinmaa et al. (2013) found that maintaining phloem transport capacity may be essential for photosynthesis; specifically, low phloem transport capacity increased viscosity build-up within the phloem, resulting in stomatal closure (and down-regulation of photosynthesis). This finding was further substantiated in a follow-up study from Nikinmaa et al. (2014), where they linked individual models of assimilation and transpiration (Mäkelä et al. 2006), xylem and phloem transport (Hölttä et al. 2006) and tree hydraulic architecture (Sievänen et al. 2008) into a comprehensive mechanistic analysis between whole-tree development and different physiological processes. This study emphasized the complexity of bark structure and function. It has only been recently that our current empirical knowledge on bark structure and function have been obtained from season-long field conditions on mature trees (Pfautsch et al. 2015). These factors alone may complicate or conflict with earlier studies under laboratory conditions or short-term measurements. The interactions between whole-tree bark processes (including growth) and its environment on an inter- and intra-annual scale remains a challenge and needs further development (Schiestl-Aalto et al. 2015). These time scales may provide significant understanding on tree-dominated ecosystems under different environmental conditions.

1.5 Motivation for the thesis and study aims

This thesis presents the interaction between bark hydraulic processes and the environmental regulation of carbohydrate production and growth on an inter- and intra-annual scale. The work was motivated by two factors: first, advances in research have paved way for new possibilities to understand the importance of bark hydraulics. The aforementioned processes can be further investigated due to the intimate linking with bark processes. Second, there is a lack of current understanding of how the bark functions as part of a whole-tree economy (Ryan and Asao 2014; Sevanto 2014). This holds especially true with studies pertaining to measured field data on mature trees. This is important because observing bark dynamics in unrestricted environments will allow us to explain uncertainties that theory nor controlled conditions may encounter; or in counterpoint, it can support these latter studies. The study will focus on non-destructive methods to analyze whole-tree bark hydraulic function and its processes on an ecologically important species in Finland, Scots pine (*Pinus Sylvestris* L.). The analysis was performed on 54-year old, 18 m. high mature trees. A unifying framework is created that links the dynamic changes of individual tree processes to the changes of the bark and stem – ultimately tying up the whole-tree context. The significance of the work lies in the relatively new concepts introduced that offers new possibilities to study growth processes and its linking to environment and tree processes. Much of the studies in this field have only been performed with theoretical models in controlled environments at short-measurement time scales. Only during the last decade have studies earnestly observed bark hydraulics in unconstrained conditions – as water stress, nutrient deficiencies and changes in irradiance add to the already complex topic.

There have been relatively few non-destructive techniques for measuring phloem flow and turgor. Pulse labelling of trees with isotopically enriched (or depleted) CO_2 and tracking the respiration stream is a method for studying the carbon transport rate in the phloem (Plain et al. 2009; Dannoura et al. 2011). Although it is a robust method, it is costly and the experimental design requires many technical details. A simpler technique that has potential is by measuring the changes in stem diameter (or radius) with dendrometers (Sevanto et al. 2003; Sevanto et al. 2011). When measured simultaneously over the xylem and inner-bark, dendrometers show great promise in differentiating changes between the xylem and phloem tissues (Sevanto et al. 2003; Sevanto et al. 2011; Mencuccini et al. 2013). Moreover, they are easier to maintain and readily available than other instruments to empirically measure changes along the stem. Stem diameter changes are influenced by both the reversible component due to water and carbon transport on one hand, and an irreversible component, which represents cambial growth (De Schepper and Steppe 2010). The water-related reversible component is caused by differences (or time lag) between leaf transpiration and root water uptake (Zweifel et al. 2005; Steppe et al. 2006; Zweifel et al. 2006), while the carbon-related reversible change is a result of movement of osmotic concentration from sources to sinks along the stem (Mencuccini et al. 2013). The growth component is due to dividing and enlarging wood and bark cells in the cambium (Zweifel 2016), and will only appear when sufficient water and sugars are available (De Schepper and Steppe 2010).

A variety of studies have used dendrometers as a tool to derive growth- and water content-related (of the xylem and phloem) changes (Sevanto et al. 2003; Zweifel et al. 2005; Deslauriers et al. 2007). In conjunction with modelling, dendrometers have supported the estimation of carbon transport, growth and water-related changes – specifically, how the hydraulic conductance between the xylem and phloem affects changes of the inner-bark

(Sevanto et al. 2011); comprehensive whole-tree transport (roots, leaves and stem) of carbon and water transport (De Schepper and Steppe 2010); and the inference of a phloem transport signal within the inner-bark (Mencuccini et al. 2013). These complex, multi-parameter approaches reveal the close hydraulic coupling between the xylem and the phloem and the processes linked to this coupling. Despite the confirmation of this theoretical framework, in-depth studies that link the signals within the bark to environmental variables are lacking. Mencuccini et al. (2013) were able to establish a relationship between gross primary production (i.e. entire photosynthetic production of organic compounds in a specific area) and sugar transport. However, additional research is needed to confirm these recent findings, and in addition, sugar compartmentation (i.e. sugar unloading processes) must be recognized. I further developed the model introduced in Mencuccini et al. (2013) by first, simplifying the model (to four parameters) to reveal a growth signal; and second, to link this signal to growth-driven environmental variables (**Study I**).

Another physiological compartment activity that occurs upon sugar unloading is aerobic respiration. Respiration is a chemical reaction that releases energy from glucose, and used to produce new organs and maintain existing ones. Respiration can be further separated into growth and maintenance respiration; with the former relating to the costs of building new biomass, and the latter to maintaining life processes of living cells (Stockfors and Linder 1998). It is clear that stem growth is correlated to stem growth respiration (Ryan 1990). Many studies have attempted to link these two by measurements from dendrometers (e.g. Stockfors and Linder (1998); Vose and Ryan (2002); Zha et al. (2004)). However, deriving growth from dendrometers has been difficult because of the tight hydraulic coupling between the xylem and phloem. In other words, measurements of radial stem growth are masked by changes due to water content, influenced by xylem water potential changes (Daudet et al. 2005; Mencuccini et al. 2013). In **Study II**, the model introduced in **Study I** was used to link daily stem growth rate to daily stem growth respiration, and observed at an intra- and inter-annual scale. In addition, it was compared to raw dendrometer measurements to further validate the model (**Study I**, **II**).

The inter- and intra-annual growth state was studied by assessing how photosynthetic and leaf respiration parameters observed in the field and near-optimal conditions are inter-connected (**Study III**). In this case, the growth state is the photosynthetic capacity or efficiency of a physiological process. This is important in the context of bark hydraulics since sugar production is required to maintain transport capacity and turgor (Nikinmaa et al. 2013), in addition for growth and for maintenance of existing biomass. By examining the intra-annual dynamics, we are able to understand: 1) how seasonality affects assimilate production; and 2) net carbon balance from the photosynthetic system over the course of the year. Photosynthesis in the dormant period is often overlooked, but conifers may compensate for low assimilation rates in the summer by photosynthesizing during the winter (Neilson et al. 1972). In addition, refilling of carbohydrate reserves in March/April (i.e. before growth occurs) may indicate photosynthetic processes (Snyder 1990). Previous studies have observed these dynamics in prevailing conditions (Kolari et al. 2007; Linkosalo et al. 2014) and in controlled laboratories (Ottander and Öquist 1991; Zarter et al. 2006), but not concurrently. By measuring in prevailing and optimal conditions, we are able to clearly understand the tree's physiological state. This includes its optimal photosynthetic capacity, and the instantaneous and slow photosynthetic responses to different environmental drivers.

Intra-annual dynamics form a multi-faceted dimension to changes in bark hydraulics, especially during the spring recovery period of the year. For example, the metabolic processes involved during the spring differ greatly than during the summer – as more

photoassimilates are required during initial growth to support the formation of structural elements (Krabel 2000). Although intra-annual variation of growth has been studied (Rossi et al. 2006b), some physiological bark mechanisms that occur at the onset of growth remain unexplored. It is seldom that studies link different whole-tree physiological processes to each other. Rather, studies have focused on physiological responses to changing environment (Rossi et al. 2006c; Čufar et al. 2008; Rossi et al. 2008; Swidrak et al. 2011), neglecting any linkages. It is important to reveal both: the bark hydraulic response to the environmental and how the changes from the bark link with other whole-tree processes at the onset of growth (**Study IV**).

The studies in this thesis were to understand the whole-tree processes linked to the dynamic variations of the bark, on an inter- and intra-annual scale. Specifically, the study focused on mature Scots pine and measured on-site in the field. To recognise this objective, the aims of the thesis were to:

1) develop a model to separate the water status-related changes from the inner-bark to reveal a growth and osmotic concentration signal (**Study I**)
2) link the signal to environmental drivers of stem growth (**Study I**) and to stem growth respiration (**Study II**), and validate the growth estimate against traditionally-acquired methods of radial stem growth (**Study I** and **II**)
3) identify the influence of seasonality on photosynthesis and leaf respiration, and quantify the photosynthetic responses measured from prevailing (field-measured) and optimal (controlled) conditions (**Study III**)
4) determine the various physiological tree responses linked to bark hydraulics before, during and after spring recovery (**Study IV**)

2. MATERIAL AND METHODS

Understanding bark hydraulics largely depends on axial water movement and the water exchange between the xylem and phloem tissues, which is highly connected to tree physiology and the environmental variables affecting it. It is important to clarify and understand the affecting quantities when analysing the processes that are supported within the bark. At SMEAR II (Station for Measuring Forest Ecosystem – Atmosphere Relations) in Hyytiälä, southern Finland, year-round, micro-meteorological and stand-level measurements are continuously monitored and provide the supporting material for this thesis. An extensive description of the measurements at the research station can be found in Vesala et al. (1998). In this section, only a broad summary of the measurements and models that were used for this thesis is described. In general, **Study I** and **II** adopted a hydraulic model based on Mencuccini et al. (2013) and with the aid of tree and environmental data, the model was used to give insight into the processes related to bark hydraulics and tree physiology. **Study III** compared continuously measured field data with response measurements conducted in laboratory conditions. Comparisons included the use of pre-existing models (e.g. Farquhar model (Farquhar et al. 1980)). **Study IV** analysed measurement data to find a correlation between different tree processes before and after spring recovery.

2.1 Experimental setup

2.1.1 Site description and environmental data

The data collection and the experiments for this thesis were conducted at the SMEAR II stand between 2007 and 2013 at Hyytiälä (61°51´N, 24°17´E, 170m a.s.l.). The study site was an even-aged, 52-year-old homogenous, naturally regenerated Scots pine forest with a mixture of various birches (*Betula* spp.), Norway spruce (*Picea abies*) and Aspen (*Populus tremula*). The understorey was mainly composed of dwarf shrubs (*Vaccinium myrtillus*, *Vaccinium vitis-idaea*) and mosses (Dicranum spp., *Pleurozium schreberi*). The parent material of the soil was composed of haplic podzol, formed from sandy and coarse silty glacial till, with a thin humus layer and low nitrogen levels. Further details about the site can be found in Hari and Kulmala (2005) and Vesala et al. (2005). The growing season in this boreal zone generally begins in late April and the ends early September. The mean annual precipitation of the area was 71.1 cm, with a mean annual air temperature of 3.5 °C (Pirinen et al. 2012). The coldest and warmest months were January (mean –8.9 °C) and July (mean +15.3 °C), respectively.

Mature, ~50-year old Scots pines were used in all experiments. The Scots pine canopy in the stand reached a height of 18 m tall, with a living canopy height of 8 m. The projected leaf area index (LAI) was 2 – 2.5 m^2 m^{-2} (Rautiainen et al. 2012). Access to the canopy was provided by a permanent scaffolding tower installation. Environmental field data used for the experiments were recorded continuously from the scaffolding tower at the study site using data loggers (Campbell Scientific Ltd., Leicester, UK) at a 1-min time resolution (**Study I**, **II**, **III**, **IV**), and turbulent fluxes (eddy covariance) at a 30-min time resolution (**Study II**). Environmental data included air temperature (**Study I**, **II**, **IV**), precipitation (**Study I**, **II**, **IV**), relative humidity (**Study I**), soil water content (and converted to soil water potential) (**Study I**) and photosynthetic photon flux density (PPFD) (**Study I**). From

eddy covariance, measurements of net ecosystem CO_2 exchange (NEE), stand gross primary productivity (GPP) and stand evapotranspiration (ET) were used (**Study II**).

2.1.2 Radial stem measurements

All radial stem measurements were conducted on-site using point dendrometers, based on linear variable-displacement transducers (LVDT; model AX/5.0/S, Solartron Inc. West Sussex, U.K.) at a height of ~15 m of the sample tree. A pair of point dendrometers was used in the study, one measuring xylem radial change and the other measuring whole stem radial change. Xylem change was measured by resting a dendrometer against a small screw that was inserted approximately 10 mm through the outer- and inner-bark, into the superficial part of the existing xylem. Whole stem change was measured by excising ~3 mm of the bark with a scalpel to expose the phloem tissue. The dendrometer head measuring whole stem change rested against the phloem. The dendrometers were secured to rigid steel frames and attached to the tree with screws using attachment plates. The two dendrometers were spaced ~30 mm apart and ran parallel to the ground surface. A detailed description of the installation can be found in **Study I** and **II**. Inner-bark radial change was required in the study, and was calculated as the difference between whole stem and xylem radial changes. Therefore, the inner-bark includes the phloem tissue produced to the outside of the pre-existing xylem tissue, the vascular cambium and the newly formed xylem (see Figure 1 in **Study I**).

2.1.3 Microcoring

Microcoring is a method to extract wood samples with the least amount of mechanical injury to a tree. These samples allow for detailed analyses of cambium activity and wood formation, and require repeated sampling to count newly formed xylem over the course of the summer growth period (Rossi et al. 2006a). Microcores were taken from four Scots pine trees within the same stand, ~20 m away from the site of the dendrometer measurements (for the years 2007 – 2009). Since the microcored trees were in similar conditions to the dendrometer site, stem cell growth derived from the microcores could be represented as proxy for the trees from the dendrometer site. The microcores were extracted using bone marrow sampling needles in 2007 and with a Trephor (Rossi et al. 2006a) in 2008 and 2009. For a detailed description of microcore slide and image preparation, see **Study I** and **II** and Jyske et al. (2014). The microcore images revealed the number of dividing, enlarging, thickening and mature cells and counted using image analysis software, Image-Pro Plus v7.0. (Media Cybernetics Inc., Bethesda, Maryland, USA). The analyses in the current studies used the first day of tracheid formation (**Study I**, **II**) and radial stem cell growth (**Study I**).

2.1.4 Gas exchange measurements

Gas exchange measurements were used for **Study II**, **III** and **IV**. Stem CO_2 efflux (**Study II**, **IV**), continuous gas exchange measurements (**Study III**), and VOC (volatile organic compounds) emissions (**Study IV**) were measured on-site using chambers and enclosures

installed onto the sample tree(s) while standard and ambient gas exchange response measurements (**Study III**) were performed in the laboratory within Hyytiälä.

To sample CO_2 efflux (E_S) from the stem, a chamber was firmly attached to the bark and a continuous air flow of 1.3 l min^{-1} was circulated through the chamber. Measurements were temperature-normalized by referencing the air temperature inside and outside the enclosure. E_S was calculated as the difference between the CO_2 concentration flowing out from the chamber and the CO_2 concentration of the ambient air into the chamber. The chamber was located within the lower part of the crown (at a height of 12 m in 2007), but was moved ± 2 m in the vertical direction in subsequent years. Additionally, the air flow rate has been changed between studies and the analyses have been adjusted to represent the new rates.

Pine shoot photosynthesis was measured using continuous gas exchange chambers that were installed in the upper canopy (Altimir et al. 2002). The chambers were open most of the time to have the shoots (and chamber interior) exposed to ambient conditions. When fluxes were measured, the chambers closed intermittently for 1 min and gas concentrations and environmental variables were recorded every 5 sec. In general, measurements of CO_2 concentration, air temperature inside the chamber and photosynthetically active radiation (PAR) outside of the chamber were sampled 50 – 80 times a day. To calculate the needle surface area of the sampled shoots, the dimensions of the needles of each shoot were measured and the equation from Tiren (1927) was used.

To analyse standard and ambient gas exchange response measurements, branches from the upper canopy of randomly selected trees were cut, placed under water and sampled at the laboratory in Hyytiälä. Prior to measuring, the branches were re-cut to re-initiate unrestricted water flow through the branch and needles. To measure photosynthesis, a portable IRGA (infra-red gas analyzer) equipped with an integrated fluorometer (Walz GFS-3000, Heinz Walz, Germany) was used. For each branch, four fascicles, totalling eight needles were enclosed inside the IRGA measuring cuvette. The needles were acclimated before measurements for 15 – 20 min. to a flow rate of 650 µmol s^{-1} and relative humidity was between 55 – 70 %. In total, three different response measurements were taken for each branch: (i) light response curve at ambient (i.e. field) temperature and ambient CO_2 concentration, (ii) light response curve at standard (i.e. controlled at 18 °C) temperature and ambient CO_2 concentration, and (iii) CO_2 response curve at standard temperature of 18 °C and saturating light (i.e. 1300 µmol m^{-2} s^{-1}). The light and CO_2 response curves were produced by changing light or CO_2, respectively, in a step-wise manner of decreasing intensity from its initial acclimated state, followed by an increasing intensity from its initial acclimated state. For light response measurements, initial PAR was at 600 µmol m^{-2} s^{-1} and for CO_2 response measurements, CO_2 concentration was 380 µmol mol^{-1}. By measuring continuous and response gas exchange measurements, we can estimate certain properties from the following models: (i) light responses from the field, (ii) biochemical Farquhar-model of photosynthesis, and (iii) instantaneous temperature responses of photosynthesis and respiration. Further descriptions of these models are explained in **Study III**. At the end of each branch measurement, fresh and dry mass and specific leaf area (SLA) were calculated.

Stem VOC emissions were measured from the sample tree at a height of 12 m., and the enclosure was firmly fixed and designed specifically to measure reactive gases with materials chemically inert to many VOCs, to avoid signal loss and noise. The enclosure was wrapped with transparent and UV-permeable FEP foil (0.05 mm thick, Fluorplast, Maalahti, Finland) and bound on both ends. Temperature within the enclosure was measured and completely protected against precipitation. VOCs within the enclosure were drawn from a heated FEP-tubing of 64 m length to the analyser with a flow rate of air at 1

dm^3 min^{-1}. The analyser, a proton transfer reaction-quadrupole mass spectrometer (PTR-QMS, Ionicon, Innsbruck, Austria; Hansel et al. (1995)), measured ten specific protonated mass ions, but for this study, ions pertaining to m/z 137 were used. This trace gas corresponded to monoterpenes in Scots pine emissions. The operating procedures are explained in detail by Taipale et al. (2008). Sampling time for VOC emissions took 2 min 45 s and was taken 24 times per day. The enclosure was flushed prior to measurement with above-canopy air at a rate of about 0.4 L min^{-1}, so as not to spoil the sample.

2.1.5 Other measurements

Sap flow was measured using a thermal dissipation probe at a height of 13 m using the Granier method (Granier 1987); see Hölttä et al. (2015) for further details. The Granier method required two needle-like probes (of 50 mm in length), each inserted into a 2 mm-wide brass cylinder and into the sapwood, vertically and spaced 10 cm apart. The upper probe was heated with continuous power (approximately 0.2 W) and the sap flux density was calculated as the difference between the two probe's temperatures.

Continuous field measurements of chlorophyll fluorescence were measured using a Monitoring PAM fluorometer system (Heinz Walz, GmbH, Germany) from the upper canopy. Roughly three to four pairs of needles were held in the measuring head and the prevailing (F') and maximal fluorescence (F'_m) were measured every 15, 30, or 60 min using the saturating pulse technique at night (see Porcar-Castell et al. (2008b) for a detailed explanation). The aim was to estimate the daily maximum $\emptyset_{PSII}$ (i.e. the operating quantum yield of photochemistry in photosystem II):

$$\emptyset_{PSII} = \frac{F'_m - F'}{F'_m}. \quad (3)$$

2.2 Modelling the estimated growth signal derived from radial stem measurements

To extract the growth signal from radial stem measurements, a hydraulic model based on the principles of Mencuccini et al. (2013) was used to separate the reversible water-induced component ($\hat{\Delta}D_b$) from inner-bark radial variation. $\hat{\Delta}D_b$ relates directly to the exchange of water between the xylem and inner-bark, driven by changes in xylem water potential. By separating $\hat{\Delta}D_b$ from the inner-bark radial variation, the residual – the component that includes irreversible stem growth and reversible changes in inner-bark osmotic concentration is revealed (hereafter estimated growth, $\hat{\Delta}G_m$). Although $\hat{\Delta}D_b$ is reversible and small on a seasonal scale, the separation of this component is important because it is typically larger than growth on a daily scale, and can therefore mask quantifiable short-term (e.g. daily) growth. This is especially important when, for example, environmental variables are studied. Therefore, to assume dendrometer measurements (with water-induced changes included) as proxy for stem growth, it can lead to misinterpretation of results.

This model approach applies Hooke's Law, which indicates that water-induced dimensional changes in the xylem reflect changes in xylem water potential (Perämäki et al. 2001). Consequently, the inner-bark would follow xylem water-related changes, as the radial exchange in water between the two tissues tend towards an equilibrium (Hölttä et al. 2009). The model requires four parameters to estimate $\hat{\Delta}D_b$: changes of xylem radius (ΔD_x), changes of inner-bark radius (ΔD_b), xylem radial hydraulic conductance (α) and the

ratio of the elastic modulus of the inner-bark to xylem (β). The radial parameters are obtained from dendrometer measurements, while α and β are estimated from the model itself. The change of the inner-bark radius that is solely affected by xylem water potential $\left(\hat{\Delta}D_b\right)$ at time $(t + \Delta t)$ (i.e. the next measuring point), can be estimated from the changes of inner-bark and xylem radii at time (t):

$$\hat{\Delta}D_b(t + \Delta t) = \hat{\Delta}D_b(t) + \alpha(\beta\Delta D_x(t) - \hat{\Delta}D_b(t))\Delta t. \quad (4)$$

Parameters α and β are estimated by first employing piecewise linear regression (between notable break points in growth) over one full growing season of measured inner-bark radial variation. This is done to remove the growth signal from these measurements (Zweifel et al. 2001; Mencuccini et al. 2013). After removing the growth signal, a least-square regression fitting is employed and iterated 100 times over Eq. (4) using ΔD_x and ΔD_b measurements at 30 minute intervals (Excel Solver, Microsoft). After the α and β parameters are obtained, Eq. (4) is then employed on the raw time series to estimate $\hat{\Delta}D_b$.

When $\hat{\Delta}D_b$ has been estimated, we can determine the estimated growth at time (t):

$$\hat{\Delta}G_m(t) = \Delta D_b(t) - \hat{\Delta}D_b(t). \quad (5)$$

$\hat{\Delta}G_m$ is therefore the radial variation due to all other processes influencing dimensional changes of the inner-bark (i.e., changes in inner-bark osmotic concentration and cambial growth) and can be used as proxy for radial stem growth. For additional detail in modelling theory, estimation and parameterization, refer to **Study I** and **II**.

2.3 Calculation of growth respiration

Measured stem CO_2 efflux (E_S) was partitioned into maintenance and growth respiration components using the mature tissue method (Amthor 2012), which assumes that the total respiration measured from mature tissue (i.e. tissue that is no longer growing) is apportioned to solely the maintenance respiration component. Therefore, maintenance respiration estimated outside of the growing season (e.g. before day 130 and after day 240) can be used to calculate annual maintenance respiration rates.

To estimate the temperature response of woody tissue respiration (R), an exponential equation was used:

$$R = E_{S10}/Q_{10}{}^{(10-T)/10}, \quad (6)$$

where Q_{10} is the temperature coefficient of respiration, T is the temperature at reference point R and E_{S10} is the CO_2 efflux at a tissue temperature of 10 °C.

Annual maintenance respiration rates were estimated by first calculating Q_{10} over a continuous interval of two weeks during a period of non-growth activity:

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\frac{10}{T_2-T_1}}, \quad (7)$$

where $R_1$and R_2 are CO_2 efflux rates at temperatures T_1 and T_2, respectively. During non-growing periods, it is assumed that changes due to temperature would also reflect similar changes during the growing season. After calculating annual maintenance

respiration rates, the difference between these values and E_S reveals estimated growth respiration.

3. RESULTS AND DISCUSSION

The main outcome from this thesis is that tree bark hydraulics offers a unifying theoretical framework to study dynamic tree-water relations that go beyond processes that are exclusively in the bark. These dynamic relations offer a holistic understanding of the interactions between various tree processes and its physiological responses to environmental change**.** Specifically in this thesis, the results from the modelling of estimated growth revealed an intricate correlation between the environmental factors that affect growth that was previously misunderstood. Furthermore, changes in tree processes such as photoassimilation and winter-summer transformation may be linked to sap flow and sugar transport dynamics. By understanding the linkages, we are closer to defining holistically, bark hydraulic function and structure. In this chapter, I present key results from the studies and focus on the interpretation of these results with a thorough discussion.

3.1 Model to estimate stem growth

The model separating changes due to water-related exchange (between the xylem and phloem) from inner-bark radial stem variations revealed a signal that can be used as proxy for stem growth (**Study I**). This signal (hereafter, estimated growth) includes cambial growth and changes in inner-bark osmotic concentration. This model demonstrates that the individual processes that drive sap flow (in the xylem) and sugar transport (in the phloem) along the stem is closely interconnected. The model assumes that water tends towards (and away from) the phloem in order to reach equilibrium between the two tissues, and consequently, sugar is transported (and deposited) along the phloem tract. This transport is driven by an osmotic concentration gradient, from areas of high osmotic potential to areas of low potential. Similarly, water in the xylem is motivated by a water potential gradient, behaving in a similar manner to osmotic gradients within phloem. The model showed that water-related changes of measured stem radius indeed mask cambial growth and osmotic concentration changes. This is evident when observing the differences between direct radial stem measurements (e.g. whole stem) and estimated growth on a daily scale (see Figure 3 in **Study I**). However, direct radial stem measurements could also be used cautiously in non-water-stressed environments for assessing growth over longer time periods (e.g. seasonal), despite that these changes include sizable water-driven changes (Klepper et al. 1971). During water-stressed periods (e.g. during prolonged precipitation), using direct radial stem measurements can be limiting because a substantial increase of stem girth may occur, which completely conceals growth and osmotic concentration changes (**Study I**). This precipitation effect can last during the whole precipitation period and up to several days after cessation (Figure 3). The result is not unique because radial stem variations are mainly related to variables linked to tree hydraulics. Radial stem variations of either xylem (Irvine and Grace 1997; Perämäki et al. 2001) or whole stem (Offenthaler et al. 2001; Zweifel et al. 2001) are approximately linearly proportional to changes in stem water potential. Therefore, radial stem variations have a high (positive) correlation to precipitation (**Study I**). The model presented in the study does not wholly remove the effects from precipitation, but effectively minimizes it. This is because the model estimates are derived from direct radial stem measurements. Comparison of the accumulation of tracheids (measured from microcores) to estimated growth showed a similar seasonal, sigmoidal curve with a slight but consistent lag (**Study I**). Estimated growth increased roughly ten days before the first tracheids were formed (in late May). Meanwhile, the maximum cumulative estimated growth occurred approx. two weeks earlier than formation

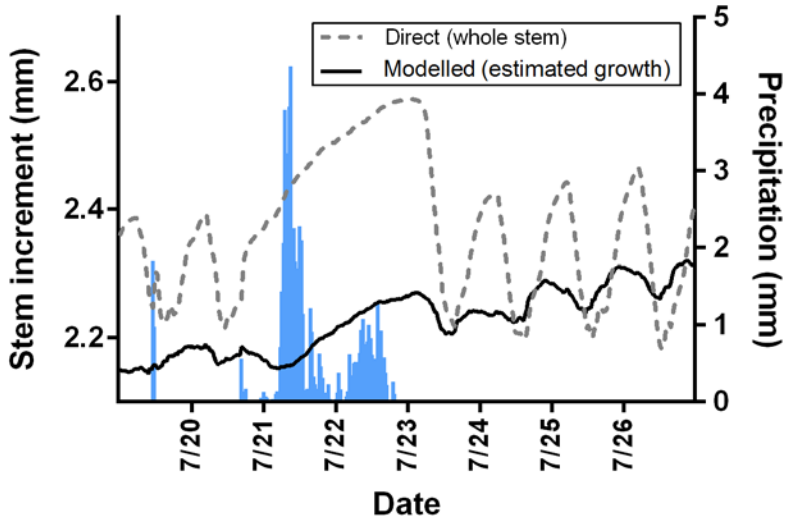

Figure 3. Daily precipitation (blue), measured direct radial variation of whole stem and modelled estimated growth (i.e. growth and changes due to osmotic concentration) during the period of July 19-26, 2008. Directly measured radial variation and estimated growth were offset to 0 on May 1.

of the last tracheids. The time lag may be due to phloem growth (in bark tissue), observed from estimated growth (and direct radial stem measurements) – tissue not measured from microcores (**Study I**). It is also plausible that the formation of the secondary cell wall occurred after the cessation of tracheid growth. These lags were observed during the early summer but largely disappeared towards the end of the summer when growth ceased. Estimated growth-derived values were always larger than those from microcores. This is because microcores do not include phloem growth and the compression of non-lignified cells when sampling (**Study I**).

During a 24 h cycle, estimated growth showed two distinct swelling and shrinking periods of change in osmotic concentration (**Study I**). The first period occurred around noon, when the stem swelled rapidly, and shrunk around the late morning of the following day. This period suggests an increase of osmotic concentration along the stem – a rapid propagation of osmotic concentration from sugar sources to sinks. The second period occurred during predawn (when the first period is still shrinking). This rapid increase and sudden decrease may be due to axial pressure propagation in the phloem, induced by the first swelling period. These findings suggest that sugar pools are accumulated during the day and translocated at night (Hölttä et al. 2006; Mencuccini et al. 2013). In addition, growth was observed to predominantly occur during the night, when transpiration has ceased and water tension has relaxed (De Schepper and Steppe 2010; Hölttä et al. 2010).

In relation to its environment, estimated growth was highly and positively correlated to temperature, PPFD and soil water potential – variables that are directly linked to metabolic activity and tree productivity (**Study I**). Moreover, estimated growth was negatively correlated to precipitation. These results sharply contrasted direct radial measurements, which showed positive correlation to precipitation and negative correlation to PPFD. Direct

radial measurements show a positive correlation to precipitation, because precipitation effects impact radial measurements almost immediately. Since estimated growth does not include water-related changes, precipitation effects are not detected. Furthermore, a negative correlation to PPFD was expected from direct radial measurements since it correlates with high transpiration and low leaf water potential. During the first half of the growing season, growth is largely dependent on temperature, while during the second half of the growing season (when cessation of tree growth and the onset of tree dormancy and winter hardiness processes have begun), both temperature and water availability were limiting factors for growth. While growth persists during this half, it is at a much lower rate than during the first half (see Table 2 of **Study I**).

3.2 Respiration

A significant correlation with stem CO_2 efflux showed the connections between estimated growth and canopy GPP to R_g (**Study II**). This was observed on a daily scale over the growing season of several years. A one-day time lag was found of estimated growth preceding R_g and supported by eddy covariance measurements (see Figure 3 in **Study II**). This time lag was not observed using direct dendrometer measurements, indicating that water-related influences may mask daily growth (**Study I**, **II**). The time lag may be partly due to within-stem diffusion resistances causing stem CO_2 efflux to lag behind actual stem respiration (**Study II**). The correlation between R_g and estimated growth differed considerably during different times of the season, which coincided well with the different phases of growth (i.e. cell division, enlargement and thickening) (Table 1) (**Study II**). During cell division, the correlation was low, but increased significantly during cell enlargement. Correlation decreased (yet still significant) during the thickening phase from when cells were enlarging. R_g-growth dynamics have also been observed in studies from Stockfors and Linder (1998) in *Picea abies* and Vose and Ryan (2002) in *Pinus strobus*. Growth rates derived from estimated growth sharply contrasted growth rates derived from raw dendrometer measurements, with the latter showing inconsistent correlation year-to-year. The stem respiration study of **Study II** found a similar decline in respiration during the late summer/early autumn to the shoot respiration study of **Study III**. This reflects growth processes declining, since much of the total CO_2 respired from trees during the summer is most apportioned to growth respiration (**Study II**). During April, an increase of respired CO_2 was observed – at a time too early to be directly linked with visible growth (**Study II**, **III**, **IV**). This could indicate metabolic processes related to the phase change from winter to summer state and the initiation of growth (**Study II**, **III**, **IV**, Lavigne et al. (2004), Gruber et al. (2009)).

3.3 Photoproduction

Photosynthetic capacity is strongly linked to prevailing temperature and results from light response measurements revealed a strong downregulation in photosynthetic capacity towards the winter and a recovery in the spring (**Study III**). However, comparisons between laboratory and field measurements revealed that instantaneous responses from the field were superimposed on the seasonal acclimation, thus overestimating the downregulation of photosynthetic capacity from the summer to winter state. Laboratory measurements indicated significant potential for photosynthetic production during the winter, with results showing up to 20% of summertime values, whilst field condition values were close to zero. This indicates that the remaining 80% of the reduction in transient photosynthetic rates is attributed to the slow changes in the capacity of light and carbon

Table 1. Squared correlation coefficient (r^2) between growth respiration (R_g) and the estimated growth rates derived from estimated growth ($\Delta\hat{\Delta}G_m$) and raw dendrometer measurements (ΔD_w).

* ($P < 0.05$), ** ($P < 0.01$)

		Spring	Early Summer	Late Summer
2007	$\Delta\hat{\Delta}G_m$	0.05*	0.30**	0.16**
	ΔD_w	0.02	0.00	0.04
2008	$\Delta\hat{\Delta}G_m$	0.02	0.36**	0.15**
	ΔD_w	0.08*	0.05*	0.03
2009	$\Delta\hat{\Delta}G_m$	0.04	0.25**	0.15**
	ΔD_w	0.00	0.01	0.00
2011	$\Delta\hat{\Delta}G_m$	0.01	0.31**	0.01
	ΔD_w	0.00	0.31**	0.04

reactions (**Study III**). Thus, the remaining 20% is due to instantaneous responses that determine how much of the capacity is realized when conditions are favourable (e.g. milder spells). Photosynthetic responses to temperature linked well with the recovery of the photosynthetic machinery during the spring, whereas the downregulation of photosynthesis was better explained with light rather than temperature during the winter.

Measurements from laboratory and field conditions also revealed that the regulation mechanisms of light and carbon reactions are different. This is especially the case during the autumn when carbon reaction-related parameters (e.g. light-saturated photosynthesis) declined steadily from summertime conditions towards autumn, while light reaction-related parameters (e.g. chlorophyll fluorescence) maintained near-summertime levels during the autumn period (**Study III**). Light reaction-related parameters are less sensitive to changes in temperature and more sensitive to changes in light (**Study III**). Both field and laboratory measurements responded similarly to changes in light, indicating low instantaneous response. This may imply that there is no pressure to downregulate light reaction-related parameters, since autumns in the boreal zone are generally very dark (**Study III**). Complete downregulation of light reactions do not occur until late winter (Porcar-Castell et al. 2008a; Porcar-Castell 2011). Carbon fixation, on the other hand, is more sensitive to temperature as it is solely controlled by biochemical reactions (**Study III**). From these results, it is clear that carbon and light-based reactions in the boreal zone behave in accordance with the changes in the environment. In the autumn and winters, low light is effectively utilized and photosynthetic capacity is not maximized since it is superfluous (**Study III**). If warm spells occur during the winter, photosynthesis could occur even at low light, but not at maximal

efficiency. It is also important to downregulate respiration during the winter to avoid excessive carbon loss (**Study III**).

3.4 Spring recovery

Immediately after spring recovery (i.e. thawing of the stem) in the spring, processes in the bark and xylem begin to occur (**Study III**, **IV**). Within the xylem, embolized vessels are replaced with new functional ones and/or conduits are refilled with water (Cochard et al. 2001). This is encouraged by osmotic water flow from the phloem being driven into embolized conduits after the unloading of sugars (Nardini et al. 2011). The unloading of sugars into the phloem allows the embolized conduit area to become strong sinks and thus generate the driving bulk water force for refilling. Radial stem variations after spring recovery, lasting 2 – 3 weeks, demonstrated irregular behaviour in comparison with the regular pattern observed during summer conditions (**Study I**, **IV**). This behaviour included inner-bark swelling and shrinking occurring before or concomitantly with xylem variations and abnormal daily amplitude changes, such as large swelling and shrinking of the inner-bark relative to xylem daily amplitude. These irregular processes indicate that forces other than transpiration drive the pattern of radial stem variations and may suggest that the inner-bark plays an active role in xylem recovery (**Study I**, **IV**). In addition, a rapid but large monoterpene emission burst (lasting several hours) was observed that coincided with the bark hydraulic recovery, shortly after the winter state. Although the cause of the burst is not fully understood, the occurrence at least coincides with, if not caused by, the recovery of hydraulic processes that precedes the physiological active summer state of the tree (**Study IV**). Spring recovery of photosynthetic parameters, initiation of growth and the successful transition from winter to summer state (indicated by regular diurnal pattern of radial stem variation and sap flow) could be related to favourable temperature for tree growth (**Study I**, **II**, **III**, **IV)**.

4. REVIEW OF PUBLICATIONS

Study I presents a model that estimates cambial growth after separating the water-related influences from dendrometer measurements. The model first estimates the influence of water-related changes of the xylem to the inner-bark and is then separated from inner-bark radial variation. The residual signal is growth and reversible changes in inner-bark osmotic concentration (hereafter, estimated growth). On a daily scale, growth was observed to increase during the night and changes in osmotic concentration occurred from noon to dawn in the morning. Comparing estimated growth with environmental factors, temperature was a limiting factor for growth in the early summer. Meanwhile, temperature and water availability were limiting factors during the late growing season. Contrasting directly measured radial stem variations, precipitation was not significantly correlated to estimated growth. These results show that the model effectively removed any influences of water-related changes from dendrometer measurements. Therefore, the use of direct dendrometer measurements as proxy for growth is problematic on a daily time scale, and analysis against environmental variables is discouraged as water-related influences mask growth at these time scales.

Study II furthers the model presented in the previous study by analyzing growth respiration dynamics in relation to estimated growth. Growth respiration was estimated from stem CO_2 efflux measured on the same tree as measured radial stem variations. Growth respiration was estimated based on the Amthor method, where maintenance respiration rates were first calculated as a function of temperature during non-growth periods (e.g. during spring or autumn), and subtracted from measured stem CO_2 efflux. Night-time values of growth respiration were used to minimize the effects of CO_2 movement along the xylem tract. A time lag study was considered and analysis included partitioning intra-annual growth into three segments according to the different phases of cellular growth (i.e., cell division, cell expansion and cell wall thickening). Growth respiration was found to follow growth derived from radial stem measurements with a one-day time lag, which was corroborated with eddy covariance measurements. The relationship between growth respiration and stem growth varied during growing season, indicated by the growth process (e.g. cell division and enlargement).

Study III is a study of the seasonality of photosynthesis by separating the slow and instantaneous responses of the state of leaf physiology to the environment. In addition, light and carbon reaction and respiration parameters were quantified. The separation of these responses was done by observing and comparing the differences between field measurements and near-optimal controlled measurements. Seasonality analysis focused on the physiological changes of the photosynthetic machinery between seasons (e.g. winter to spring and summer to autumn). A model expressing the acclimation of the photosynthetic machinery to temperature was employed to understand the slow photosynthetic responses to environmental drivers. Over the course of the year, light and carbon reaction-related parameters varied differently from one another, largely due to temperature sensitivity. In the winter, Scots pine is able to rapidly benefit from warmer conditions. Recovery of photosynthetic parameters could be linked with light during the autumn and winter, whereas temperature explained the recovery during the spring. Finally, the seasonal patterns of photosynthesis and respiration arise from the instantaneous responses superimposed on the slow seasonal temperature acclimation.

Study IV focuses on the physiological responses relating to the bark after spring recovery. The observations are explained using radial stem measurements, sap flow and shoot and stem gas exchange (including emissions of monoterpenes). Analysis of spring recovery included days before, during and after the last freeze-thaw period in the spring. The aim was to develop a holistic view of tree stem physiology during the springtime phase change from the winter state to summer state, and its implication for water transport capacity dynamics. This was realized when observed processes coincided with the recovery of xylem transport capacity concomitantly with the increase in temperature. Irregular variations between both xylem and inner-bark before and during the spring recovery period indicated that forces other than transpiration drive radial stem variations.

5. CONCLUDING REMARKS

Recent studies about the role that dynamic variations of the bark have on tree processes have paved the way to focus on the interconnections between various tree components and processes. While this thesis does not encompass all whole-tree processes, the processes that have been specifically observed in these studies further develop the result that the dynamic variations of the bark influence the changes to the metabolic, physiological and physical state of the tree. This was achieved by analysing with field measurements and modelling of bark dynamics. Based on the understanding of tree water relations, a relatively simple theoretical framework linking growth, respiratory and spring recovery processes was established. This theoretical framework is valuable for classifying and quantifying observations, creating new opportunities to study hydraulic properties, but also challenging previously-accepted opinions.

The modelling performed in **Study I** revealed how both internal and external factors influence intra-annual cambial growth dynamics. **Study II** further explores cambial growth dynamics by showing how it coincides with assumed stem growth respiration. From the observations of **Study III**, a connection with the changes of the bark in respect to the seasonality of photosynthesis was found. This connection was also observed in **Study I**, **II** and **IV** during the spring, when photoproduction was initiated following the recovery of the xylem transport capacity. **Study IV** continues the spring studies by revealing a link between seemingly separate individual tree processes to the recovery of the bark from winter dormancy. Stem CO_2 respiration results from **Study II**, **III** and **IV** revealed a strongly coupling between tree respiration and to the changes in bark hydraulics. Interestingly in April for **Study II**, **III**, **IV**, each study independently confirmed that measured respiration increased prior to growth, which may reflect the biochemical processes related to spring recovery and the initiation of growth.

As a whole, this study showed that the changes within the bark are inextricably linked with tree processes due to the tight coupling with sap flow-related changes of the xylem. State-of-the-art modelling in tandem with long-established field measurements has allowed for new possibilities to explore bark dynamics. Further development of a holistic eco-physiological approach to understanding bark hydraulics has yet to reach its full potential and merits future research.

REFERENCES

Altimir N., Vesala T., Keronen P., Kulmala M., Hari P. (2002). Methodology for direct field measurements of ozone flux to foliage with shoot chambers. Atmospheric Environment 36(1): 19-29.
http://dx.doi.org/10.1016/S1352-2310(01)00478-2

Amthor JS. (2012). *Respiration and crop productivity*: Springer Science & Business Media.

Bel A., Gamalei YV. (1992). Ecophysiology of phloem loading in source leaves. Plant, Cell & Environment 15(3): 265-270.
http://dx.doi.org/10.1111/j.1365-3040.1992.tb00973.x

Blechschmidt-Schneider S. (1990). Phloem transport in Picea abies (L.) Karst. in mid-winter. Trees 4(4): 179-186.
http://dx.doi.org/10.1007/BF00225313

Bostwick DE., Dannenhoffer JM., Skaggs MI., Lister RM., Larkins BA., Thompson GA. (1992). Pumpkin phloem lectin genes are specifically expressed in companion cells. The Plant Cell 4(12): 1539-1548.
http://dx.doi.org/10.1105/tpc.4.12.1539

Chapin FS., Schulze E-D., Mooney HA. (1990). The ecology and economics of storage in plants. Annual review of ecology and systematics: 423-447.

Cochard H., Lemoine D., Améglio T., Granier A. (2001). Mechanisms of xylem recovery from winter embolism in Fagus sylvatica. Tree Physiology 21(1): 27-33.
http://dx.doi.org/10.1093/treephys/21.1.27

Čufar K., Prislan P., de Luis M., Gričar J. (2008). Tree-ring variation, wood formation and phenology of beech (Fagus sylvatica) from a representative site in Slovenia, SE Central Europe. Trees 22(6): 749-758.
http://dx.doi.org/10.1007/s00468-008-0235-6

Dannoura M., Maillard P., Fresneau C., Plain C., Berveiller D., Gerant D., Chipeaux C., Bosc A., Ngao J., Damesin C. (2011). In situ assessment of the velocity of carbon transfer by tracing 13C in trunk CO2 efflux after pulse labelling: variations among tree species and seasons. New Phytologist 190(1): 181-192.
http://dx.doi.org/10.1111/j.1469-8137.2010.03599.x

Daudet F-A., Améglio T., Cochard H., Archilla O., Lacointe A. (2005). Experimental analysis of the role of water and carbon in tree stem diameter variations. Journal of Experimental Botany 56(409): 135-144.
http://dx.doi.org/10.1093/jxb/eri026

Daudet F., Lacointe A., Gaudillere J., Cruiziat P. (2002). Generalized Münch coupling between sugar and water fluxes for modelling carbon allocation as affected by water status. Journal of Theoretical Biology 214(3): 481-498.
http://dx.doi.org/10.1006/jtbi.2001.2473

De Schepper V., Bühler J., Thorpe M., Roeb G., Huber G., van Dusschoten D., Jahnke S., Steppe K. (2013a). 11C-PET imaging reveals transport dynamics and sectorial plasticity of oak phloem after girdling. Frontiers in plant science 4.
http://dx.doi.org/10.3389/fpls.2013.00200

De Schepper V., De Swaef T., Bauweraerts I., Steppe K. (2013b). Phloem transport: a review of mechanisms and controls. Journal of Experimental Botany 64(16): 4839-4850.
http://dx.doi.org/10.1093/jxb/ert302

De Schepper V., Steppe K. (2010). Development and verification of a water and sugar transport model using measured stem diameter variations. Journal of Experimental Botany 61(8): 2083-2099.
http://dx.doi.org/10.1093/jxb/erq018

Denny M. (2012). Tree hydraulics: how sap rises. European Journal of Physics 33(1): 43.

Deslauriers A., Rossi S., Anfodillo T. (2007). Dendrometer and intra-annual tree growth: what kind of information can be inferred? Dendrochronologia 25(2): 113-124.
http://dx.doi.org/10.1016/j.dendro.2007.05.003

Dickson R (1989). Carbon and nitrogen allocation in trees. Annales des sciences forestières: EDP Sciences. 631s-647s.

Ehlers K., Knoblauch M., Van Bel A. (2000). Ultrastructural features of well-preserved and injured sieve elements: minute clamps keep the phloem transport conduits free for mass flow. Protoplasma 214(1-2): 80-92.
http://dx.doi.org/10.1007/BF02524265

Farquhar G., von Caemmerer Sv., Berry J. (1980). A biochemical model of photosynthetic CO2 assimilation in leaves of C3 species. Planta 149(1): 78-90.
http://dx.doi.org/10.1007/BF00386231

Ferrier JM., Christy AL. (1975). Time-dependent Behavior of a Mathematical Model for Munch Translocation Application to Recovery from Cold Inhibition. Plant Physiology 55(3): 511-514.
http://dx.doi.org/10.1104/pp.55.3.511

Fetene M., Benker C., Beck E. (1997). The Pathway of Assimilate Flow from Source to Sink in Urtica dioica L., Studied with14C under Ambient Atmospheric Conditions. Annals of botany 79(6): 585-591.
http://dx.doi.org/10.1006/anbo.1996.0392

Fisher DB. (1978). An evaluation of the Münch hypothesis for phloem transport in soybean. Planta 139(1): 25-28.
http://dx.doi.org/10.1007/BF00390805

Franceschi VR., Tarlyn NM. (2002). L-Ascorbic acid is accumulated in source leaf phloem and transported to sink tissues in plants. Plant Physiology 130(2): 649-656.

http://dx.doi.org/10.1104/pp.007062

Franck N., Vaast P., Génard M., Dauzat J. (2006). Soluble sugars mediate sink feedback down-regulation of leaf photosynthesis in field-grown Coffea arabica. Tree Physiology 26(4): 517-525.
http://dx.doi.org/10.1093/treephys/26.4.517

Granier A. (1987). Evaluation of transpiration in a Douglas-fir stand by means of sap flow measurements. Tree Physiology 3(4): 309-320.
http://dx.doi.org/10.1093/treephys/3.4.309

Gruber A., Wieser G., Oberhuber W. (2009). Intra-annual dynamics of stem CO2 efflux in relation to cambial activity and xylem development in Pinus cembra. Tree Physiology 29(5): 641-649.
http://dx.doi.org/10.1093/treephys/tpp001

Hall A., Minchin P. (2013). A closed-form solution for steady-state coupled phloem/xylem flow using the Lambert-W function. Plant, Cell & Environment 36(12): 2150-2162.
http://dx.doi.org/10.1111/pce.12125

Ham B-K., Lucas WJ. (2013). The angiosperm phloem sieve tube system: a role in mediating traits important to modern agriculture. Journal of Experimental Botany: ert417.
http://dx.doi.org/10.1093/jxb/ert417

Hansel A., Jordan A., Holzinger R., Prazeller P., Vogel W., Lindinger W. (1995). Proton transfer reaction mass spectrometry: on-line trace gas analysis at the ppb level. International Journal of Mass Spectrometry and Ion Processes 149: 609-619.
http://dx.doi.org/10.1016/0168-1176(95)04294-U

Hari P., Kulmala M. (2005). Station for measuring ecosystem-atmosphere relations. Boreal Environ. Res 10(5): 315-322.

Hölttä T., Kurppa M., Nikinmaa E. (2013). Scaling of xylem and phloem transport capacity and resource usage with tree size. Frontiers in plant science 4.
http://dx.doi.org/10.3389/fpls.2013.00496

Hölttä T., Linkosalo T., Riikonen A., Sevanto S., Nikinmaa E. (2015). An analysis of Granier sap flow method, its sensitivity to heat storage and a new approach to improve its time dynamics. Agricultural and Forest Meteorology 211: 2-12.
http://dx.doi.org/10.1016/j.agrformet.2015.05.005

Hölttä T., Mäkinen H., Nöjd P., Mäkelä A., Nikinmaa E. (2010). A physiological model of softwood cambial growth. Tree Physiology 30(10): 1235-1252.
http://dx.doi.org/10.1093/treephys/tpq068

Hölttä T., Mencuccini M., Nikinmaa E. (2009). Linking phloem function to structure: Analysis with a coupled xylem–phloem transport model. Journal of Theoretical Biology 259(2): 325-337.
http://dx.doi.org/10.1016/j.jtbi.2009.03.039

Hölttä T., Mencuccini M., Nikinmaa E (2014). Ecophysiological aspects of phloem transport in trees. *Trees in a Changing Environment*: Springer, 25-36.

Hölttä T., Vesala T., Sevanto S., Perämäki M., Nikinmaa E. (2006). Modeling xylem and phloem water flows in trees according to cohesion theory and Münch hypothesis. Trees - Structure and Function 20(1): 67-78.
http://dx.doi.org/10.1007/s00468-005-0014-6

Imaji A., Seiwa K. (2010). Carbon allocation to defense, storage, and growth in seedlings of two temperate broad-leaved tree species. Oecologia 162(2): 273-281.
http://dx.doi.org/10.1007/s00442-009-1453-3

Irvine J., Grace J. (1997). Continuous measurements of water tensions in the xylem of trees based on the elastic properties of wood. Planta 202(4): 455-461.
http://dx.doi.org/10.1007/s004250050149

Jyske T., Mäkinen H., Kalliokoski T., Nöjd P. (2014). Intra-annual tracheid production of Norway spruce and Scots pine across a latitudinal gradient in Finland. Agricultural and Forest Meteorology 194: 241-254.
http://dx.doi.org/10.1016/j.agrformet.2014.04.015

Klepper B., Browning VD., Taylor HM. (1971). Stem Diameter in Relation to Plant Water Status. Plant Physiology 48(6): 683-685.
http://dx.doi.org/10.1104/pp.48.6.683

Knoblauch M., Knoblauch J., Mullendore DL., Savage JA., Babst BA., Beecher SD., Dodgen AC., Jensen KH., Holbrook NM. (2016). Testing the Münch hypothesis of long distance phloem transport in plants. eLife 5: e15341.
http://dx.doi.org/10.7554/eLife.15341

Kolari P., Lappalainen HK., Hänninen H., Hari P. (2007). Relationship between temperature and the seasonal course of photosynthesis in Scots pine at northern timberline and in southern boreal zone. Tellus B 59(3): 542-552.
http://dx.doi.org/10.1111/j.1600-0889.2007.00262.x

Krabel D. (2000). Influence of sucrose on cambial activity. Savidge, R, A,, Barnett, J, R,, Napier, R ed (s). Cell and molecular biology of wood formation. Experimental Biology Reviews... BIOS Scientific Publishers Ltd.: Oxford, UK: 113-125.

Lacointe A. (2000). Carbon allocation among tree organs: a review of basic processes and representation in functional-structural tree models. Annals of Forest Science 57(5): 521-533.
http://dx.doi.org/10.1051/forest:2000139

Lacointe A., Minchin PE. (2008). Modelling phloem and xylem transport within a complex architecture. Functional Plant Biology 35(10): 772-780.
http://dx.doi.org/10.1071/FP08085

Lang A. (1979). A relay mechanism for phloem translocation. Annals of botany 44(2): 141-145.

Lavigne M., Little C., Riding R. (2004). Changes in stem respiration rate during cambial reactivation can be used to refine estimates of growth and maintenance respiration. New Phytologist 162(1): 81-93.
http://dx.doi.org/10.1111/j.1469-8137.2004.01004.x

Lewis SL., Lopez-Gonzalez G., Sonké B., Affum-Baffoe K., Baker TR., Ojo LO., Phillips OL., Reitsma JM., White L., Comiskey JA. (2009). Increasing carbon storage in intact African tropical forests. Nature 457(7232): 1003-1006.
http://dx.doi.org/10.1038/nature07771

Linkosalo T., Heikkinen J., Pulkkinen P., Mäkipää R. (2014). Fluorescence measurements show stronger cold inhibition of photosynthetic light reactions in Scots pine compared to Norway spruce as well as during spring compared to autumn. Frontiers in plant science 5.
http://dx.doi.org/10.3389/fpls.2014.00264

Lucas WJ., Groover A., Lichtenberger R., Furuta K., Yadav SR., Helariutta Y., He XQ., Fukuda H., Kang J., Brady SM. (2013). The plant vascular system: evolution, development and Functions. Journal of integrative plant biology 55(4): 294-388.
http://dx.doi.org/10.1111/jipb.12041

Mäkelä A., Kolari P., Karimäki J., Nikinmaa E., Perämäki M., Hari P. (2006). Modelling five years of weather-driven variation of GPP in a boreal forest. Agricultural and Forest Meteorology 139(3): 382-398.
http://dx.doi.org/10.1016/j.agrformet.2006.08.017

Mencuccini M., Hölttä T. (2010). The significance of phloem transport for the speed with which canopy photosynthesis and belowground respiration are linked. New Phytologist 185(1): 189-203.
http://dx.doi.org/10.1111/j.1469-8137.2009.03050.x

Mencuccini M., Hölttä T., Sevanto S., Nikinmaa E. (2013). Concurrent measurements of change in the bark and xylem diameters of trees reveal a phloem-generated turgor signal. New Phytologist 198(4): 1143-1154.
http://dx.doi.org/10.1111/nph.12224

Münch E. (1930). Stoffbewegungen in der Pflanze.

Nardini A., Gullo MAL., Salleo S. (2011). Refilling embolized xylem conduits: is it a matter of phloem unloading? Plant Science 180(4): 604-611.
http://dx.doi.org/10.1016/j.plantsci.2010.12.011

Neilson R., Ludlow M., Jarvis P. (1972). Photosynthesis in sitka spruce (Picea sitchensis (Bong.) Carr.). II. Response to temperature. Journal of Applied Ecology: 721-745.

Nikinmaa E., Hölttä T., Hari P., Kolari P., Mäkelä A., Sevanto S., Vesala T. (2013). Assimilate transport in phloem sets conditions for leaf gas exchange. Plant, Cell & Environment 36(3): 655-669.
http://dx.doi.org/10.1111/pce.12004

Nikinmaa E., Sievänen R., Hölttä T. (2014). Dynamics of leaf gas exchange, xylem and phloem transport, water potential and carbohydrate concentration in a realistic 3-D model tree crown. Annals of botany 114(4): 653-666.
http://dx.doi.org/10.1093/aob/mcu068

Offenthaler I., Hietz P., Richter H. (2001). Wood diameter indicates diurnal and long-term patterns of xylem water potential in Norway spruce. Trees - Structure and Function 15(4): 215-221.
http://dx.doi.org/10.1007/s004680100090

Ottander C., Öquist G. (1991). Recovery of photosynthesis in winter-stressed Scots pine. Plant, Cell & Environment 14(3): 345-349.
http://dx.doi.org/10.1111/j.1365-3040.1991.tb01511.x

Pallardy SG. (2010). *Physiology of woody plants*: Academic Press.

Patrick J. (1997). Phloem unloading: sieve element unloading and post-sieve element transport. Annual review of plant biology 48(1): 191-222.
http://dx.doi.org/10.1146/annurev.arplant.48.1.191

Perämäki M., Nikinmaa E., Sevanto S., Ilvesniemi H., Siivola E., Hari P., Vesala T. (2001). Tree stem diameter variations and transpiration in Scots pine: an analysis using a dynamic sap flow model. Tree Physiology 21(12-13): 889-897.
http://dx.doi.org/10.1093/treephys/21.12-13.889

Pfautsch S., Hölttä T., Mencuccini M. (2015). Hydraulic functioning of tree stems—fusing ray anatomy, radial transfer and capacitance. Tree Physiology: tpv058.
http://dx.doi.org/10.1093/treephys/tpv058

Phillips RJ., Dungan SR. (1993). Asymptotic analysis of flow in sieve tubes with semi-permeable walls. Journal of Theoretical Biology 162(4): 465-485.
http://dx.doi.org/10.1006/jtbi.1993.1100

Pirinen P., Simola H., Aalto J., Kaukoranta J-P., Karlsson P., Ruuhela R. (2012). *Tilastpja Suomen ilmastosta 1981-2010*: Citeseer.

Plain C., Gerant D., Maillard P., Dannoura M., Dong Y., Zeller B., Priault P., Parent F., Epron D. (2009). Tracing of recently assimilated carbon in respiration at high temporal resolution in the field with a tuneable diode laser absorption spectrometer after in situ 13CO2 pulse labelling of 20-year-old beech trees. Tree Physiology 29(11): 1433-1445.
http://dx.doi.org/10.1093/treephys/tpp072

Porcar-Castell A., Juurola E., Ensminger I., Berninger F., Hari P., Nikinmaa E. (2008a). Seasonal acclimation of photosystem II in Pinus sylvestris. II. Using the rate constants

of sustained thermal energy dissipation and photochemistry to study the effect of the light environment. Tree Physiology 28(10): 1483-1491.
http://dx.doi.org/10.1093/treephys/28.10.1483

Porcar-Castell A., Pfündel E., Korhonen JF., Juurola E. (2008b). A new monitoring PAM fluorometer (MONI-PAM) to study the short-and long-term acclimation of photosystem II in field conditions. Photosynthesis Research 96(2): 173-179.
http://dx.doi.org/10.1007/s11120-008-9292-3

Porcar-Castell A. (2011). A high-resolution portrait of the annual dynamics of photochemical and non-photochemical quenching in needles of Pinus sylvestris. Physiologia Plantarum 143(2): 139-153.
http://dx.doi.org/10.1111/j.1399-3054.2011.01488.x

Rautiainen M., Heiskanen J., Korhonen L. (2012). Seasonal changes in canopy leaf area index and MODIS vegetation products for a boreal forest site in central Finland. Boreal Environment Research 17(1): 72-85.

Rossi S., Anfodillo T., Menardi R. (2006a). Trephor: a new tool for sampling microcores from tree stems. Iawa Journal 27(1): 89.
http://dx.doi.org/10.1163/22941932-90000139

Rossi S., Deslauriers A., Anfodillo T. (2006b). Assessment of cambial activity and xylogenesis by microsampling tree species: an example at the Alpine timberline. Iawa Journal 27(4): 383-394.
http://dx.doi.org/10.1163/22941932-90000161

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 Phytologist 170(2): 301-310.

Rossi S., Deslauriers A., Griçar J., Seo JW., Rathgeber CB., Anfodillo T., Morin H., Levanic T., Oven P., Jalkanen R. (2008). Critical temperatures for xylogenesis in conifers of cold climates. Global Ecology and Biogeography 17(6): 696-707.
http://dx.doi.org/10.1111/j.1466-8238.2008.00417.x

Ryan MG. (1990). Growth and maintenance respiration in stems of Pinus contorta and Picea engelmannii. Canadian Journal of Forest Research 20(1): 48-57.
http://dx.doi.org/10.1139/x90-008

Ryan MG., Asao S. (2014). Phloem transport in trees. Tree Physiology 34(1): 1-4.
http://dx.doi.org/10.1093/treephys/tpt123

Sala A., Woodruff DR., Meinzer FC. (2012). Carbon dynamics in trees: feast or famine? Tree Physiology 32(6): 764-775.
http://dx.doi.org/10.1093/treephys/tpr143

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.

http://dx.doi.org/10.1111/nph.13275

Schnyder H. (1993). The role of carbohydrate storage and redistribution in the source-sink relations of wheat and barley during grain filling-a review. New Phytologist: 233-245. http://dx.doi.org/10.1111/j.1469-8137.1993.tb03731.x

Sevanto S. (2014). Phloem transport and drought. Journal of Experimental Botany: ert467. http://dx.doi.org/10.1093/jxb/ert467

Sevanto S., Hölttä T., Holbrook NM. (2011). Effects of the hydraulic coupling between xylem and phloem on diurnal phloem diameter variation. Plant, Cell & Environment 34(4): 690-703. http://dx.doi.org/10.1111/j.1365-3040.2011.02275.x

Sevanto S., Vesala T., Perämäki M., Nikinmaa E. (2003). Sugar transport together with environmental conditions controls time lags between xylem and stem diameter changes. Plant, Cell & Environment 26(8): 1257-1265. http://dx.doi.org/10.1046/j.1365-3040.2003.01049.x

Sievänen R., Perttunen J., Nikinmaa E., Kaitaniemi P. (2008). Toward extension of a single tree functional–structural model of Scots pine to stand level: effect of the canopy of randomly distributed, identical trees on development of tree structure. Functional Plant Biology 35(10): 964-975. http://dx.doi.org/10.1071/FP08077

Snyder MC. (1990). *Seasonal patterns of carbohydrate reserves within red spruce seedlings in the Green Mountains of Vermont*.

Steppe K., De Pauw DJW., Lemeur R., Vanrolleghem PA. (2006). A mathematical model linking tree sap flow dynamics to daily stem diameter fluctuations and radial stem growth. Tree Physiology 26(3): 257-273. http://dx.doi.org/10.1093/treephys/26.3.257

Stockfors J., Linder S. (1998). Effect of nitrogen on the seasonal course of growth and maintenance respiration in stems of Norway spruce trees. Tree Physiology 18(3): 155-166. http://dx.doi.org/10.1093/treephys/18.3.155

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. http://dx.doi.org/10.1093/treephys/tpr034

Taipale R., Ruuskanen T., Rinne J., Kajos M., Hakola H., Pohja T., Kulmala M. (2008). Technical Note: Quantitative long-term measurements of VOC concentrations by PTR-MS–measurement, calibration, and volume mixing ratio calculation methods. Atmospheric Chemistry and Physics 8(22): 6681-6698.

Thompson M., Holbrook N. (2003). Scaling phloem transport: water potential equilibrium and osmoregulatory flow. Plant, Cell & Environment 26(9): 1561-1577.

http://dx.doi.org/10.1046/j.1365-3040.2003.01080.x

Thorpe M., Minchin P., Gould N., McQueen J., Holbrook N., Zwieniecki M. (2005). The stem apoplast: a potential communication channel in plant growth regulation. Vascular transport in plants: 201-220.

Tiren L. (1927). Om barrytans storlek hos tallbestånd.

Turgeon R. (2010). The puzzle of phloem pressure. Plant Physiology 154(2): 578-581. http://dx.doi.org/10.1104/pp.110.161679

Turgeon R., Wolf S. (2009). Phloem transport: cellular pathways and molecular trafficking. Annual review of plant biology 60: 207-221. http://dx.doi.org/10.1146/annurev.arplant.043008.092045

Tyree MT., Christy AL., Ferrier JM. (1974). A simpler iterative steady state solution of Münch pressure-flow systems applied to long and short translocation paths. Plant Physiology 54(4): 589-600. http://dx.doi.org/10.1104/pp.54.4.589

van Bel AJ. (2003a). The phloem, a miracle of ingenuity. Plant, Cell & Environment 26(1): 125-149. http://dx.doi.org/10.1046/j.1365-3040.2003.00963.x

van Bel AJ. (2003b). Transport phloem: low profile, high impact. Plant Physiology 131(4): 1509-1510.

Vesala T., Haataja J., Aalto P., Altimir N., Buzorius G., Garam E., Hämeri K., Ilvesniemi H., Jokinen V., Keronen P. (1998). Long-term field measurements of atmosphere-surface interactions in boreal forest combining forest ecology, micrometeorology, aerosol physics and atmospheric chemistry. Trends in Heat, Mass and Momentum Transfer 4: 17-35.

Vesala T., Suni T., Rannik Ü., Keronen P., Markkanen T., Sevanto S., Grönholm T., Smolander S., Kulmala M., Ilvesniemi H. (2005). Effect of thinning on surface fluxes in a boreal forest. Global Biogeochemical Cycles 19(2). http://dx.doi.org/10.1029/2004GB002316

Vose JM., Ryan MG. (2002). Seasonal respiration of foliage, fine roots, and woody tissues in relation to growth, tissue N, and photosynthesis. Global Change Biology 8(2): 182-193. http://dx.doi.org/10.1046/j.1365-2486.2002.00464.x

Wilson JB. (1988). A review of evidence on the control of shoot: root ratio, in relation to models. Annals of botany 61(4): 433-449.

Windt CW., Vergeldt FJ., De Jager PA., Van As H. (2006). MRI of long-distance water transport: a comparison of the phloem and xylem flow characteristics and dynamics in poplar, castor bean, tomato and tobacco. Plant, Cell & Environment 29(9): 1715-1729. http://dx.doi.org/10.1111/j.1365-3040.2006.01544.x

Woodruff DR. (2014). The impacts of water stress on phloem transport in Douglas-fir trees. Tree Physiology 34(1): 5-14.
http://dx.doi.org/10.1093/treephys/tpt106

Yakushiji H., Nonami H., Fukuyama T., Ono S., Takagi N., Hashimoto Y. (1996). Sugar accumulation enhanced by osmoregulation in Satsuma mandarin fruit. Journal of the American Society for Horticultural Science 121(3): 466-472.

Zarter CR., Demmig-Adams B., Ebbert V., Adamska I., Adams WW. (2006). Photosynthetic capacity and light harvesting efficiency during the winter-to-spring transition in subalpine conifers. New Phytologist 172(2): 283-292.
http://dx.doi.org/10.1111/j.1469-8137.2006.01816.x

Zha T., Kellomäki S., Wang K-Y., Ryyppö A., Niinistö S. (2004). Seasonal and Annual Stem Respiration of Scots Pine Trees under Boreal Conditions. Annals of Botany 94(6): 889-896.
http://dx.doi.org/10.1093/aob/mch218

Zweifel R. (2016). Radial stem variations – a source of tree physiological information not fully exploited yet. Plant, Cell & Environment 39(2): 231-232.
http://dx.doi.org/10.1111/pce.12613

Zweifel R., Item H., Häsler R. (2001). Link between diurnal stem radius changes and tree water relations. Tree Physiology 21(12-13): 869-877.
http://dx.doi.org/10.1093/treephys/21.12-13.869

Zweifel R., Zimmermann L., Newbery DM. (2005). Modeling tree water deficit from microclimate: an approach to quantifying drought stress. Tree Physiology 25(2): 147-156.
http://dx.doi.org/10.1093/treephys/25.2.147

Zweifel R., Zimmermann L., Zeugin F., Newbery DM. (2006). Intra-annual radial growth and water relations of trees: implications towards a growth mechanism. Journal of Experimental Botany 57(6): 1445-1459.
http://dx.doi.org/10.1093/jxb/erj125