Intra-annual tree growth response to warming along an elevational gradient

Inauguraldissertation
der Philosophisch-naturwissenschaftlichen Fakultät
der Universität Bern

vorgelegt von
Gregory M. King
von Nova Scotia, Canada

Leiter der Arbeit:

Prof. Dr. Fortunat Joos
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Dr. David C. Frank
Eidgenössische Forschungsanstalt für Wald, Schnee und Landschaft (WSL)

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Summary

Tree growth response along an elevational gradient: climate or genetics?

Environment and genetics combine to influence tree growth and should therefore be jointly considered when evaluating forest responses to a warming climate. Here, we combine dendroclimatology and population genetic approaches with the aim of attributing climatic influences on growth of European larch (*Larix decidua*) and Norway spruce (*Picea abies*). Increment cores and genomic DNA samples were collected from populations along a ~900-meter elevational transect where the air temperature gradient encompasses a ~4 °C temperature difference. We found that low genetic differentiation among populations indicates gene flow is high, suggesting that migration rate is high enough to counteract the selective pressures of local environmental variation. We observed lower growth rates towards higher elevations and a transition from negative to positive correlations with growing season temperature upward along the elevational transect. With increasing elevation there was also clear increase in the explained variance of growth due to summer temperatures. Comparisons between climate sensitivity patterns observed along this elevational transect with those from *Larix* and *Picea* sites distributed across the Alps reveal good agreement and suggest that tree-ring width (TRW) variations have a more climate-driven than genetics-driven response at regional and larger scales. We conclude that elevational transects are an extremely valuable platform for understanding climatic-driven changes over time and can be especially powerful when working within an assessed genetic framework.

Climatic drivers of hourly to yearly tree radius variations along a 6°C natural warming gradient

Climate affects the timing, rate and dynamics of tree growth, over time scales ranging from seconds to centuries. Monitoring how a tree's stem radius varies over these time scales can provide insight into intra-annual stem dynamics and improve our understanding of climate impacts on tree physiology and growth processes. Here, we quantify the response of radial conifer stem size to environmental fluctuations via a novel assessment of tree circadian cycles. We analyze four years of sub-hourly data collected from 56 larch and spruce trees growing along a natural temperature gradient of ~6°C in the central Swiss Alps. During the growing season, tree stem diameters were greatest at mid-morning and smallest in the late evening, reflecting the daily cycle of water uptake and loss. Along the gradient, amplitudes calculated from the stem radius cycle were ~50% smaller at the upper site (~2200 m a.s.l.) relative to the lower site (~800 m a.s.l.). We show changes in precipitation, temperature and cloud cover have a substantial effect on typical growing season diurnal cycles; amplitudes were nine times smaller on rainy days (>10 mm), and daily amplitudes are approximately 40% larger when the mean daily temperature is 15-20°C than when it is 5-10°C. We find that over the growing season in the sub-alpine forests, spruce show greater daily stem water movement than larch. However, under projected future warming, larch could experience up to 50% greater stem water use, which may severely affect future growth on already dry sites. Our data further indicate that because of the confounding influences of radial growth and short-term water dynamics on stem size, conventional methodology probably overstates the effect of water-linked meteorological variables (i.e. precipitation, relative humidity, etc.) on intra-annual tree growth. We suggest future studies use intra-seasonal measurements of cell development and consider whether climatic factors produce reversible changes in stem diameter. These study design elements may help researchers more accurately quantify and attribute changes in forest productivity in response to future warming.

Convergence of conifer leaf and cambial phenology sensitivity to temperature

Despite many studies of external plant phenology, there are fewer studies concerned with the phenology and dynamics of secondary growth, the primary driver behind biomass storage in forests. Greater understanding of how plant growth will respond to climate change is essential from both an ecosystem and management perspective. However, methods used to quantify these trends are under scrutiny; artificial warming experiments appear to have under-estimated the influence of warming on plant communities, while observational studies have limited control over conditions. Here, we present outcomes from four years of weekly observation of tree-ring formation for two different conifers along a 1400-m elevational transect to assess the timing, rate and duration of leaf phenology and xylem cell development. The resumption of secondary growth is most closely linked to early season air temperatures following snowmelt and comparing observations across elevations or between years, we find a convergence of ecosystem sensitivity to warming, implying temperature response overrides other factors. Our results suggest that under moderate warming scenarios the onset of secondary tree growth will occur 4-6 days earlier per °C. However, we also find that the dynamics of growth cessation appear to be more complex than initiation, meaning an earlier start does not necessarily infer a longer season. Our data show that results from natural elevational gradients are extremely valuable to further understanding of forest response to projected warming and are well suited to parameterize improved ecosystem models.

Intra-annual dynamics of non-structural carbohydrates in the cambium of mature conifer trees reflect radial growth demands

The presence of soluble carbohydrates in the cambial zone, either from sugars recently produced during photosynthesis or starch remobilized from storage organs, is necessary for radial tree growth. However, considerable uncertainties on carbohydrate dynamics and the consequences on tree productivity exist. This study aims to better understand the variation of the different carbon pools at intra-annual resolution by quantifying how cambial zone sugar and starch concentrations fluctuate over the season and in relation to cambial phenology. A comparison between two physiologically different species growing at the same site, i.e., the evergreen *Picea abies* and the deciduous *Larix decidua* and between *Larix decidua* from two contrasting elevations is presented to identify mechanisms of growth limitation.

Results indicate that the annual cycle of sugar concentration within the cambial zone is coupled to the process of wood formation. The highest sugar concentration is observed when the number of cells in secondary wall formation and lignification stages is at a maximum, subsequent to most radial growth. Starch disappears in winter while other freeze-resistant non-structural carbohydrates (NSC) increase. Slight differences in NSC concentration between species are consistent with the differing climate sensitivity of the evergreen and deciduous species investigated. The general absence of differences between elevations suggest that the cambial activity of trees growing at the treeline was not limited by the availability of carbohydrates at the cambial zone but instead by environmental controls on growing season duration.

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Chapter 1

Introduction

Research Context and Rationale

A consensus has formed over the past three decades that the Earth's climate is warming and that anthropogenic activities are the primary drivers of this warming (IPCC 2013). The continued warming is now linked to unprecedented changes in the abiotic environment and in ecosystem state and functioning (Serreze et al. 2000; Parmesan 2006; Butchart et al. 2010; Doney et al. 2012). Despite broad scientific agreement that the climate will continue to warm well into the next century, significant uncertainties remain regarding long-term impacts on the Earth's biosphere. Uncertainties include feedback loops, ecosystem resilience, possible species assemblage transitions, and the coherence of observational and experimental evidence concerning response sensitivity (Bonan 2008; Lenton 2011; Wolkovich et al. 2012). Understanding the impacts of warming on forest ecosystems is fundamental as forests contain the majority of terrestrial biomass, act as an essential link regulating earth-atmosphere fluxes and provide extensive, although often poorly acknowledged, services for human populations around the globe (Myneni et al. 2001; Seneviratne et al. 2006; Pan et al. 2011; de Groot et al. 2012).

This dissertation uses high-resolution, intra-annual measurements of tree growth, including weekly leaf phenology and radial growth observations, sub-hourly dendrometer readings, and *in-situ* meteorological measurements to improve understanding of the mechanisms that influence tree and forest response to climate warming. Uniquely, I collect these data along a 1400-m elevational transect located in the central Swiss Alps, an ecosystem within a limited spatial extent where the principal driver of environmental conditions along the gradient is temperature. The range of temperatures experienced by the forested ecosystems along this gradient encompasses temperature change projected for the next century providing a unique opportunity to assess possible responses to future climatic conditions. This long-term sensitivity can also be compared with inter-annual responses collected over four years of measurements. The scientific research in this dissertation contributes to bridging the decadal to centennial "dendrochronological" and the laboratory-based "physiological" time-scales of tree response. Collected data provide insight into and new quantification of forest response to climate change.

This thesis consists of six chapters: an introduction, four scientific manuscripts that have either been published or will shortly be submitted for publication and a final synthesis. This introductory chapter provides an overview of warming impacts on forest growth, reviews basic tree-ring structure, discusses the methods to observe intra-annual growth dynamics, and finally situates my dissertation by explaining the theory behind the experimental design and introducing the study site.

Global Warming and Impacts on Forests

A Warming Climate

Together, instrumental and paleoclimate records provide a broad understanding of the variability and long-term trends of the global climate system. Global temperatures are rising (0.85°C over the past 132 years), and the coherence, magnitude and rate of warming appears to be unprecedented over the past 1400 years (IPCC 2013). It has become clear that anthropogenic activities, including land use change and the burning of fossil fuels (He et al. 2014), are the dominant drivers of warming in the atmosphere, the oceans and the cryosphere. Atmospheric CO_2 concentrations have moved beyond levels recorded on the planet in the last 800,000 years (Augustin et al. 2004) and are continuing to rise, along with other greenhouse gases, influencing ensemble climate models that project future warming (IPCC 2013). In the near-future (from 2016-2035), there is a high likelihood of mean global air surface temperatures continuing to increase, with medium confidence that projected increases will be between 0.3 to 0.7°C relative to the period 1985-2006, a much more rapid pace (Kirtman et al. 2013). Looking further ahead to the end of the 21st century, warming is expected to continue, and under the most pessimistic emission pathway 2081-2100 surface temperatures are expected to be between 2.6 to 4.8°C greater than the 1986-2005 baseline (Collins et al. 2013). All of the projected scenarios reveal warming will not be uniform and that the land surface is likely to warm 1.4 to 1.7 times the rate of the ocean. Additionally it is virtually certain there will be increases in the frequency, duration and magnitude of hot extremes on both a daily and seasonal scale (Collins et al. 2013). This dissertation deals explicitly with forest response to experienced warming (specifically focused on temperate latitudes), and the following sections explore observed and expected changes to trees, which are essential components of our terrestrial ecosystems.

Warming Impacts on Temperate Forests

Trees found in temperate forests appear to have three fundamental pathways or scales on which warming temperatures can play a role; i) the response of physiological growth processes within season, ii) shifts in the timing of the growing season, and iii) long-term genetic adaptation. Here, I provide a comprehensive overview of these topics.

Anchored to a single location and extending into the atmosphere, trees are greatly influenced by both soil and air temperatures. It is well-known that most biochemical processes proceed at increased rates under warmer temperatures (Hegarty 1973); however, specific focus on the physiological processes of photosynthesis and respiration is important for tree growth (Saxe et al. 2001). Studies have generally identified 0°C and somewhere between 25-40°C as the lower and upper limits, respectively, for tree biological activity (Kozlowski et al. 1997). While this may seem a relatively wide range, growth morphology and other pathways can regulate internal tree temperatures, especially at the leaf surface, preventing photosynthetic declines at both low and high temperatures. Overall, increases in temperature over the next century are likely to increase the rates of photosynthesis at temperate latitudes. However, this does not necessarily translate into increased growth as autotrophic respiration, which increases more than photosynthesis at different points during the growing season, may consume up to 70% of photosynthetic production (Ryan et al. 1997; Piao et al. 2008). Over time, respiration increases will likely acclimate and become more closely linked to substrate availability (Landsberg and Sands 2010). This balance between photosynthesis and respiration means that temperature increases of less than 2°C (over a baseline of the year 2000) are likely to lead to increased growth although species-specific responses are expected (Saxe et al. 2001).

Closely linked with a warming influence on tree growth are related changes in water availability. The growing parts of a living tree are 80-90% water, and a tree's trunk is more than half water (Kozlowski 1962). Water is required for photosynthesis, acts as the primary solvent for moving nutrients and gases around a tree and also maintains the turgor necessary for cell enlargement, preserving the shape of newly formed structures and keeping stomata open during gas exchange. The main pathway of water movement is transpiration. Although this represents water loss, it also establishes a potential gradient for water uptake from the soil, driving large amounts of water through mature trees. Absorption occurs via roots and water ascends to the leaves via the xylem (Kozlowski et al. 1997; Tyree and Zimmermann 2002). Overall, trees maintain a water budget, and deficits can have severe impacts including greater rates of respiration and decreases in rates of cell enlargement. Although they are linked with increased growth in many areas, increased temperatures may also induce greater water stress on trees in dry, moisture-limited sites (Saxe et al. 2001). Additionally, alongside warming average temperatures, forests will need to adapt to increased climatic variability, often in the form of extreme events such as drought, that will have large impacts on sensitivity and landscape processes (Lindner et al. 2010; Vasseur et al. 2014). These events have been linked with temperature-induced droughts that lead to hydraulic stress within the branches and roots of individual trees (Anderegg et al. 2012; Choat et al. 2012).

Warming temperatures also play a role in the timing of seasonal growth onset and cessation. Increased temperatures appear to have a significant effect on growing season length, prominently influencing earlier budburst dates due to warmer springs (Menzel et al. 2006). Other processes such as growth cessation, frost hardening, winter chilling requirements, breaking dormancy and leaf and stem growth onset will all be advanced or delayed by warming temperatures, although the links among the various processes are still uncertain (Rossi et al. 2008b; Way 2011;

Richardson et al. 2013). An increase in growing season length means that critical temperatures for plant growth (6-7°C) will often be exceeded at higher latitudes and elevations (Körner and Paulsen 2004). Following the hypothesis of climatic niche conservatism (Zimmermann et al. 2009; Amano et al. 2014), the distributions of some species will likely shift, invoking the possibility of novel species assemblages (Thuiller et al. 2008).

As long-lived organisms, trees often tolerate short-term variability, but under a prolonged directional pressure such as climate warming, it is essential to consider the longer-term implications for tree genetic adaptation and possible evolutionary changes. Tree growth response to environmental signals is mediated, amplified or altered by an individual's genetics, each exhibiting a range of possible expressions known as phenotypes. The ability of an individual to express a wide range of different phenotypes depending on the environment reduces severe effects of warming. Called phenotypic plasticity (Bradshaw 2006), conifer traits such as xylem morphology can reveal large variations that contribute to an ability to survive a wide range of conditions (Martinez-Meier et al. 2009; Bryukhanova and Fonti 2013). Conversely, the adaptive capacity of a species strongly depends on its length of time in a region, selective pressures and gene flow (Aitken et al. 2008). For example, trees that under-utilize changing resources through delayed growth onset or premature dormancy will likely be replaced over time by individuals taking advantage of a longer growing season (Saxe et al. 2001). However, the relative importance of plasticity and adaptation in response to warming remains uncertain and it is unclear if the rate of warming may overwhelm an individual's capacity to adapt (Jump and Peñuelas 2005).

In this dissertation, I investigate response across all of these major pathways. Physiological processes such as temperature and moisture limitation on tree growth are studied using increment cores for a decadal-scale perspective (CHAPTER II) and high-resolution dendrometers to offer an intra-annual perspective on the water continuum within mature alpine conifers (CHAPTER III). I also provide measurements of growing season length based on both leaf and cambial phenology to investigate the sensitivity of those processes to variations in temperature inter-annually and on a decadal scale (CHAPTER IV). Finally, I assess population-level tree genetics, measure gene flow across the elevational gradient and consider the importance of genetic and climatic drivers on tree growth (CHAPTER II).

Tree-ring Structure and Measurement Techniques

In temperate regions, climatic variation driven by annual cycles produces a periodic dormancy of tree growth, leading to the creation of distinct annual rings (Schweingruber 1996). Trees therefore provide records at an annual resolution, covering long periods of time, and with broad spatial distribution, providing a valuable method for the retrospective quantification of tree growth (Fritts 1976). Generally tree rings are treated as an integrated record of environmental conditions over a growing season (MacDonald et al. 1993; Büntgen et al. 2011). Yet, many processes underlying trees' physiological response to climate operate on significantly shorter time-scales (i.e. hourly or daily) which are difficult to resolve using standard dendrochronological techniques, and require data collection on finer scales. To provide greater context regarding the intra-annual scale of response, I provide a brief overview of the process of tree-ring formation in conifers, the group of trees studied in this dissertation and an introduction to how these processes can be moniotored.

The Process of Xylem Formation

The physiological basis of tree growth is the assimilation of raw materials resulting in cell division within the meristematic tissues of the plant (Kozlowski 1962). Meristems contain undifferentiated cells responsible for two different types of growth within a tree. Primary growth, the lengthening of stems and roots, results from apical meristem division, while secondary growth is the result of lateral meristem division and produces an increase in diameter. The vascular cambium, a lateral meristem, has been identified as the source of conductive tissues for about three hundred years, although Hartig (1853) was the first to suggest its physical structure. This concept

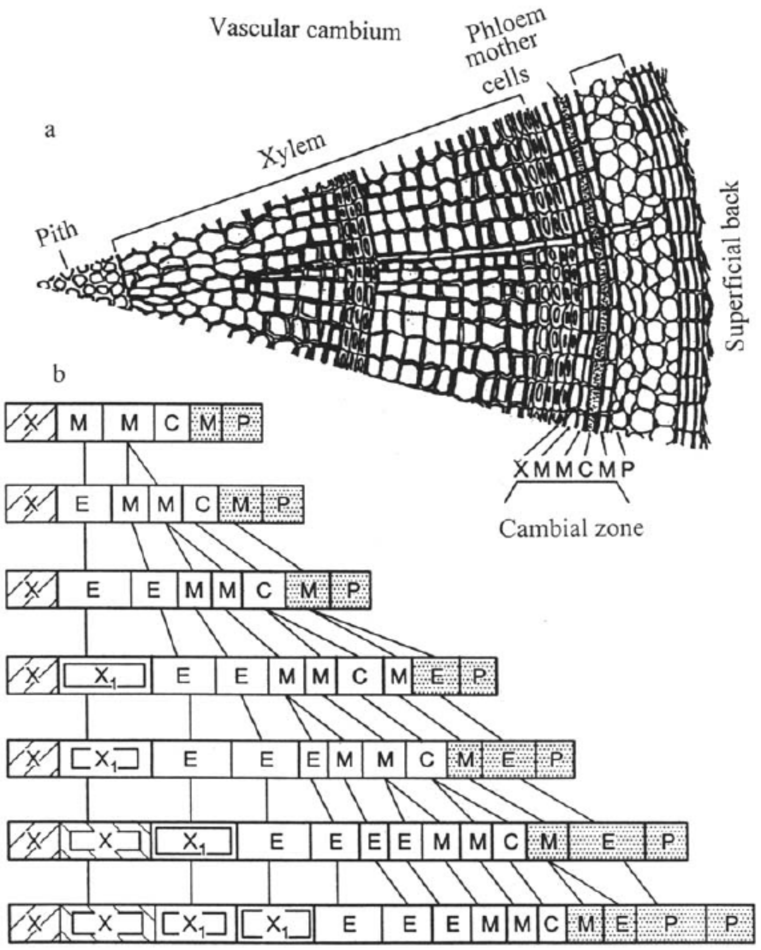

Figure 1.1. Wood cross-section of pine (a) and scheme illustrating production in the cambial zone (b); C (cambial initial), M (mother xylem and phloem cells), X (mature xylem cells), P (mature phloem cells), and E (cells in the enlarging phase) (from Vaganov et al. 2006)

has been refined from a biserate layer of cells to a self-perpetuating single layer of cells, which divide to produce mother cells that subsequently divide once or twice and mature into either xylem or phloem (Sanio 1873; Fraser 1952; Bannan 1955; Bannan 1968). Throughout this dissertation I follow the nomenclature proposed by Wilson et al. (1966), who define the cambial zone as including all cells capable of division, including cambial initials and both phloem and xylem mother cells (Figure 1.1). The phloem conducts sugars and resources produced by photosynthesis throughout the tree, while xylem transports water and nutrients, provides structural support and is a storage reservoir for the tree (Kozlowski et al. 1997). Conifer xylem is composed of two cell types: paranchyma and tracheids (Tyree and Zimmermann 2002). Paranchyma cells form rays that provide carbohydrate storage and transport, and resin ducts that function as wound response (Spicer and Holbrook 2007). These cells can be extremely long-lived and make up between 5-10% of wood volume in conifers. Tracheids, the main focus of my work, are the primary building blocks that make up more than 90% of wood volume and are organized into regular radial files (Vaganov et al. 2006).

Each xylem tracheid differentiates following an ordered sequence of enlargement, wall-thickening and maturation (Kramer 1979). Radial enlargement is driven by water absorption in cellular vacuoles, and the resulting turgor pressure leads to cell expansion (Abe et al. 1997). Expansion takes place while tracheid cells have a thin primary wall that can be stretched. Once expansion is complete, tracheids begin to deposit a more rigid secondary cell wall on top of the primary walls. Directionally oriented cellulose microfibrils are laid down and filled with the organic

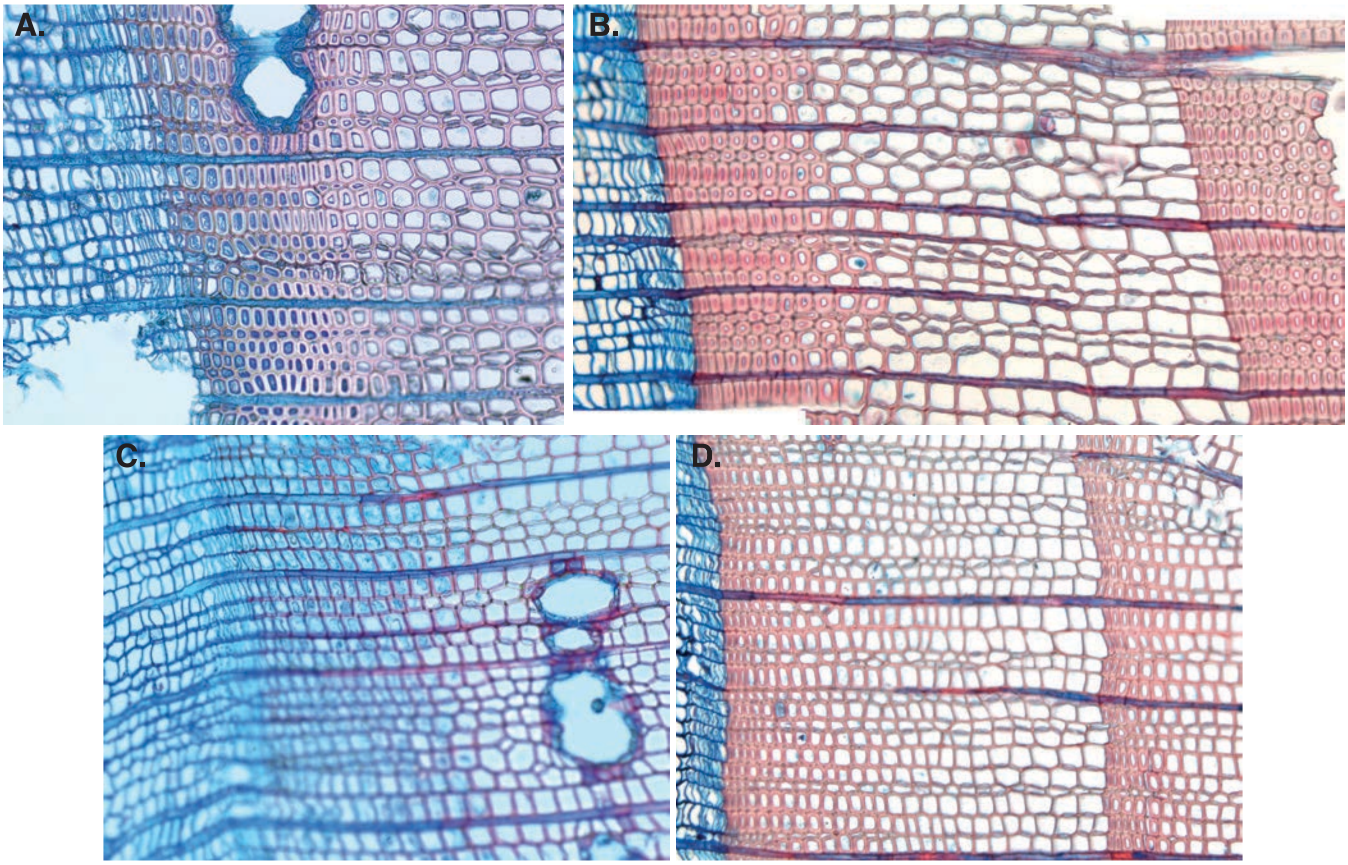

Figure 1.2. Microsection photographs of *Larix decidua* (A & B) and *Picea abies* (C & D). Sections are from a still-forming ring (A & C) and trees that have completed growth (B & D).

polymer lignin, although substances such as hemi-cellulose, pectin and some proteins are also involved (Kramer 1979). Secondary walls in conifers are heavily lignified, both to provide structural support and to maintain form under large water pressure differentials. The final stage of differentiation is the autolysis of the nucleus and all cellular organelles. Once dead, the cell is considered mature and assumes its primary function of water transport (Wilson et al. 1966). Tracheid development follows a seasonal progression, transitioning from the earlywood, with large radial cell diameters (7-8 times the size of a cambial initial) and thin cell walls, to latewood, with small radial diameters (2-3 times the size of a cambial initial) and thicker walls (Vaganov et al. 2006). These properties produce the internal structure of rings, which can be analyzed for width, density, or cell anatomical characteristics (Schweingruber 1996). Based on the limitations of dendrochronology and full ring analysis discussed above, I use the anatomy of ring formation to examine the processes and drivers underlying a single year of growth.

Monitoring Intra-annual Tree Growth

Significant interest in how trees grow has led to advances in several methods for measuring and monitoring growth each with their own advantages and disadvantages. Below is an introduction to the development of methods used in this dissertation along with highlights of several key findings following their use. The research contributing to this dissertation captured information about the seasonal progression of cambial cell development using two primary methods, microcoring and dendrometers.

Microcoring involves the incremental collection of forming wood over the length of the growing season (Figure 1.2). Early intra-annual tree growth research using this method was coarse and required large samples, which both crushed thin-walled forming xylem cells and limited the number of samples that could be taken without severely impacting subsequent tree growth.

Figure 1.3. Pictures of a band or circumerfence dendrometer (A), a point or radial dendrometer (B) and installed dendrometers at a site used in my dissertation (C).

This early research focused was placed on the way in which growth varies within a tree and on comparisons among species (Hartig 1878; Mischke 1890; Brown 1912; Kienholz 1934). Improved experimental work on xylem differentiation led to the development of many ideas regarding controls on growth and rates of differentiation, while also establishing terminology that set the basis for further intra-annual work (Larson 1960; Kozlowski 1962; Wodzicki 1964; Wilson et al. 1966). Clarity and consensus on the physical structure and role of the cambium led to studies investigating the environmental factors that influence wood formation (Denne 1971; Antonova et al. 1983). More recently, sample collection advanced to the use of small surgical bone punches (Antonova and Stasova 1993; Deslauriers et al. 2003a; Makinen et al. 2003) or specifically designed tools, including the TREPHOR (Forster et al. 2000; Rossi et al. 2006a). These new instruments allow more frequent sampling, conforming the large role that temperature plays in tracheid development, although several studies have identified other factors such as photoperiod, tree age, tree size and social status that also appear to influence cambial dynamics (Rossi et al. 2006b; Rossi et al. 2008a; Rathgeber et al. 2011; Cuny et al. 2012). Most analysis of intra-annual data in the last fifteen years has used a Gompertz sigmoid function to model the data (Camarero et al. 1998; Rossi et al. 2003), but recent work has suggested general additive models are capable of improved insight (Cuny et al. 2013).

Dendrometers, an alternative to direct samples causing cambial damage, are sensors placed on the exterior of the tree to observe tree growth dynamics and have been in use since the late 19th century (Christison 1889; Jost 1892; Friedrich 1897). Dendrometers are either band-type, measuring changes around the circumference of the stem or radial, point-type (Figure 1.3; Reineke 1932; Daubenmire 1945).

Although observations were hindered by lower resolution, early researchers recognized that dendrometers do not measure just growth, but also reversible changes in the stem driven by internal water movement (MacDougal 1921). Early dendrometers required visual collection of observations and had a number of disadvantages, including their tendency to record artifacts not related to growth. In the 1950s, recording dendrometers were designed, inscribing data onto a revolving barrel (Fritts and Fritts 1955) or electronically (Phipps and Gilbert 1960), which permitted continuous observation of the tree stem. During this period, several studies used dendrometers to investigate drivers of diurnal variations and to make comparisons between different species and tree growing in different environments (Daubenmire 1949; Fritts and Fritts 1955; Bormann and Kozlowski 1962; Kozlowski and Winget 1964). Continued technology advancements have contributed to the ability to collect stem measurements at a very high spatial and temporal resolution and to store these large amounts of data electronically (Drew and Downes 2009). Attempting to isolate a growth signal from large amounts of data has proven challenging. The most common approach has been the stem cycle method which defined growth as the cumulative positive increment after splitting each diurnal cycle into three component parts: shrinkage, recovery and growth increment (Downes et al. 1999). Many researchers have subsequently used daily weather variables to explain variability of the isolated "growth" (Deslauriers et al. 2003b; Gruber et al. 2009; Krepkowski et al. 2011; Duchesne et al. 2012). However, the focus on understanding growth has led researchers to overlook the reversible fluctuations of the tree stem as a source of information about water balance. The diurnal water movement has been linked to lag effects between transpiration and soil water uptake (Lassoie 1973; Lassoie and Salo 1981; Herzog et al. 1995; Zweifel and Häsler 2001). Further work involving simple physiological models and increased consideration of stomatal regulations has also expanded our understanding of tree water deficits, providing insight into the efficiency of species response to drought conditions (Zweifel et al. 2005; Zweifel et al. 2007). Despite suggestions that stem hydration patterns obscure the onset, cessation and rates of growth, several authors have tested the role of stem rehydration as a signal for growth onset (Makinen et al. 2008; Turcotte et al. 2009; Korpela et al. 2010).

Substantial advances have been made in the ability to assess tree growth *in-situ* and over the course of the entire year. Understanding the controls on cambial division and tree growth is essential for forecasting how trees will respond to projected climate change and the development of mechanistic models can provide a more complete picture of forest growth (Anchukaitis et al. 2006; Vaganov et al. 2006). Cambium growth models, which inherently require a thorough understanding of influences, provide a more complete basis for data interpretation for those who use tree rings as an archive of environmental information (Breitenmoser et al. 2013). Model output can also improve subsequent ecosystem process models, of which tree growth is often an essential component.

Establishment of an Elevational Gradient in the Swiss Alps

My dissertation is primarily concerned with alpine ecosystems, as they are particularly sensitive to increases in temperature and provide insight into the effects of future warming on temperate forests. In this section I discuss utilizing elevation as a platform to study the influence of temperature warming on trees, provide background on the significant warming experienced by alpine ecosystems and finally situate this dissertation within the unique research environment of the Lötschental.

Harnessing Vegetation Changes Across Elevation

Mountain vegetation zones have stimulated the investigation of controls on plant distribution for several centuries (Von Humboldt and Bonpland 1807; Tranquillini 1979; Körner 2003). Different climatic environments located in close proximity represent an excellent opportunity to elucidate major influences on growth and provide an exceptional platform for insight into both the evolutionary and ecological basis of an ecosystem. The primary driver of change with elevation is considered to be temperature and, to a lesser degree, atmospheric pressure and radiation (Körner 2007a). Close links between temperature and alpine tree growth are suggested by research linking the upper limits of global treelines to a mean growing-season temperature of approximately 6-7°C (Korner 1998; Körner and Paulsen 2004; Körner 2007b) while also observing that warming temperatures are contributing to longer growing seasons which, especially at lower elevations, has led to increased evapotranspiration and associated drought-induced mortality (Allen et al. 2010; Lévesque et al. 2013). Furthermore, the range of temperatures experienced from valley bottom to treeline represent analogues of past and future climate conditions (CHAPTER II). Thus, this dissertation uses a steep elevational gradient as an opportunity to harness an exceptional "natural laboratory" to assess tree- and forest-level response to projected conditions and provide a perspective on what they may resemble in the future.

A Focus on Alpine Regions

Along with coastal and high latitude regions, mountains are expected to be among the areas most highly impacted by increased temperatures (Beniston 2003). Mountains play a large role in carbon sequestration and storage, as 28% of the world's forests are located in alpine environments (Fischlin, A et al. 2007). They contain exceptional species richness and provide ecosystem services such as water filtration that extend well beyond their boundaries. Mountains appear to be particularly sensitive to warming, and several studies and models have observed greater rates of warming in alpine regions than on other land surfaces, especially in temperate mountain regions such as the Swiss Alps (Figure 1.4; Böhm et al. 2001; Ceppi et al. 2010). The drivers behind these trends remain uncertain,

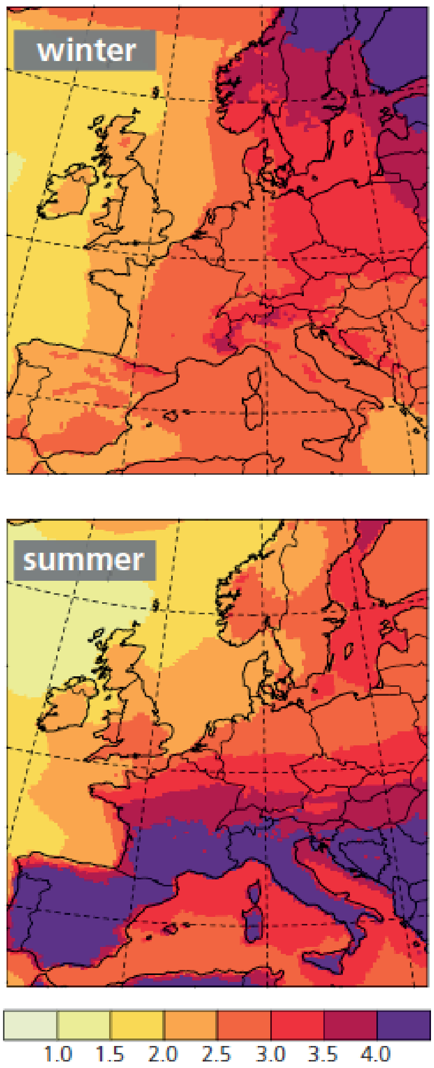

Figure 1.4. Air temperature increases projected from climate model simulations across Europe for the period 2070-2099 for both winter and summer. Greater warming is visible across the greater alpine arc, especially prominent in winter. Figure from Swiss Climate Change Scenarios Report (CH2011 2011)

but are likely related to heat flux dynamics, resulting in increased heat retention during the day and an associated increase of long-wave radiation at night, especially during the winter and spring (Keller et al. 2005; Rangwala and Miller 2012). As vegetation distributions in mountain environments are closely coupled with climate, they are expected be significantly affected by increased warming. Plant communities will likely undergo upward migration, shifting toward higher altitudes in pursuit of appropriate temperatures, compressing them into smaller land areas, and making them more vulnerable to further pressures. In fact, alpine plant and animal species appear to be extremely sensitive to warming as current populations contain genetic signatures indicative of pressures linked to past climatic change (Bonin et al. 2006). Based on models of species response, projected warming could result in 60% extinction rates of alpine plant species across Europe (Thuiller et al. 2005).

The Lötschental

The Lötschental is an inner-alpine, east-west situated glacially formed valley. The top of the valley remains glaciated (Langgletscher) and is a part of the Aletsch-Jungfrau-Bietschhorn UNESCO world heritage site. Initial settlement

Figure 1.5. Mixed coniferous forest (primarily spruce and larch) found on the slopes of the Lötschental, here above the village of Ferden. The Chastlara avalanche path can be seen on the left of the picture.

of the valley likely took place during the Roman period, but remained isolated due to geographic barriers that make access to the valley difficult. Close links exist between the four main villages spread along the bottom of the valley and the steep forested slopes above them (Figure 1.5). The mixed spruce and larch forests served as an essential construction and cultural resource for the population and remain an important physical barrier to potentially destructive rockfalls and avalanches.

The Lötschental has been the site of numerous tree-ring-based investigations including the climate response of tree growth (Neuwirth et al. 2004; Frank and Esper 2005b), climate reconstruction from tree-ring width (Büntgen et al. 2005; Frank and Esper 2005a), maximum latewood density (Frank and Esper 2005a; Büntgen et al. 2006b), and stable isotopes (Treydte et al. 2001; Kress et al. 2010). Tree rings were also used to date historical buildings (Büntgen et al. 2006a) and insect outbreaks over the past centuries (Esper et al. 2007; Büntgen et al. 2009; Johnson et al. 2010). These investigations provide an extensive knowledge base for the research in this dissertation. At the same time, the research conducted in this dissertation contributes to an improved mechanistic understanding for the interpretation of these dendrochronological investigations, whose length ranges from centuries to millennia.

Thesis Structure & Objectives

The following four chapters are a cumulative work that advances knowledge of different aspects of intra-annual growth analysis. Chapters two through five are manuscripts that have been accepted or are being prepared for publication in peer-reviewed literature. Each chapter analyzes different components of tree growth response to warming across an elevational gradient, providing understanding of how mountain forests could respond in the future. CHAPTER II investigates decadal to centennial climate response of conifers growing along the elevational gradient in the Swiss Alps. Most importantly, it investigates the possible confounding factors of local adaptation and gene flow of individuals along the transect and provides a basis for attributing growth to genetic or climatic drivers. CHAPTER III focuses on a high-resolution, extensive network of dendrometer data collected along the elevational gradient. I introduce a novel technique to assess and quantify tree's diurnal stem water movement. I investigate the influence of weather and climate on these dynamics and also compare responses across elevation and between species. CHAPTER IV compares weekly observations of leaf and cambial phenology collected along the transect. Using an improved modeling approach to provide accurate estimates of onset, cessation and rates of xylem formation, I investigate environmental drivers and compare inter-annual and multi-decadal sensitivities. CHAPTER V traces the movements of non-structural carbohydrates in two conifer species throughout a growing season. The chapter presents these results within the context of cambial phenology data collected along the elevational gradient and suggest links between the dynamics of wood formation and the presence and ratios of several mobile carbohydrates. CHAPTER VI provides a synthesis of the dissertation, linking the completed work into a cohesive picture of advances in our understanding of intra-annual growth. I discuss associated ongoing projects that will complement our research and provide several suggestions for future questions that can be addressed with this extensive dataset. The total work provides a body of knowledge that moves us further toward bridging the gap between two relevant scales of inquiry into tree growth response to climate change.

Project Funding

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Chapter 2

Tree growth response along an elevational gradient: climate or genetics?

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Introduction

Recent climatic warming has been implicated as a driver in shifts of physiological and ecological processes (e.g. timing and rates of growth, species range shifts) of both plants and animals worldwide (Parmesan 2006). These changes may reflect the inherent ability of individuals to plastically respond to environmental variation, but it remains unclear if some of these observations point to genetic adaptation and possible evolutionary change within species (Hoffmann and Sgrò 2011; Donnelly et al. 2012). Further understanding the interaction between genetics and plant response to environmental drivers is crucial to improve forecasts of species survival and provide accurate conservation planning under increasing environmental pressure.

The question of genetic versus plastic response is of particular relevance for trees because they are long-lived, stationary, and spend relatively long periods of time in juvenile (non-reproductive) states (Jump et al. 2006). Recent rapid climate change has led to uncertainty about the ability of some tree species to keep pace with their changing environmental envelopes with unknown consequences for future forest composition (McLachlan et al. 2005; Visser 2008). Because about 30 percent of the Earth's land surface is covered by forests (FAO 2010), these ecosystem changes will have consequences for carbon and water cycles, rates of net primary production and many other ecosystem services that human societies rely upon (Courbaud et al. 2011).

Dendrochronology is a frequently used method in tree and forest growth research. Annual growth rings are used to interpret the biological response to climate and the stability of this response across inter-annual to centennial time-scales. Although tree-ring data are commonly employed to reconstruct climate variability (e.g. Frank et al. 2010; Büntgen et al. 2011), dendrochronology has also been used to evaluate how the growth of tree populations might respond under future climate scenarios. For example, Goldblum (2010) used white oak chronologies distributed across its range in North America to model the response to past climate, and in tandem with temperature projections, predicted radial growth decreases across much of the species' northern range. Similarly, Pasho et al. (2011) analyzed the influence of water availability on eight Mediterranean tree species across a climatic gradient in north-eastern Spain. The authors found high variability in species responses across the gradients investigated, and concluded that under more frequent and severe droughts most trees within the study area would be adversely affected. While we do not wish to review all dendrochronological articles that have not explicitly considered the genetic composition of the populations under investigation, these two studies are representative of many previous works that implicitly assume observations were driven solely by physiological responses to climatic change with no genetic contribution.

The importance of considering the underlying genetic composition of populations is demonstrated by Ohsawa and Ide (2008), who found differences in genetic diversity with altitude in 70% of studies they reviewed, with more than 50% of these studies observing inter-population genetic differentiation. The presence of differentiated populations is an indication that natural selective pressures and in situ adaptive response may exist, casting doubt on the ability to assess future species response to climate variability without consideration of the genetics of populations under study. However, it should be considered that genetic differentiation among populations is a direct function of migration, i.e. gene flow, and random genetic drift. Proximity of populations and large effective population size thus likely prohibit genetic differentiation. Moreover, gene flow counteracts local adaptation, so that only strong selection may lead to adaptation under high migration rates.

Two prominently used approaches to study the genetics of populations are common gardens and molecular markers. Common garden (provenance) tests are classic experiments that attempt to separate climatic and genetic signals by growing plants from different environments in a single location and measuring their growth response (Kawecki and Ebert 2004). Considering trees, these studies require a significant amount of time, as a second generation is required to measure the true heritability of traits. More recently, studies have turned to neutral molecular markers (e.g. microsatellites) to investigate genetic variability and differentiation within and between tree populations (Holderegger et al. 2010). A shift to molecular methods is being prompted by faster processing times, the ability to sample a far greater number of individuals within a population and lower costs (Selkoe and Toonen 2006). Clearly,

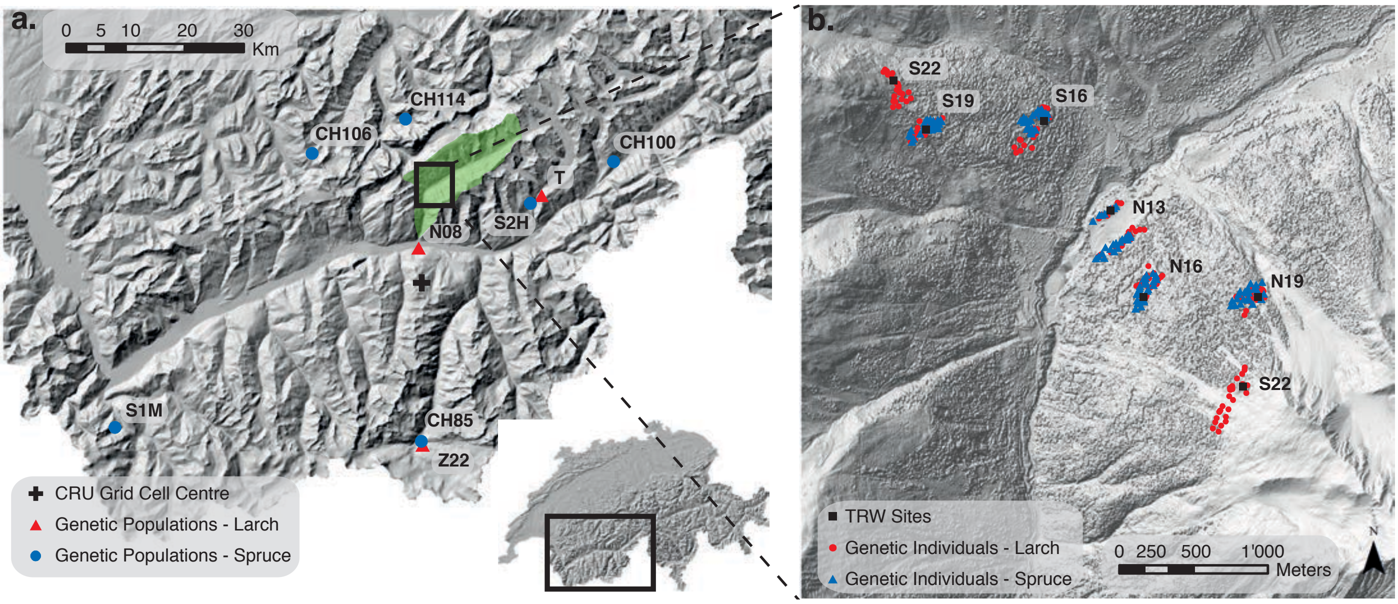

Figure 2.1: (a) Regional relief map showing the location of the sampling area (black rectangle) and the regional genetics sampling populations in filled black circles (*Larix decidua*) and filled black triangles (*Picea abies*). The black cross represents the grid point center for the CRU data covering the period 1901-2009. (b) Hillshade digital elevation model of the Lötschental indicating the tree-ring sampling locations (white squares) and the location of each individual for genetic analysis with light grey circles (*L. decidua*) and dark grey triangles (*P. abies*).

these markers do not directly inform about adaptive responses of populations (or absence thereof), but are taken as indicators of demographic processes and of gene flow. Accordingly, one may interpret measures of extensive gene flow, as obtained from neutral molecular markers, as indirect evidence of reduced chances for local adaptation, because very strong selective pressures would be required to overcome the constant immigration of non-local alleles (Savolainen et al. 2007).

Here, we perform a comprehensive study of climatic influences on tree growth after considering possible genetic effects. Increment cores and tissue samples were collected from two conifer species (*Picea abies* and *Larix decidua*) along a 900-meter elevational transect. We first test if populations are genetically well-mixed along the length of the elevational gradient. We then aim to quantify the climatic controls on intra-annual tree growth and assess if this transect may be used to further investigate alpine conifer response to projected climate warming. We compare our results from the elevational gradient to a dataset of sites from across the Alps to evaluate tree growth response across larger spatial scales and hypothesize what role genetics may play in these relationships.

Methods

Study Area

Our study location was the Lötschental, an inner-alpine valley located in the central Swiss Alps (46°23’40”N, 7°45’35”E; Figure 2.1). The valley is oriented along a northeast-southwest direction and connects to the Rhône Valley (canton of Valais). Sampling sites were established on the forested slopes around the town of Ferden, consisting of primarily evergreen Norway spruce [*Picea abies (L.)* Karst.] and deciduous European larch [*Larix decidua* Mill.] extending from 1300 to ~2300 m a.s.l. (within the manuscript, species are addressed by genus names). The valley has a cool, dry climate with a mean annual temperature of 6°C, ranging from -3°C (January) to 15°C (July) and a mean annual precipitation exceeding 800 mm (data from MeteoSwiss surface observation network). The valley soils are formed from calcareous-free substrate, including moraines and crystalline bedrock (gneiss and granite) from the Aar massif. This contributes to acidic soils characterized by coarse stone content and low amounts of clay. Several soil types were observed, ranging from Leptosol, Cambisol to Podzol, with intermediates.

Climate and Meteorological Data

In-situ air temperatures (Onset, U23 Pro loggers) were recorded at 15-minute intervals beneath the canopy at each sampling site as part of an ongoing research along this elevational gradient (Moser et al. 2010; King et al. 2013). Temperature differences along the gradient were calculated with monthly averages over a four-year period (2008-2011). Gridded (0.5° x 0.5°) historical temperature and precipitation records (Climatic Research Unit (CRU) TS 3.10; Brohan et al. 2006) were used to correlate with the tree-ring records from the Lötschental. The CRU data were extracted from the closest grid point to our field site (grid center: 46.25° W, 7.75° E) and covered the period 1901-2009. Anomalies were calculated on a monthly basis with respect to 1971-2000 mean conditions. Additional downscaled climate data at a spatial resolution of 1 km2 was used to calculate annual temperature and precipitation amounts for comparisons between our transect sites and sampling sites across Europe (WorldClim; Hijmans et al. 2005).

Tree-Ring Data and Analyses

Seven sites were selected along the 900-m elevational transect; a single site at the valley bottom (~1300 m a.s.l.) and three sites at ~300-m elevation intervals along both the north and south slope aspects to ~2200 m a.s.l., just below the upper forest limit. Codes for aspect (N and S) and elevation (in hundreds of meters) were assigned to each site, e.g. N16 for northern aspect at 1600 m a.s.l. At each site, with the exception of N22 and S22, which contain no *Picea*, two radii from mature trees of both species were sampled at breast height (~1.3 m). An average of 23 ± 4 trees (43 ± 7 series) were sampled per site for a total of 272 trees (518 series) sampled overall.

Standard dendrochronological techniques were used to collect and prepare samples for tree-ring width (TRW) measurement (Schweingruber 1996). Ring variations were visually cross-dated and measured using a LINTAB tree-ring measuring system (precise to 0.001 mm). Two radii per tree were measured in all but a few cases. Cross-dating accuracy was verified with the software program COFECHA (Holmes 1983). Comparisons among different detrending methods (e.g., regional curve standardization, negative exponential, varying spline lengths) were conducted, but no significant differences in the climate sensitivity/response among techniques were found (data not shown). Age-related trends were ultimately removed from individual raw data using a 100-year spline to preserve inter-annual to multi-decadal variability. Mean site chronologies were calculated using the robust bi-weight mean. Chronologies were well replicated over time, with the common period of all sites (minimum of five series) beginning in 1876. If the increment cores did not contain the innermost ring, pith-offset estimates were made using standard techniques based upon ring curvature, multiple cores per tree and a transparency with concentric circles. The time taken for trees to reach coring height (~1.3 meters above the ground) makes the dendrochronological ages younger than the germination age. We use the term tree age to refer to the estimated age of the tree at breast height.

Coherence, trends and absolute rates of growth were assessed and calculated from raw TRW measurements aligned by tree age. Average growth over the first 50 years for each species at each elevation was calculated. Sample depth, expressed population signal (EPS) and running Rbar (mean inter-series correlation) statistics were calculated for each site (Supp. Figure 2.1 and Supp. Table 2.1). Following detrending, pairwise correlations among all trees were calculated and plotted as function of elevation difference. Further comparisons among the standardized site chronologies revealed strong agreement between slopes and subsequent climate-growth correlations were performed with chronologies grouped by elevation for each species.

Pearson correlations between elevation chronologies for each species and gridded temperature and precipitation data were computed for all months from previous June to current December and included two seasonal averages of April-September (AMJJAS) and June, July and August (JJA). To compare spatial data patterns, *Picea* and *Larix* JJA temperature correlations from each site in the Lötschental were plotted against mean annual temperature and then compared with data from the same species (*Larix decidua*, n=65; *Picea abies*, n=220) from a network of published

TRW chronologies from across the Greater Alpine Region (4–19 °E, 43–49 °N; Babst et al., 2013). Linear models were fit to each dataset for both species.

Genetic Sampling and Molecular Analyses

To assess genetic differentiation among tree populations along the elevational gradient, we collected living plant material from 30 individuals of each species at each sampling elevation and aspect. There was significant overlap between the trees sampled for genetics and increment core collection. Four cambial probes per individual were collected with a TREPHOR puncher (Rossi et al. 2006) and immediately dried on silica gel. Each individual's geographic position was collected using a handheld GPS unit. To avoid parental influence through spatial autocorrelation in relatedness, individuals were located a minimum of 30 m from each other.

Needle samples from additional populations outside the study area were included to test if more distant populations showed higher genetic differentiation than that comprised within a single valley. We selected three population samples of *L. decidua*, one of which represented a subsample of the population described in Pluess (2011), and five locations of *P. abies* (Gugerli et al. 2001; C. Sperisen, unpublished data).

We extracted genomic DNA from isolated cambial tissues or needles with the 96-well DNeasy Plant Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. Multiplex polymerase chain reactions (PCRs) for nuclear microsatellite (nSSR) genotyping comprised PCR Multiplex Kit (Qiagen, Hilden, Germany), primer premix with amounts per primer adjusted to obtain balanced amplifications (Supp. Table 2.2), water and about 5ng DNA. On the basis of previous studies (Tollefsrud et al. 2009; Pluess 2011), the following loci were selected: bcLK189, bcLK228, bcLK229, bcLK235, bcLK253, bcLK263, bcLK211(Isoda and Watanabe 2006), and UAKLLy6 (Khasa et al. 2000) for *L. decidua* and EATC1B02, EATC1E03, EATC2B02, EATC1G05 (Scotti et al. 2002), and SpAC1F7 (Pfeiffer et al. 1997) for *P. abies*. Three loci were excluded from the final data set of *L. decidua* because of inconsistent amplification (bcLK211) and the likely presence of null alleles (significant deviation from Hardy–Weinberg equilibrium in each population; bcLK263, UAKLLy6), which left us with five loci for data analysis in each species.

Amplified fragments were electrophoretically separated on an ABI3130 automated capillary sequencer (Applied Biosystems, Foster City, USA) with GeneScan 400HD [ROX™] (Applied Biosystems, Foster City, USA) as an internal size standard. Allele calling and binning was manually performed using Genemapper 3.7 (Applied Biosystems, Foster City, USA).

After confirming that there were no consistent deviations from HWE, except for the two loci excluded from the *L. decidua* data set (see above), and testing for possible linkage disequilibrium (LD), we calculated expected heterozygosity (HE) per locus, averaged over all populations, an analysis of molecular variance (AMOVA), and matrices of pairwise genetic differentiation (FST) among populations per species using Arlequin 3.5.1.3 (Excoffier and Lischer 2010). Statistical significance was assessed at the 0.05 level by generating a null distribution over 1000 permutations. In addition, nSSR data were used to calculate pairwise relatedness among all individuals on the transect (Lynch and Ritland 1999), using GenAlEx 6.4 (Peakall and Smouse 2006). Each of these values was then multiplied by 2 to create a scale ranging from -1 to 1.

Results

Climatic Change and Elevational Transects

Gridded, long-term, instrumental climate records (CRU TS 3.10, Figure 2.2a and b) revealed warming air temperatures and no trend in precipitation sums over the past century. A linear model of climatic anomalies over

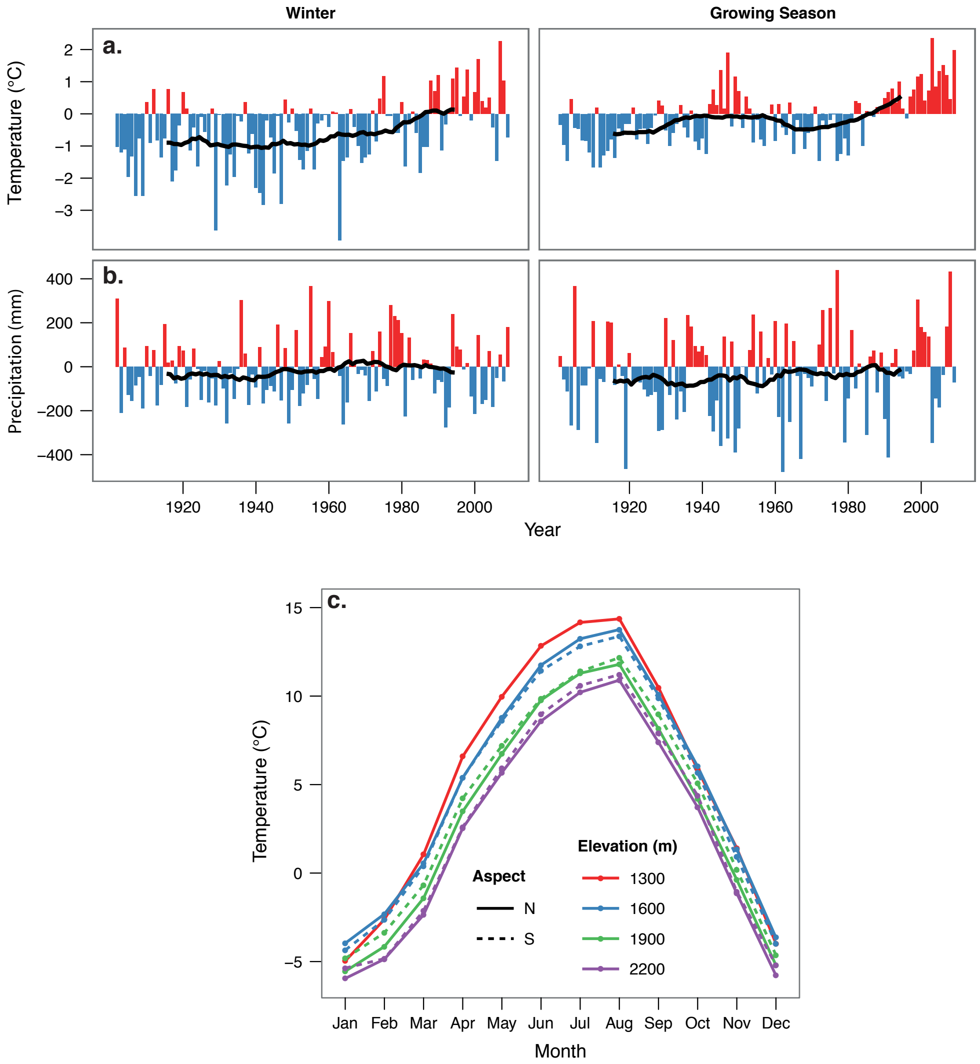

Figure 2.2: (a) Temperature and (b) precipitation anomalies for the period 1901-2009 (departure from 1971-2000 average) for winter (Dec-Feb) and growing season (Apr-Sep). Light grey and dark grey bars show negative and positive anomalies, respectively. Black lines show 30-year running means. (c) Seasonal course of air site temperatures averaged over the period 2008-2011. Elevations are presented as different line types and slope aspects are presented as different colours (black = north aspect, grey = south aspect).

the period 1901-2009 showed that growing season (AMJJAS) temperatures had a significant positive trend of 0.13°C/decade ($F_{1,107} = 39$, $P < 0.001$), while winter temperatures (December-February) showed a similar positive trend of 0.14°C/decade ($F_{1,106} = 20.8$, $P < .001$). These rates correspond to a 1.4°C temperature increase over the 109-year record. The temperature records also revealed an accelerating warming trend during the period 1975-2009, with more frequent positive anomalies both during winter and the growing season. Over these 35 years, the decadal warming trends increased almost five-fold to 0.6°C/decade in the growing season, and increased by about 40% during the winter. An examination of the range of seasonal anomalies provided an assessment of the inherent climatic variability at our sites. The warmest growing season occurred in 2003 (2.4°C departure from mean) while the warmest winter on record (2.3°C), took place four years later in 2007. The lowest winter temperature anomaly of -3.9°C was in 1963 and the coldest growing season in 1910 (-1.7°C). Taking into account these extremes, temperatures showed an approximate range of 4.0 ± 0.8°C during the growing season and 6.2 ± 1.1°C for the winter.

The sampled elevational gradient showed a maximum air temperature difference of 4.2°C between the lowest and

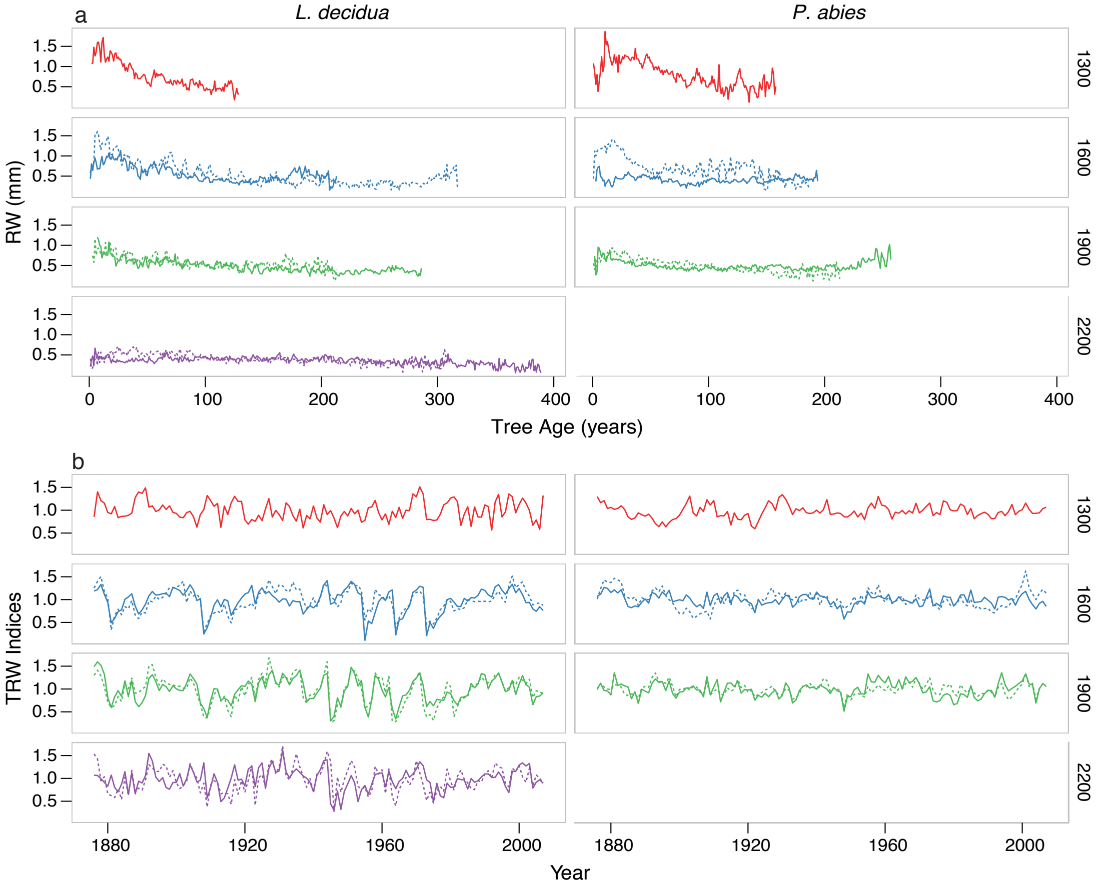

Figure 2.3: (a) Site averaged raw tree-ring width (TRW) chronologies over the complete record, aligned by tree age. (b) Site averaged standardized TRW indices (using a 100-year spline) for the complete period of series overlap (1876-2007), truncated at a minimum of five series. Chronologies are split by species (*L. decidua* in left panels, *P. abies* in right panels) and elevation, and by slope (black = north aspect, grey = south aspect).

highest elevation (Figure 2.2c). This difference is slightly greater than the 4.0°C range of temperature anomalies from 1901-2009. Inter-site differences were more prominent during the growing season than during winter when absolute temperatures along the transect were generally within 2.0°C. Notably, there was greater temperature variability over elevation than between the slope aspects, although the southern aspect was slightly warmer (~ 0.3°C) for most elevations.

Radial Tree Growth Along the Transect

To better understand the growth trends of trees along the transect, we plotted raw TRW and made comparisons between species, slope aspects and elevations. These data show that tree age increased with elevation, that trees are generally older on the north aspect and that *Larix* are older than *Picea*. *Larix* showed consistently higher series inter-correlation and mean sensitivity than *Picea*, and EPS for most sites was above the commonly applied threshold of 0.85 (Supp. Figure 2.1 and Supp. Table 2.1).

Alignment of site TRW chronologies by tree age reveals that radial growth broadly decreased as elevation increased, and that radial growth was generally greater on the southern aspect (Figure 2.3a). Absolute growth rates over the first 50 innermost rings (tree age 1-50) ranged from 1.09 mm/year (1300 m) to 0.44 mm/year (2200 m) for *Larix* and 1.14 mm/year (1300 m) to 0.65 mm/year (1900 m) for *Picea*.

Comparisons of standardized TRW chronologies over the common period (1876-2007) revealed differences

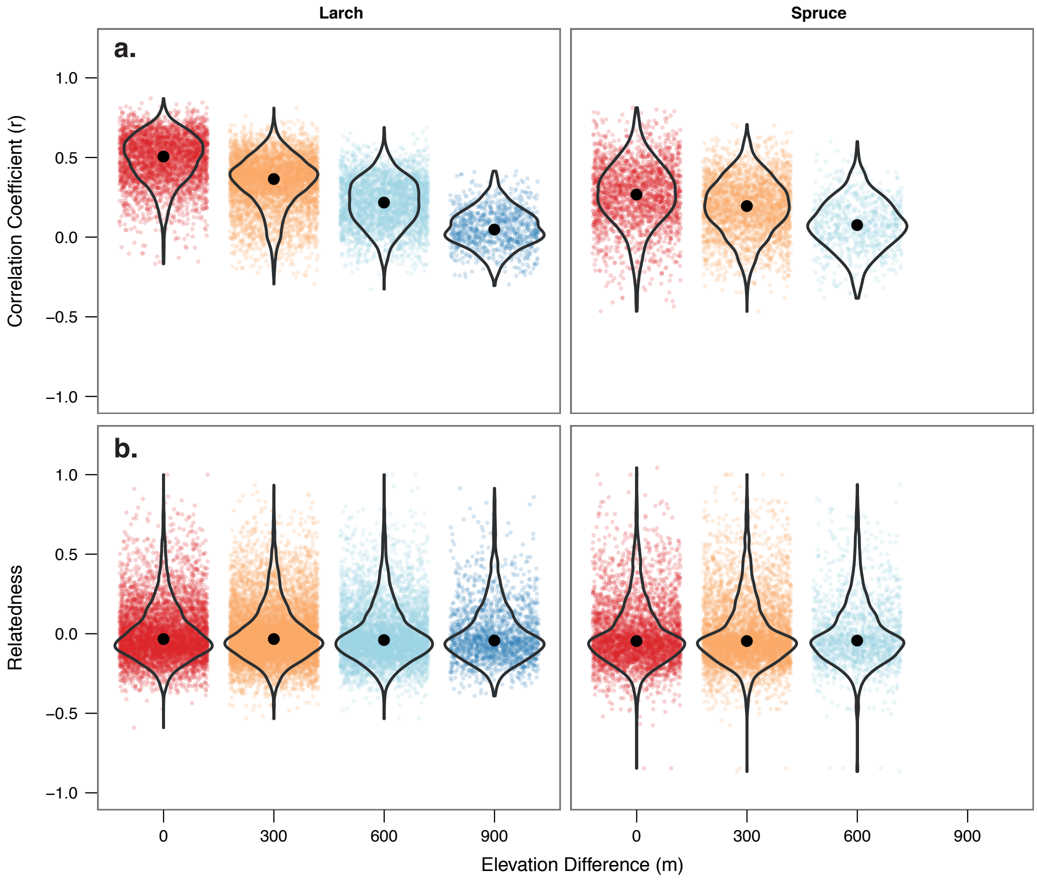

Figure 2.4: (a) Pearson correlation of pairwise individual tree standardized TRW series and (b) pairwise genetic relatedness of individual trees across both slopes. Both are plotted as function of difference in elevation across a transect of *Larix decidua* and *Picea abies*. In all panels, points (jittered with random noise and plotted with a transparency of 20%) represent single comparisons, black dots show the median of the data for each elevation grouping, and black violin plots represent the probability density of observations as calculated from a kernel density estimator.

between species and among elevations, but very high similarity between slope aspects (Figure 2.3b). The most conspicuous patterns were intermittent, abrupt reductions in ring width of *Larix* occurring approximately every decade (especially prominent between 1940 and 1980). These patterns were generally synchronous among elevations above 1300 m and contributed to the higher mean sensitivity and Rbar in *Larix*.

Growth and Genetic Coherence Along Transect

To assess the common signal of the standardized TRW chronologies along the elevational gradient, we computed the pairwise correlations of inter-individual TRW records. These demonstrated a notable decrease in median correlation as elevational distance between individuals increased (Figure 2.4a). Overall, *Larix* showed greater inter-tree correlation than *Picea*, but both species had their highest median correlations between trees at the same elevation. Additionally, computed correlations between *Larix* and *Picea* were all extremely low (< 0.1) and were consistent across all elevations (data not shown). The variability of the correlations decreased in both species with greater elevation difference, although there were also fewer pairwise observations.

An assessment of pairwise genetic relatedness for each individual across all sites (including both slopes) revealed that both species have high genetic similarity (Figure 2.4b). Additional comparisons were made between each Lötschental population and other populations of both *Larix* and *Picea* collected around the canton of Valais (Figure 2.1) in an attempt to provide additional evidence that gene flow at the landscape scale faces barriers. Values

Table 2.1: List of *Larix* and *Picea* populations sampled for analysis in the study.

									Proportion F_{ST} (P<0.05) *		
Species	Location	Code	Elevation (m)	Latitude [°N]	Longitude [°E]	N	Mean H_E ± SD	Overall F_{ST}	L'tal		outside
Larix decidua								0.016			
	Lötschental[1]	N13	1300	*46.39*	*7.76*	30	0.669 ± 0.199		0.00	<	1.00
		N16	1600			30	0.678 ± 0.160		0.00	<	0.67
		N19	1900			30	0.727 ± 0.090		0.00	<	0.67
		N22	2200			30	0.749 ± 0.100		0.00	<	0.33
		S16	1600			30	0.719 ± 0.104		0.17	<	0.33
		S19	1900			30	0.718 ± 0.092		0.00	<	0.67
		S22	2200			30	0.700 ± 0.178		0.17	<	0.67
	Gampel[1]	N08	800	*46.30*	*7.74*	30	0.713 ± 0.088				0.71
	Aletsch[2]	T	1900	*46.38*	*8.01*	30	0.736 ± 0.071				0.14
	Zermatt[3]	Z22	2170	*46.00*	*7.75*	30	0.732 ± 0.142				1.00
Picea abies								0.013			
	Lötschental[1]	N13	1300	*46.39*	*7.76*	30	0.559 ± 0.203		0.25	<	0.50
		N16	1600			30	0.527 ± 0.212		0.00	<	0.67
		N19	1900			30	0.583 ± 0.203		0.00	=	0.00
		S16	1600			30	0.631 ± 0.134		0.00	<	0.50
		S19	1900			30	0.610 ± 0.205		0.00	<	0.17
	Schlettere[4]	CH100	1625	*46.43*	*8.17*	16	0.574 ± 0.197				0.10
	Sillere[4]	CH106	1900	*46.45*	*7.51*	14	0.633 ± 0.229				0.00
	Findelbach[4]	CH85	1800	*46.01*	*7.75*	16	0.604 ± 0.197				0.10
	Oeschinensee[4]	CH114	1580	*46.50*	*7.72*	15	0.629 ± 0.154				0.60
	Val d'Arpette[5]	S1M	1960	*46.03*	*7.08*	30	0.596 ± 0.156				0.40
	Aletsch[5]	S2H	1630	*46.37*	*7.99*	30	0.557 ± 0.199				0.50

*number of occurrences that population shows significant (P<0.05) genetic differentiation to other population, related to number of respective comparisons;
Sample Providers: [1] G. King/WSL; [2] A. Plüss/ETHZ; [3] S. Brodbeck/WSL; [4] C. Sperisen/WSL; [5] F. Gugerli/WSL

of pairwise FST showed that there is a lower degree of genetic differentiation within the Lötschental than between the Lötschental populations and additional populations outside of the valley (Table 2.1).

An analysis of molecular variance (AMOVA) showed that both *Picea* and *Larix* have greater genetic variance estimated to occur within populations (99.56% and 99.43%, respectively) than partitioned between slope aspect groups (0% for both species) or among populations within groups (0.46% and 0.80%, respectively).

Climate Response Along the Transect

To better understand the climate (precipitation and temperature) response of each sampling elevation across the transect, we calculated Pearson's correlations over the 1901-2007 period (Figure 2.5). Several patterns emerged, including a tendency for higher correlations in both species as elevation increased. At all elevations, *Picea* responded positively to precipitation with significant correlation coefficients during both the previous and current summer.

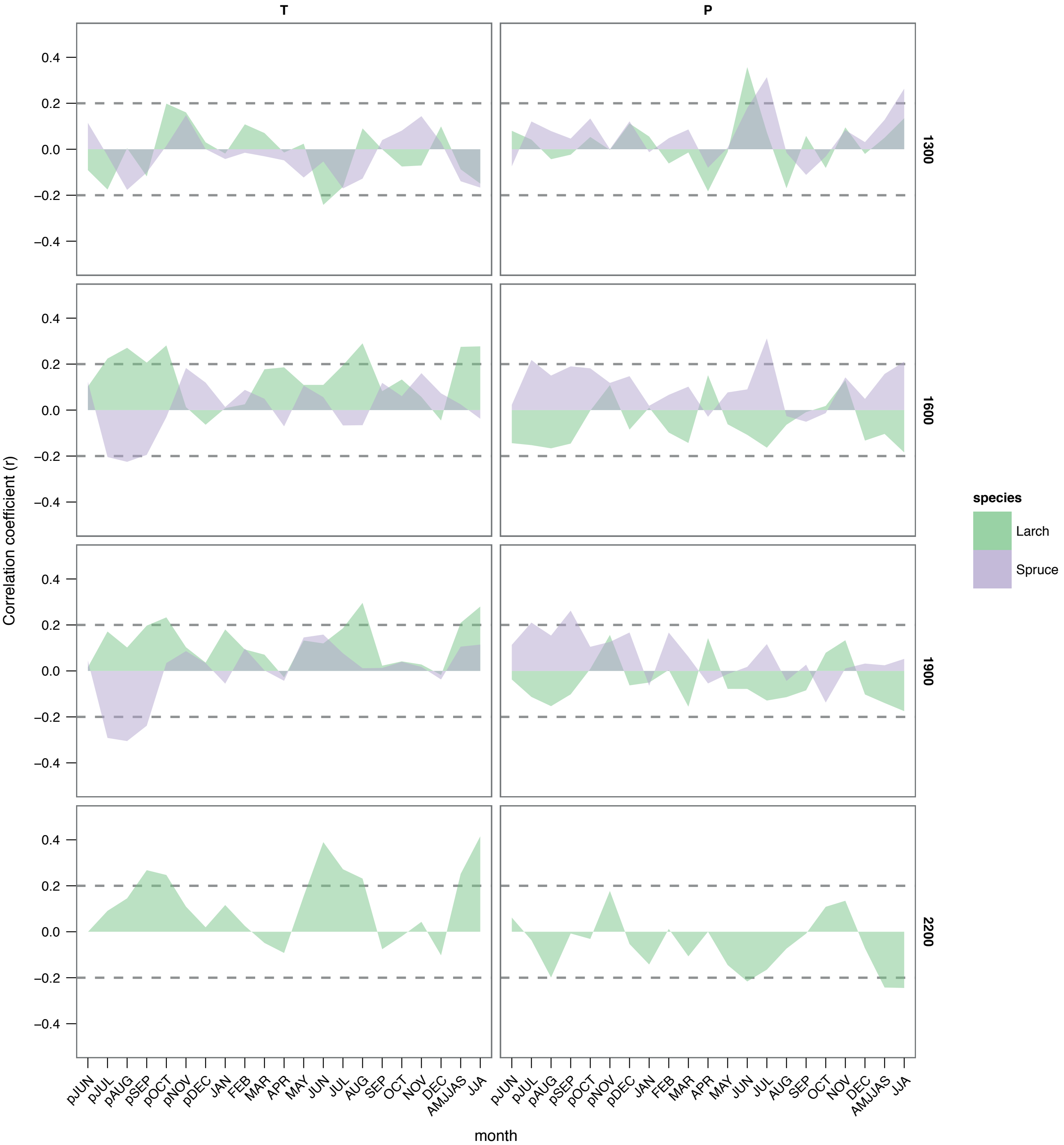

Figure 2.5: Pearson correlation between standardized TRW indices averaged by elevation and species (*Larix decidua* – dark grey, *Picea abies* – light grey) and gridded climate data for the period 1901-2007. Correlations are for a series of months, from previous June to current December as well as two seasonal averages (April–September (A-S), and June–August (JJA)). Dashed lines represent 95% confidence limits (t = 0.02, P = 0.05).

The positive precipitation response of *Picea* in the year prior to ring formation increased towards high elevations – a response contrasted with a decrease of sensitivity to current year precipitation. *Larix* tended to show negative correlations to precipitation with the exception of the lower-most elevation where significant positive correlations (r_{105} = 0.36, $P < 0.05$) with precipitation in June were obtained. Conversely, *Larix* response to temperature was generally positive and significant for current summer conditions, the exception being the lowest site, where response to current June temperature was significantly negative (r_{105} = -0.24, $P < .05$). During the growth year, both single-

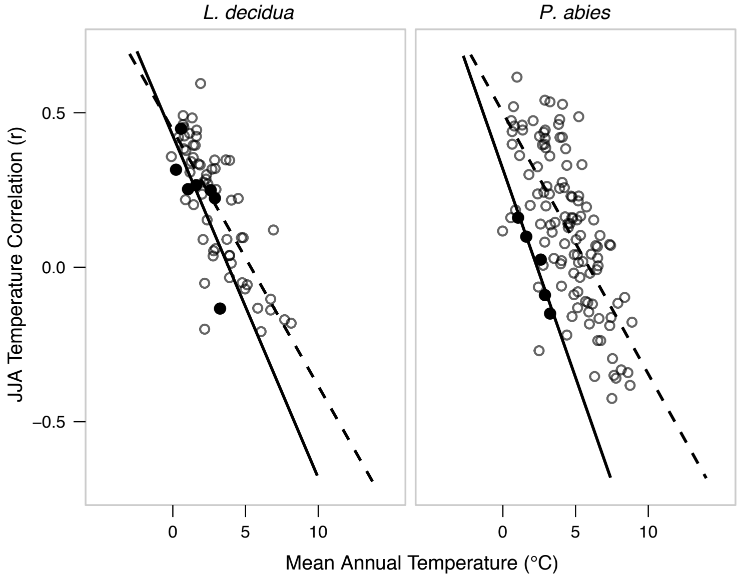

Figure 2.6: Pearson correlations for June–August (JJA) temperatures plotted as a function of mean annual temperature for sites across Europe (open circles) and within the Lötschental (filled circles) for both *Larix decidua* and *Picea abies*. Trend lines are a linear model fit to European and Lötschental data (dashed and solid, respectively).

month and aggregated summer temperature correlations (JJA & AMJJAS) reached a maximum at the highest site on the transect. Although not significant, *Picea* also revealed stronger response to current summer temperatures as elevation increased. Considering the influence of the previous growing season, *Picea* showed an increasingly negative correlation with previous July and August temperatures as elevation increased, whereas *Larix* responded similarly at the lowest site for both previous year temperature and precipitation, but revealed diverging responses with increasing elevation.

Climate Response Comparison to Larger Site Network

To assess signal similarity across a broader scale, *Picea* and *Larix* temperature response from the Lötschental was compared against a network of TRW chronologies from across the Alps (Figure 2.6). Sites located in both the Lötschental and across the Alps revealed increasingly negative correlations with JJA temperatures as mean annual temperature increased. There was good agreement in trend between the Lötschental and Alpine datasets as revealed by similar slopes for both *Larix* (-0.11 and -0.083, respectively) and *Picea* (-0.13 and -0.085). However, comparison of intercepts between the Lötschental and Alpine datasets revealed a much closer agreement for *Larix* (0.43 and 0.44, repsectively) than *Picea* (0.31 versus 0.50), demonstrative for the Lötschental *Picea* to have lower JJA temperature correlations for a given mean annual temperature than the regional counterparts.

Discussion

Long-Term Change in the Context of Elevation

Annual temperature increases for the study region indicate climate warming consistent with data from the Greater Alpine Region (Auer et al. 2007) and double the global land-surface temperature warming over the past century (IPCC 2007). Based on the extent of growing season temperature differences along the elevational transect in the Lötschental, this long-term climate trend can be translated into an approximate 300-m elevation increase. The temperature gradient is also equivalent to the inter-annual variability of growing season temperature anomalies experienced over the past century. Both of these observations suggest populations in this ecosystem appear capable of tolerating a range of environmental conditions. However, with the increased speed of warming over the past 35 years it is unknown if these populations can continue to keep pace and might instead result in distributional or evolutionary changes (Jump and Peñuelas 2005). Additionally, the temperature differences across the elevational transect (~4°C) also approach the projected temperature increases (~5°C) over the next century for Switzerland (CH2011 2011). The projected 5°C warming would equate to a more than 900-m elevation increase and demonstrates a link between different elevations and changing climates.

Elevational transects provide a powerful observational study as tree growth can be analyzed within the context of experienced long-term variability (Swetnam et al. 1999). In fact, recent results indicate that observational studies provide the best measure of response to climate change as warming experiments appear to underestimate plant response to global climate change (Wolkovich et al. 2012). A proposed explanation is that experiments attempt to isolate temperature effects where observational studies tend to integrate the complex effects of multiple climatic drivers. With the well-described link between altitude and temperature, an elevational transect offers the possibility to separate interrelated climate effects while still making observations on a natural environment (Körner 2007a; b). Considering the ability to encompass both the historical and predicted future climate, given sufficient gene flow, this transect is a valuable platform for understanding temperature-driven changes over time.

Gene Flow Across Elevational Gradients

To fully endorse an elevational gradient as a space-for-time analogue of future climate conditions, it is essential to consider gene flow across the landscape, as significantly different biological responses (e.g. tree growth) may actually only represent the noise of local adaptation based on site conditions. FST estimates, which represent the historical genetic divergence between populations of a species (Wright 1950), support our hypothesis that tree populations along the transect show no significant genetic differentiation. Additionally, relatedness data reveals high genetic similarity among the sampled trees regardless of the among-population distance (in elevation) between individuals. This evidence suggests that historical gene flow is high, presuming that migration rate is high enough to counteract the selective pressures of local environmental variation (Savolainen et al. 2007). A review of literature considering trees' genetic variation with altitude found a lack of genetic differentiation at only about half of the studies considered (Ohsawa and Ide 2008). These conflicting results within the literature indicate the importance of taking gene flow into consideration in any study comparing populations within a landscape.

Possible reasons that both *Larix* and *Picea* populations did not exhibit any significant genetic differentiation in the neutral markers across the elevational gradient in the Lötschental could include their predominant out-crossing breeding system, wind pollination, air-borne seed dispersal, and a long lifespan. Conifers, including both *Larix* and *Picea*, have both male and female reproductive structures on a single tree and attempt to avoid self-pollination by relying on differential maturity of reproductive structures and wind pollination (Williams 2009). The spread of pollen is the major mechanism of gene flow, and studies have indicated that along steep elevational gradients pollen dispersal can exceed 1000 meters aided by upslope thermal winds and even greater distances downslope due to convection cell circulation (Ebell and Schmidt 1964).

Significant genetic differentiation between pairs of tree populations within and outside of the valley was found; indicating low genetic differentiation is not a regional population characteristic (Table 2.1). The genetic differentiation we observed is probably the result of both the distance between populations and the presence of physical barriers (e.g. mountain ridges, hanging valleys) that separate the Lötschental from other sampled populations. Our results lead us to conclude that the presence of high amounts of gene flow across the elevational transect are likely to counteract local adaptation and allow us to consider that signals detected in tree growth are more likely a response to climate. However, this is not to say that local adaptation can be completely ruled out. As emphasized by Kawecki and Ebert (2004), only reciprocal transplant experiments covering two generations may substantiate if local adaptation occurs along such an elevation-related climatic gradient at short distance.

Tree Growth Patterns Across Elevational Gradients

Standardized TRW series from both species revealed synchronous growth between trees growing at the same elevation, regardless of aspect (Figure 2.3b). This is juxtaposed with previous dendroclimatology work that has suggested aspect, and in turn the radiation budget, is a primary driver of growth response (Villalba et al. 1994). However, given that air temperatures along the transect show greater similarity between aspect than among elevations (Figure 2.2), our observation of growth similarity is consistent and allows us to combine sites into elevation bands.

Growth differences are greater along the length of the transect, as productivity (TRW) decreases with elevation (Figure 2.3a). In the absence of genetic differentiation and the established link between altitude and air temperature, we can interpret greater growth as primarily a temperature-driven response. Results from a previous study of conifer productivity across a longer, but lower elevational transect (700 – 1500 m) agree with our observations, finding that trees at ~1300 m show the highest growth rates with decreasing values towards the tree-line due to temperature limitations and below 1000 m because of moisture limitations (Neuwirth et al. 2004; Frank and Esper 2005; Affolter et al. 2010). Additionally, Paulsen et al. (2000) found that *Picea* tree-ring widths from transects within treeline ecotones in the Alps decreased with elevation until 1940, but were similar for the last 60 years. They agree that the primary driver of radial stem growth is temperature, but suggest that inter-annual climate variability and threshold temperatures play an important role.

The tree-ring data reveals a general age increase with elevation which could be linked to human management (Büntgen et al. 2006), or may provide support for research that has found a link between tree age and initial growth rate (Bigler and Veblen 2009). The data also show periodic, abrupt growth decreases in *Larix* that are not observed in *Picea*, a disturbance signal likely the result of the larch bud moth (Zeiraphera diniana Gn.), a defoliating insect that has shown continuous cyclic outbreaks (approximately every 9 years) over the past 1200 years (Esper et al. 2007). The presence of cyclic local disturbances within *Larix* populations contributes to a greater mean sensitivity and higher inter-series correlations than *Picea*. However, similar climate responses were observed in high elevation *Picea abies*, *Larix decidua*, and Pinus cembra (Frank and Esper 2005) and removal of this biotic signal in maximum latewood density measurements did not significantly increase the climate sensitivity (Büntgen et al. 2006b).

Growth trends and disturbance signals fit within the linear aggregate model of TRW, a conceptual model developed by Cook (1985) to attribute the influence of different processes on ring width at a given time (t):

$$(1)\ R_t = A_t + C_t + \delta D1_t + \delta D2_t + E_t$$

where R_t is the observed ring width series; A_t is the age-related trend; C_t is the climatic/environmental signal; $\delta D1_t$ and $\delta D2_t$ represent disturbances on a local or stand- wide scale, respectively; and E_t represents any unattributed variability (an error term). We can hypothesize that expression of the four growth terms is controlled in part by the genotype frequencies of a population. The genotype interacts with the environment to produce an observed TRW and allows us to propose a conceptual modification of the linear aggregate model as:

$$(2)\ R_t = g_1 A_t + g_2 C_t + g_3 \delta D1_t + g_4 \delta D2_t + E_t$$

where the terms g_1 – g_4 represent the genetic influence on radial growth processes. Model comparisons among populations with high rates of gene flow, which likely counteract local adaptation, suggest g-terms would be approximately constant. Understanding the g_2 term would be of particular relevance for elucidating the genetic regulation on tree growth response to climatic variability.

Larix and *Picea* show stronger responses to climatic variables with increasing elevation (Figure 2.5). In detail, *Larix*

TRW reveals a positive response to temperature during both current and previous growing seasons at elevations above 1300 m a.s.l., although the response is stronger earlier in the current growing season (May-Aug) and later in the previous growing season (Sep-Oct). Comparatively, *Picea* shows a stronger response to current growing season temperatures (Jun-Jul) and a more negative response to previous growing season temperatures, although this is likely linked with a positive signal of precipitation in the previous season. These results agree with work published on high-elevation *Larix* and sub-alpine *Picea* chronologies across the Alps (Frank and Esper 2005; Büntgen et al. 2005; Carrer and Urbinati 2006). Significant correlations with climate conditions from the previous growing season are found in both species. The persistence of climatic signals into subsequent years is linked with the accumulation of reserves for the subsequent growing season. *Larix* is a deciduous conifer with a deep root system favouring water uptake, and research has shown that the storage of carbohydrate reserves from one summer is important for wood production in the following year (Kagawa et al. 2006). Conversely, *Picea* has a shallower rooting system but retains needles necessary for photosynthesis for several years, suggesting a greater emphasis on water availability for early-season growth. Support for this conclusion is provided by King et al. (2013), who show that *Picea* appear to be much more sensitive to short-term water stress than *Larix*.

The climatic response of *Larix* at the lowest study site is generally the inverse of the observed response at higher elevations. This response is interpreted as evidence of moisture limitations as a consequence of thermal stress from the higher temperature at the lower elevations. Although *Larix* can be found within the alpine arc at elevations below 1300 m, indications are that these populations are under intensive drought stress and are not well-adapted to warmer lower slopes which may exceed species climatic thresholds (Eilmann and Rigling 2012). As temperatures are projected to continue warming, this may lead to an increase in tree mortality at lower range limits (Allen et al. 2010). These observations support the hypothesis that although populations are currently established at these sites and have tolerated environmental fluctuations, projected rapid warming may have a distinct influence on future species distribution and, accordingly, community composition.

Lötschental versus Alpine-wide Climate Responses

To determine if the differences in climatic response observed within the genetically well-mixed Lötschental populations are representative of larger-scale patterns, communities and ecosystems, we compared climate sensitivities in a broader spatial context. Against a *Picea abies* and *Larix decidua* dataset from across the Alps consisting of June, July and August (JJA) temperature response, the data from the Lötschental shows good agreement with climate response as a function of mean annual absolute temperature. *Picea* chronologies from the Lötschental show lower JJA temperature correlation for a given mean annual temperature, but the reasons for this relationship are unclear. Given its East-West orientation and position as an interior valley with climate systems moving from the south-west, it has been recognized that the Lötschental sits in a rain-shadow position. In combination with higher JJA precipitation correlations from the sites in the Lötschental compared to those across the Alps (not shown), lower correlations suggest that the Lötschental trees tend to be more drought stressed than those in the surrounding regions. A greater frequency of significant correlations between *Picea* and precipitation than *Picea* and temperature also support this possibility. The overall response similarity shown by the elevational transect data with high amounts of gene flow and the Alps dataset with greater genetic diversity suggest that the genetic influence on climate response (g2 term in the conceptual model) is small and that ambient climate conditions are the most important factor in determining TRW variations across the Alps.

Conclusions and Opportunities

Our study highlights the value of using elevational gradients to quantify the response of forests under natural conditions. We emphasize the importance of considering genetic differences between study sites and we were able to demonstrate high gene flow and thus presumably low local adaptation within the Lötschental. Similarities in the relationships between climate response and mean climatology from the genetically well-mixed data in this study with those from the surrounding European Alps implies that the genetic component of the inter-annual growth response is likely small in comparison to climatic influences at the regional scale and possibly beyond. Future investigations of high-resolution wood formation across an elevational transect combined with our comprehension of gene flow and climatic influences on tree growth will enhance our understanding of forest response to continued climatic change. Additional studies linking the range of observed TRW under various environmental conditions and a known genetic framework could provide improved understanding of tree plasticity under projected climatic changes and if thresholds or limits exist for future adaptation.

Acknowledgements

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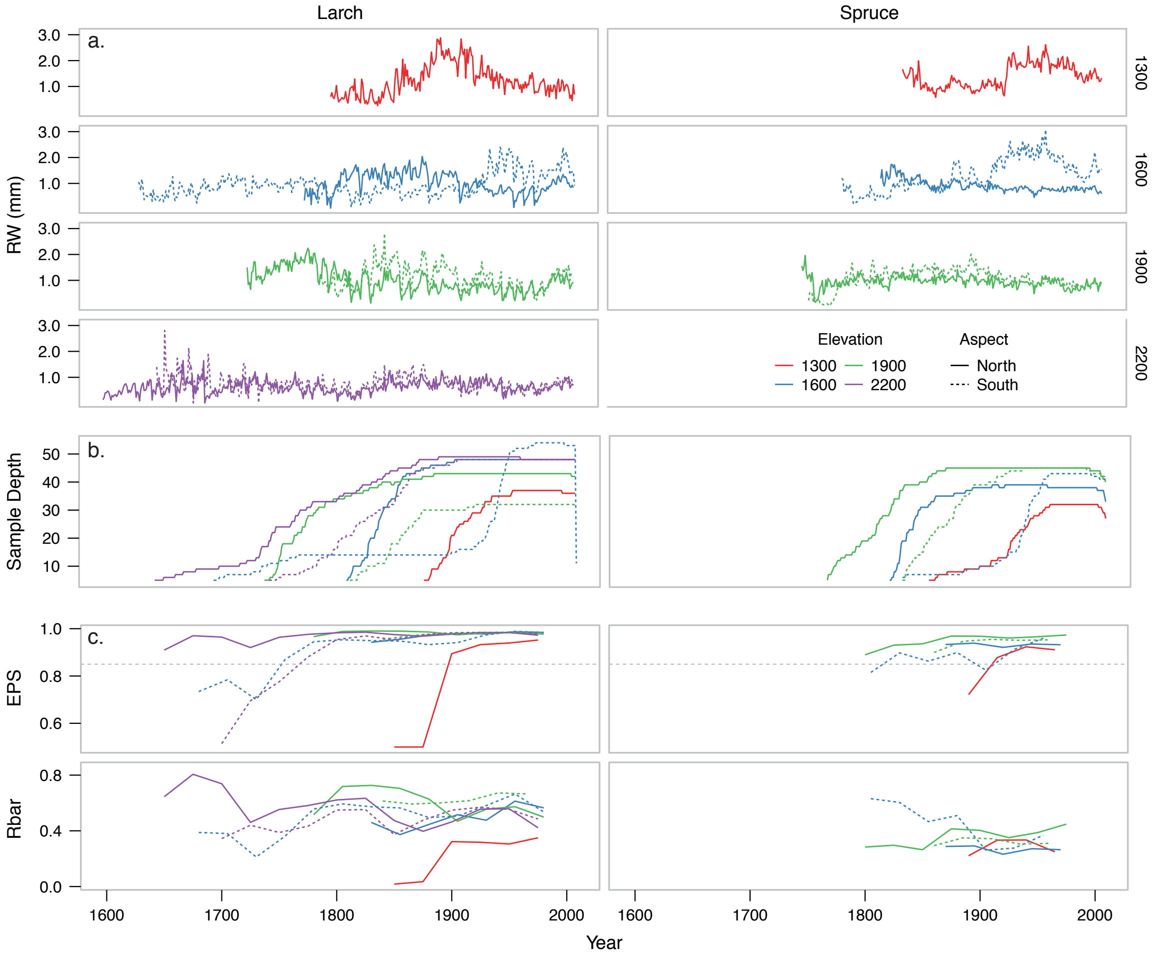
Spplemental Material to Chapter 2



Supplementary Figure 2.1. (a) Raw ring width series plotted by calendar year, (b) sample replication, (c) expressed population signal (EPS) and running Rbar for site mean chronologies. European larch (Larix decidua) are plotted in the left panels and Norway spruce (Picea abies) in the right panels. All plots follow the same legend with colours for elevation (1300 – red, 1600 – blue, 1900 – green, 2200 – purple) and different linetypes for slope aspect (north – solid lines, south – dashed lines).

Supplementary Table 2.1. Lötschental elevational transect tree-ring sampling sites with corresponding chronology information both Larix decidua and Picea abies. Sampling locations are coded for aspect (N: north; S: south) and elevation (in hundreds of meters).

Species	Site Code	Elevation	Latitude [°N]	Longitude [°E]	No. Series / Trees	First Year (≥ 1 Series)	First Year (≥ 5 Series)	Mean Series Length (years)	Series Inter-correlation	Mean Sensitivity
Larix decidua										
	N13	1300	*46.39*	*7.76*	37 / 19	1795	1876	110.3	0.600	0.316
	N16	1600	*46.39*	*7.76*	48 / 25	1772	1809	171.0	0.720	0.264
	N19	1900	*46.39*	*7.77*	44 / 23	1722	1738	225.6	0.723	0.298
	N22	2200	*46.38*	*7.77*	50 / 28	1596	1643	248.9	0.734	0.335
	S16	1600	*46.40*	*7.75*	54 / 28	1627	1694	129.7	0.630	0.247
	S19	1900	*46.40*	*7.75*	32 / 16	1786	1812	161.7	0.736	0.304
	S22	2200	*46.40*	*7.74*	48 / 24	1643	1742	194.1	0.720	0.359
Picea abies										
	N13	1300	*46.39*	*7.76*	32 / 17	1833	1854	97.6	0.497	0.188
	N16	1600	*46.39*	*7.76*	40 / 22	1814	1820	162.1	0.599	0.166
	N19	1900	*46.39*	*7.77*	45 / 24	1745	1766	203.1	0.707	0.186
	S16	1600	*46.40*	*7.75*	43 / 22	1780	1831	93.1	0.557	0.171
	S19	1900	*46.40*	*7.75*	45 / 24	1751	1831	144.0	0.589	0.176

Supplementary Table 2.2. Loci selected for use in the genetic processing from both *Picea abies* and *Larix decidua*.

					Genetic diversity		
Species	Code	Label	MM	Primer [µL]	*A*	H_E	Source
Larix decidua	bcLK189	FAM	1	0.2	11	0.748	Isoda & Watanabe 2006
	bcLK228	HEX	1	0.1	14	0.806	Isoda & Watanabe 2006
	bcLK229	HEX	1/2	0.2/0.2	5	0.527	Isoda & Watanabe 2006
	bcLK235	NED	1	0.3	13	0.683	Isoda & Watanabe 2006
	bcLK253	FAM	2	0.1	13	0.806	Isoda & Watanabe 2006
	bcLK263*	NED	2	0.2			Isoda & Watanabe 2006
	UAKLLy6*	FAM	1	0.2			Khasa et al. 2000
	bcLK211*	HEX	2	0.2			Isoda & Watanabe 2006
Picea abies	EATC1B02	HEX	1	0.4	7	0.327	Scotti et al. 2002
	EATC1E03	NED	1	0.2	7	0.537	Scotti et al. 2002
	EATC2B02	FAM	2	0.4	15	0.587	Scotti et al. 2002
	EATC1G05	HEX	2	0.4	16	0.871	Scotti et al. 2002
	SpAC1F7	HEX	1/2	0.4/0.4	9	0.729	Pfeiffer et al. 1997

MM	multiplex mix for PCR
Primer	amount of each primer (10µM) per PCR multiplex
A	number of alleles
H_E	expected heterozygosity
*	excluded from final analyses

Supplementary Table 2.3. Full Pearson correlation coefficients (r) between elevation averaged tree-ring width chronologies from both Larix decidua and Picea abies and monthly temperature values over the period 1901-2007 from the CRU 3.1 gridded dataset. Significant correlations (t = 0.2, P = 0.05) are marked bold with an asterisk (*).

Species	Elev.	pJUN	pJUL	pAUG	pSEP	pOCT	pNOV	pDEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	AMJJAS	JJA
Larix decidua																						
	1300	-0.091	-0.175	0.004	-0.118	0.199	0.16	0.03	-0.019	0.108	0.071	-0.014	0.024	**-0.242***	-0.164	0.091	0	-0.075	-0.071	0.099	-0.087	-0.149
	1600	0.102	**0.224***	**0.271***	**0.207***	**0.281***	0.013	-0.064	0.009	0.025	0.177	0.186	0.11	0.11	0.195	**0.29***	0.082	0.133	0.057	-0.045	**0.275***	**0.277***
	1900	0.016	0.171	0.102	0.197	**0.233***	0.103	0.036	0.18	0.093	0.071	-0.028	0.132	0.12	0.186	**0.296***	0.023	0.042	0.028	-0.018	**0.209***	**0.28***
	2200	-0.001	0.091	0.145	**0.268***	**0.247***	0.109	0.019	0.116	0.026	-0.049	-0.092	0.155	**0.39***	**0.272***	**0.231***	-0.076	-0.02	0.043	-0.103	**0.252***	**0.415***
Picea abies																						
	1300	0.115	-0.028	-0.177	-0.101	0.015	0.148	0.001	-0.042	-0.015	-0.031	-0.048	-0.123	-0.053	-0.17	-0.128	0.04	0.081	0.144	0.03	-0.139	-0.167
	1600	0.123	**-0.204***	**-0.225***	-0.195	-0.031	0.183	0.12	0.013	0.088	0.049	-0.071	0.107	0.056	-0.067	-0.066	0.118	0.061	0.161	0.073	0.025	-0.038
	1900	0.045	**-0.292***	**-0.305***	**-0.238***	0.034	0.086	0.034	-0.055	0.097	0.002	-0.043	0.146	0.158	0.077	0.012	0.013	0.039	0.018	-0.037	0.106	0.115

Supplementary Table 2.4. Full Pearson correlation coefficients (r) between elevation averaged tree-ring width chronologies from both Larix decidua and Picea abies and monthly precipitation values over the period 1901-2007 from the CRU 3.1 gridded dataset. Significant correlations (t = 0.2, P = 0.05) are marked bold with an asterisk (*).

Species	Elev.	pJUN	pJUL	pAUG	pSEP	pOCT	pNOV	pDEC	JAN	FEB	MAR	APR
Larix decidua												
	1300	0.08	0.042	-0.043	-0.024	0.053	-0.003	0.112	0.055	-0.062	-0.014	-0.183
	1600	-0.144	-0.153	-0.166	-0.146	-0.003	0.109	-0.085	0.011	-0.098	-0.143	0.152
	1900	-0.037	-0.113	-0.153	-0.102	0.009	0.157	-0.063	-0.051	0.004	-0.156	0.143
	2200	0.062	-0.037	-0.199	-0.008	-0.031	0.177	-0.053	-0.142	0.012	-0.107	0
Picea abies												
	1300	-0.074	0.121	0.079	0.046	0.134	0	0.121	-0.013	0.048	0.086	-0.08
	1600	0.023	**0.218***	0.15	0.19	0.182	0.118	0.148	0.019	0.066	0.102	-0.029
	1900	0.113	**0.21***	0.154	**0.262***	0.105	0.126	0.167	-0.065	0.167	0.062	-0.055

Species	Elev.	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	AMJJAS	JJA
Larix decidua											
	1300	-0.012	**0.358***	0.074	-0.17	0.058	-0.081	0.095	-0.02	0.05	0.135
	1600	-0.062	-0.108	-0.164	-0.064	-0.009	0.018	0.13	-0.133	-0.104	-0.184
	1900	-0.078	-0.078	-0.129	-0.114	-0.085	0.079	0.133	-0.102	-0.14	-0.175
	2200	-0.145	**-0.216***	-0.165	-0.073	-0.008	0.109	0.135	-0.072	**-0.243***	**-0.245***
Picea abies											
	1300	0.006	0.179	**0.314***	-0.015	-0.112	-0.031	0.083	0.031	0.128	**0.264***
	1600	0.077	0.09	**0.312***	-0.026	-0.051	-0.013	0.142	0.049	0.157	**0.211***
	1900	-0.014	0.018	0.117	-0.044	0.027	-0.138	0.011	0.032	0.025	0.052

Chapter 3

Climatic drivers of hourly to yearly tree radius variations along a 6°C natural warming gradient

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Introduction

Trees play a key role in the dynamics and coupling of the carbon and hydrological cycles (Hutjes et al., 1998; Prentice et al., 2000). In forested regions, approximately half of the precipitation is returned to the atmosphere through tree stems (Oki and Kanae, 2006). In parallel, tree photosynthetic activity constitutes approximately 50% of the total terrestrial net primary production (Bonan, 2008). These characteristics, together with additional ecosystem services, make the quantification of tree growth and its environmental interactions an important task, especially under continuing global change (IPCC, 2007).

Atmosphere-landscape feedbacks are an example of important regional to global phenomena well addressed by large-scale data sets and modelling approaches (Seneviratne et al., 2006; Frank et al., 2010; Jung et al., 2010), although tree-based measurements are still necessary to monitor and attribute growth. Repeated observations (e.g., tree diameter and height) are commonly employed to track biomass accumulation (Husch et al., 2003), whereas increment cores provide retrospective quantification of annual radial growth (Fritts, 1976). These methods offer the basis to statistically link inter-annual to long-term growth with, at best, monthly climatic variables. To improve our understanding of the physiological mechanisms driving tree growth, sub-daily and cellular scale data are required.

Dendrometers meet these conditions by recording stem radius at sub-hourly and micrometer resolution (Daubenmire, 1945; Bormann and Kozlowski, 1962; Drew and Downes, 2009), with observed variations representing a combination of growth together with water transport and storage (Herzog et al., 1995; Zweifel and Häsler, 2001). Automatic dendrometers are now widely used to provide data on coniferous and broadleaf trees from boreal to tropical forests (Perez et al., 2009; Biondi and Hartsough, 2010; Nabeshima et al., 2010; Oberhuber and Gruber, 2010; Duchesne and Houle, 2011). For example, dendrometers have been used to quantify and date the seasonal progress of wood formation, providing the basis for intra-annual tracing of isotope (e.g. $\delta^{13}C$ and $\delta^{18}O$) signatures from source to sink for an entire growing season (Gessler et al., 2009; Offermann et al., 2011). However, most studies have focused on isolating tree growth, typically via the so-called "daily" (Tardif et al., 2001; Bouriaud et al., 2005), or "stem cycle" (Downes et al., 1999; Deslauriers et al., 2003) approaches, to explore the influence of environmental variables. Over three years Deslauriers et al. (2003) attributed daily radial growth of balsam fir (*Abies balsamea (L.)* Mill.) in the boreal forest primarily to water availability and demands. Similarly, daily radial changes of tree species from Ecuador and Ethiopia measured with dendrometers were explained by available moisture with the vapour pressure deficit and precipitation being the most important factors (Bräuning et al., 2009; Krepkowski et al., 2011). The daily increment of Pinus cembra (L.) at the alpine ecotone (Gruber et al., 2009) has also been positively correlated with changes in tree water status (relative humidity and precipitation). Less emphasis has been placed on the stem water signal and how it actually may confound the attribution of growth (Zweifel et al., 2001; Makinen et al., 2003). Consequently, investigations focusing on tree water balance will contribute to improving the isolation of the growth signal, quantifying processes such as transpiration rates and water-use efficiency, and increasing understanding of soil-plant-atmosphere coupling.

Here, we present high-resolution stem radius measurements collected over four years (2007-2010). We use a novel analysis technique to i) isolate diurnal cycles, ii) examine the stem water signal and iii) attribute the influence of environmental drivers. These analyses are examined in two different common alpine species: Norway spruce (*Picea abies* Karst.) and European larch (*Larix decidua* Mill.), along a temperature gradient over a complete year. To this end, sub-hourly stem radius measurements from 56 trees at eight sites along a 1400-m altitudinal gradient were collected with associated instrumental data. The altitudinal transects provide a ~6°C growing season temperature difference, roughly corresponding to the projected increase of Switzerland summer temperatures to the year 2100 under scenario A1B (CH2011, 2011) and allowing us to make inferences as to the impacts of a warming climate.

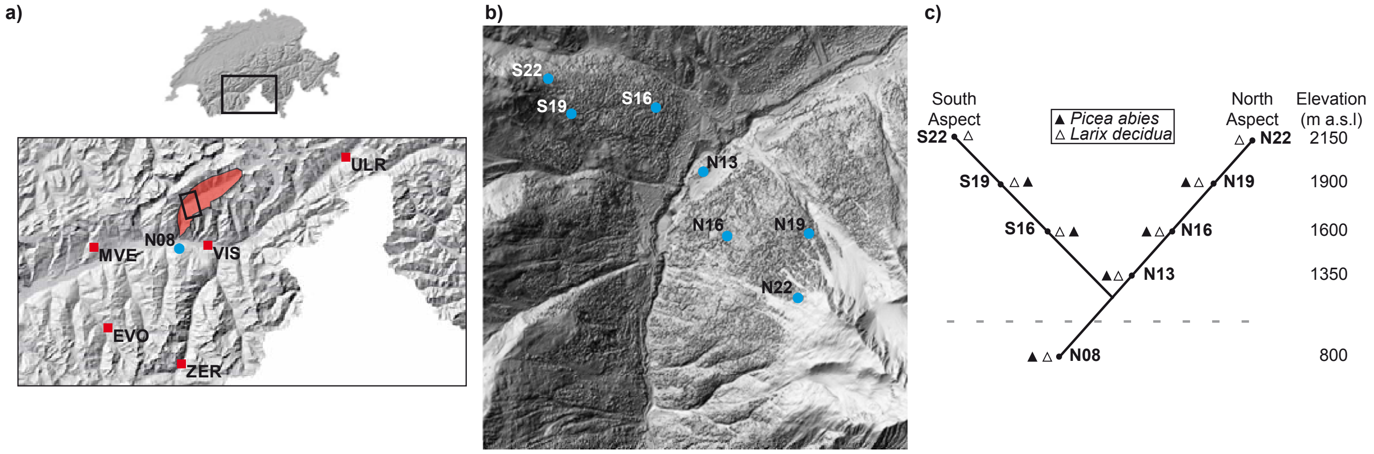


Figure 3.1: Study location. (a) Regional map showing the Lötschental (shaded red polygon) and the location of the five MeteoSwiss meteorological stations (red squares) of Evolene-Villa (EVO), Crans-Montana (MVE), Ulrichen (ULR), Visp (VIS) and Zermatt (ZER). (b) Digital elevation model (DEM) of the Lötschental centered around the village of Ferden showing the sampling sites (blue circles); the 800 m a.s.l. site is located just outside the valley is in 1a (blue circle). (c) Schematic diagram of the transect showing site elevations, codes and sampled species.

Material and Methods

2.1. Study Area

Our study was conducted across two altitudinal transects centered in the Lötschental (46°23’40”N, 7°45’35”E), an inner-alpine valley located in the central Swiss Alps (Figure 2.1a). The valley is enclosed by steep forested slopes (mostly >60%) primarily composed of mixed, evergreen Norway spruce and deciduous European larch extending to ~2300 m a.s.l. The soils along the transect are acidic and characterized by a large stone content and low amounts of clay. Different soil types were observed, ranging from Leptosol, Cambisol to Podzol, with intermediates (IUSS Working Group WRB, 2006). The soils were formed from calcareous-free parent material, including moraines and crystalline bedrock (gneiss and granite) from the Aar massif (Jemelin et al., 1985). The region has a cool climate with a mean annual temperature of 5.7°C, ranging from -3.3°C (January) to 14.8°C (July). As a relatively dry region, the mean annual precipitation is 825 mm and is well distributed throughout the year with precipitation falling predominantly as snow between November and March (MeteoSwiss sites, Figure 2.1a). During the study (2007-2010) conditions were broadly similar to the long-term average (1987-2006) except for a slightly warmer spring (attributable to a very warm 2007) and more precipitation received over the late spring and summer and less in the early autumn (Figure 2.2).

Measurements were performed at eight sites (Figure 2.1). Seven sites were established at 300-m elevation intervals starting from the valley bottom, located at ~1300 m a.s.l., along both the northwest and southeast slope aspects until ~2200 m a.s.l., just below the upper forest limit. To further extend the altitudinal gradient, an additional site was established in early 2008 just outside of the Lötschental in the main Rhône valley on a north-facing slope at an elevation of ~800 m a.s.l. Codes for aspect and altitude (in hundreds of meters) were assigned to each site (e.g. N13 or S22). Site temperatures decline by 0.47 °C/100 m on the south- and 0.42 °C/100 m on the north-facing slope and between-slope differences were generally absent.

2.2. Data Collection

2.2.1. Dendrometers

At each site, with the exception of N22 and S22 which are above the local altitudinal range of spruce, four mature, dominant and healthy trees of each species (*Picea abies* and *Larix decidua*) were selected for a total of 56 trees. The sampled trees have average heights of 27 m and diameters of 44 cm, and were similar for both species. Trees located

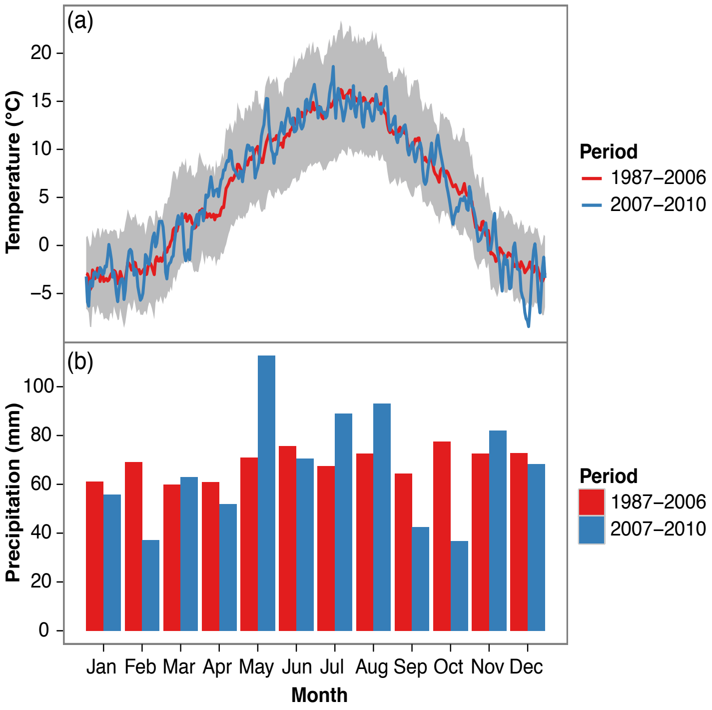


Figure 3.2: Comparison of mean daily average temperatures (a), and monthly sums of precipitation (b) for the monitoring years 2007-2010 (blue) and the 1987–2006 average (red). The grey shading in (a) indicates the mean minimum and maximum temperatures averaged over the 1987–2006 period. Data were calculated as an average from a network of five MeteoSwiss weather stations in the region.

along the two transects are generally older at higher elevations (e.g., average of 251 years at 2200 m a.s.l. and 116 at N13), with larch and spruce exhibiting similar ages at the same site (Bechet, 2010). Automatic dendrometers were installed on all trees to continuously measure stem radius changes (DR, Difference in Radius over time). Two different dendrometer types were equally distributed among species and sites: a point dendrometer and a circumference dendrometer, models DR and DC-1, respectively (Ecomatik, Munich, Germany). The measurement of stem radius requires the conversion of a distance change in the sensor rod for the point dendrometers (band length for the circumference dendrometers) into an electrical signal using a linear variable differential transformer. Signals were corrected for thermal sensitivity according to the manufacturer's specifications.

Data were recorded from October 2006 to October 2010 with a sampling resolution of hourly or better. The resulting data were processed to identify and correct errors due to factors such as animals eating wires, moisture entering the data logger housing, fallen trees, and on one occasion an errant (or precisely placed) hunting bullet. In addition, tension on the dendrometer sensor rods and wire bands was periodically adjusted to remain within the manufacturer's measurement range as the trees grew. Gaps (which remained unfilled) and abrupt jumps were corrected considering series from neighboring trees without the corresponding inhomogeneity. Sub-hourly DR values were averaged to hourly resolution. To investigate diurnal cycles, the overall growth trend was removed from the data by calculating daily means for each sensor and subtracting it from the measurements.

2.2.2. Meteorological Measurements

Site conditions were continuously monitored using a variety of sensors. From 2006 onwards stem temperatures at each site were measured every 15 minutes with a temperature logger (Onset, Tidbit) installed on a single tree at about 1.6 m above the ground and protected from direct solar radiation. Starting in the spring of 2008, air temperature, relative humidity (RH) and dew point were recorded at 15 min resolution using a sensor (Onset, U23 Pro) placed beneath the canopy at each site. Furthermore, temperature, precipitation and radiation records

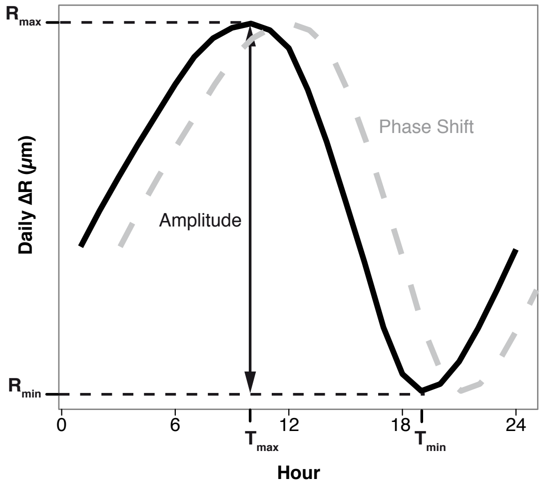


Figure 3.3: A schematic of the diurnal cycle of stem radius (DR). The local maximum (R_{max}) and minimum (R_{min}) stem radius, and the daily amplitude (ΔR), which is difference between the R_{max} and R_{min} are indicated. The T_{max} and T_{min} indicate the respective times when the local maximum and minimum radius occur. A phase shift is represented by the grey dashed line.

were compiled using a network of MeteoSwiss sites in the region (Figure 3.1a). This network was selected based on multiple comparisons including those with a temporary meteorological station installed in the Lötschental near the village of Wiler. This network provided a historical baseline (1987-2006) of hourly precipitation and temperature observations for our study.

2.3. Data Analysis

To explore the characteristic diurnal cycle of DR, which resembles a sinusoid waveform, we define several metrics based on characteristic data patterns (Figure 3.3). The local maximum (R_{max}) and minimum (R_{min}) are found at the critical points of the wave. From these points, the timing of the daily maximum (T_{max}) and minimum (T_{min}) were calculated, as well as the peak-to-peak amplitude ($\Delta R = R_{max} - R_{min}$). On rare days when stem size increased monotonically, no values were assigned.

To investigate the influence of air temperature on diurnal amplitude over the growing season we averaged observations over all years and used site as our unit of replication ($n = 8$). As we are interested in a general response across all elevations, a linear mixed-effects model (R package lme4) was fit to the data considering air temperature as a fixed effect and site as a random effect. To compare species response we fit larch and spruce independent of each other. As there is no consensus as to the best method for calculating an R^2 for this type of regression, we instead calculate the correlation between observed and predicted values (Byrnes et al., 2006) for an intuitive measure of how well our proposed model explains observed variation in the data.

To further explore circadian cycle shifts during the growing season, we classified and averaged daily changes in stem size according to meteorological conditions as reported in the regional network. We calculated different classes for daily precipitation, temperature, sunshine percentage as well as a weather index combining precipitation and sunshine amounts. Each of these variables was selected based upon previous research that identified their importance for tree transpiration processes (Jarvis and McNaughton, 1986).

All analyses were performed using the R statistical environment (R Development Core Team, 2012).

3. Results

3.1. Stem radius variations

From 2007-2010, the stem radius variations (DR) showed annual and daily cycles characteristic of trees growing in temperate regions (Figure 3.4). Trees generally reveal a synchronous response, although more coherence is found at the site-level than across the altitudinal transect. The annual variability is characterized by a progressive increase

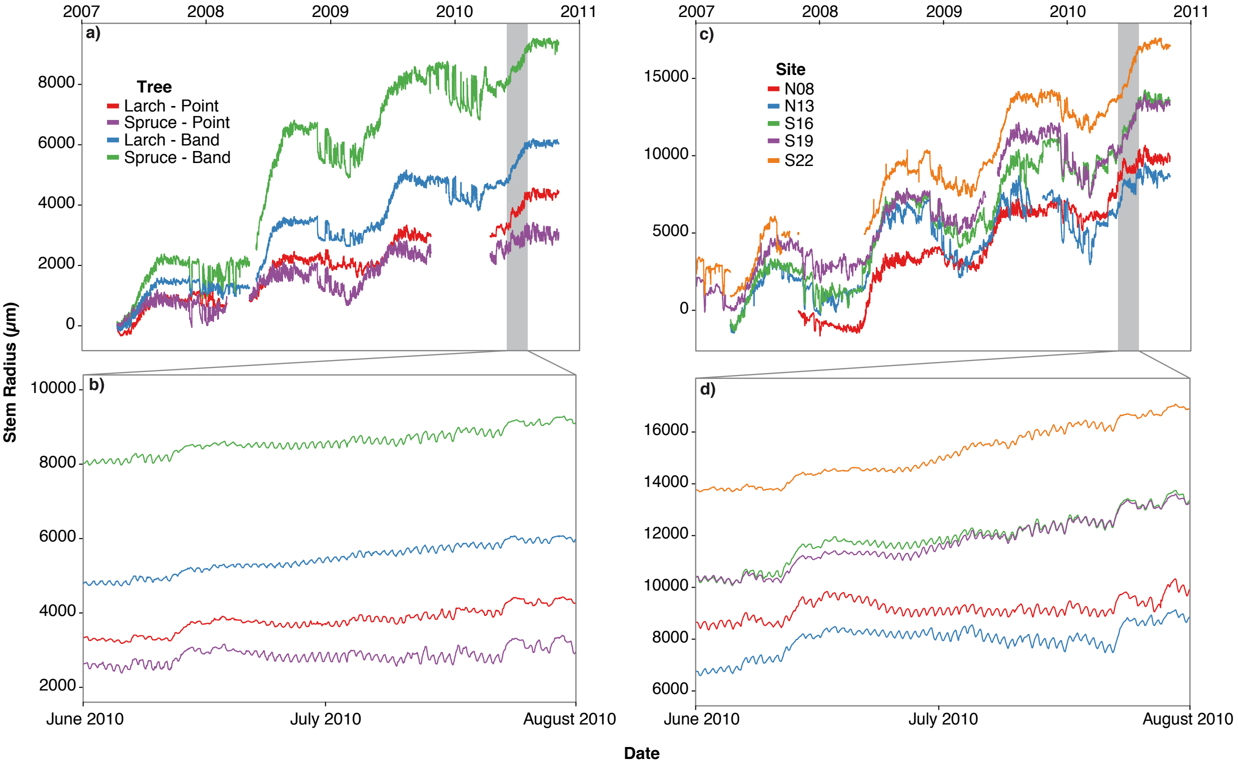


Figure 3.4: Hourly DR records of: (a-b) four trees located at a single site (S16), and (c-d) site averages along a 1400-m altitudinal gradient. The top panels show four complete growing seasons, from 2007 until 2010; and the bottom panels show the same data for a two month period from June 2010 to August 2010.

of stem radius beginning in the spring, a plateau in late summer and a decrease during the winter months (Figure 3.4a and 3.4c). The stem radius increase is closely linked with the main period of xylogenesis. The subsequent plateau in stem size suggests a reduction in cellular division and expansion, while the winter decrease indicates stem desiccation. At our sites, the amplitude of this seasonal cycle is in the range of several thousand micrometers. Within these annual cycles we also observe more abrupt stem size variations in both larch and spruce, usually on the order of hundreds of micrometers. Decreases during the wintertime are related to freezing events, while increases during the growing season are associated with precipitation. At higher resolution during the growing season (Figure 3.4b and 3.4d), the DR variations show a clear diurnal cycle that appears to be primarily elastic. Obscuration of this daily cycle by stem expansion during precipitation events is readily visible. The daily amplitude during the growing season is about 100 µm (0.1 mm). Figure 3.5 provides a close-up of the stem contraction events from December 2009 and shows their relation to site-specific air temperatures below -5°C (shaded in red). During this period, the “threshold” temperature is reached at all sites around December 14 and remains low for between 5 and 7 days, with much colder temperatures, a longer duration and larger stem contractions at higher elevations (e.g. S19 drops below -15°C and has a stem contraction of about 2000 µm). Stem size at N08 (the lowest elevation) recovers more quickly and does not record the subsequent temperature decrease and corresponding stem contraction around 28 December.

Comparisons of monthly mean diurnal cycles over a full year reveal variation with the seasons and with site elevation (Figure 3.6 and Table 3.1). The amplitude and phase of the cycles were found to vary along the course of 12 months with notable differentiation between summer and winter. The summer and winter patterns contain a local amplitude maximum in July (99.8 ± 31.4 µm) and February (30.5 ± 18.0 µm), respectively, with the amplitudes being much larger during the summer than the winter. The summer and winter patterns are most characteristically differentiated by a phase shift in the diurnal cycle. During the summer, a local maximum occurs between 0700 and 0900 Central European Time (CET; i.e., between the 8th and 10th hours of the day) followed

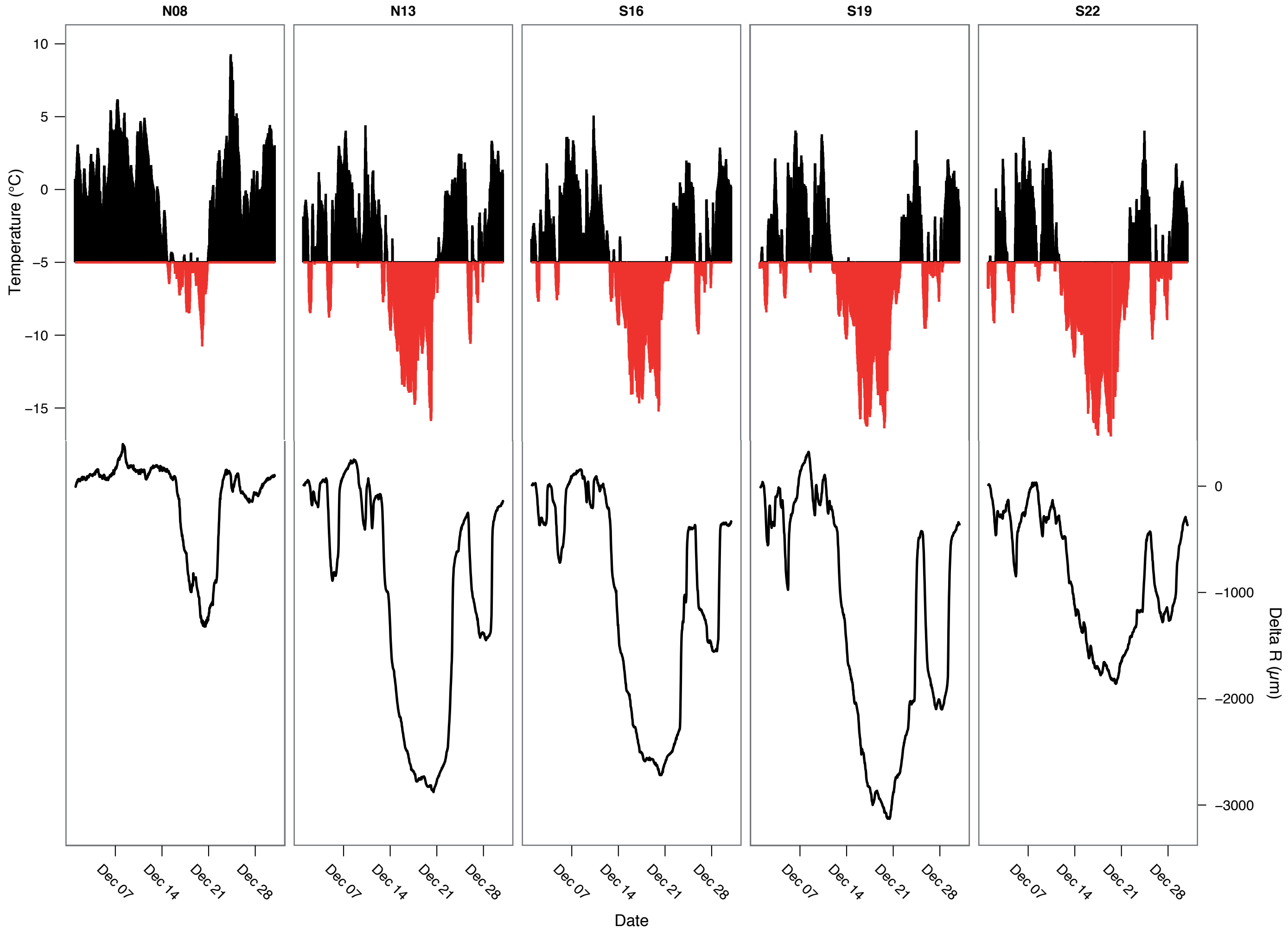


Figure 3.5: Stem contraction events and air temperatures (shaded red below -5°C) from each site along the altitudinal gradient (increasing elevation from left to right). Data are from December 2009 and represents site averages of all trees. Absolute differences between sites have been removed by setting all stem radiuses to 0 for the first measurement of the month.

by a local minimum between 1700 and 2000 CET. Conversely, in the winter the maximum is found between 1700 and 1900 CET, while the local minimum occurs between 0900 and 1100 CET. In addition to the seasonal influence, the characteristics of the daily cycle also change along the altitudinal gradient. As site elevation decreases from 2200 m (orange line) to 800 m (red line) a parallel increase in amplitude is observed, consistent with the observation of the greatest amplitudes during the warmest months. Similarly, as elevation increases, the phase shift in the diurnal cycles from the winter to summer pattern takes place at a later date. These observations of the diurnal amplitude and phase and their modulation with site elevation and the seasonal progression suggest that these characteristics are driven by climatic controls and also provide insight about water movement and storage in trees.

Air temperature is the primary environmental factor that changes with seasons and along the gradient. To investigate its influence on daily cycle amplitudes we plotted monthly mean amplitudes against mean monthly temperatures (April to October) for each site (Figure 3.7). The linear mixed effects model revealed a positively correlated increase in the amplitude of the diurnal cycle with increased air temperatures for both species (Larch: R^2_{COR} = 0.868, d.f. = 56; Spruce: R^2_{COR} = 0.766, d.f. = 40). Each species had very different intercept values (55.88 and 6.804 µm, for spruce and larch respectively), indicating that within the current temperature range, spruce experience larger peak-to-peak amplitudes than larch (e.g. at a temperature of 10°C, expected amplitude of spruce is 118.4 µm, whereas larch is 94.4 µm). The slopes of the species regressions were also different (6.252 µm for spruce and 6.804 µm for larch), which under projected scenarios of warming replicated by the altitudinal gradient would mean an

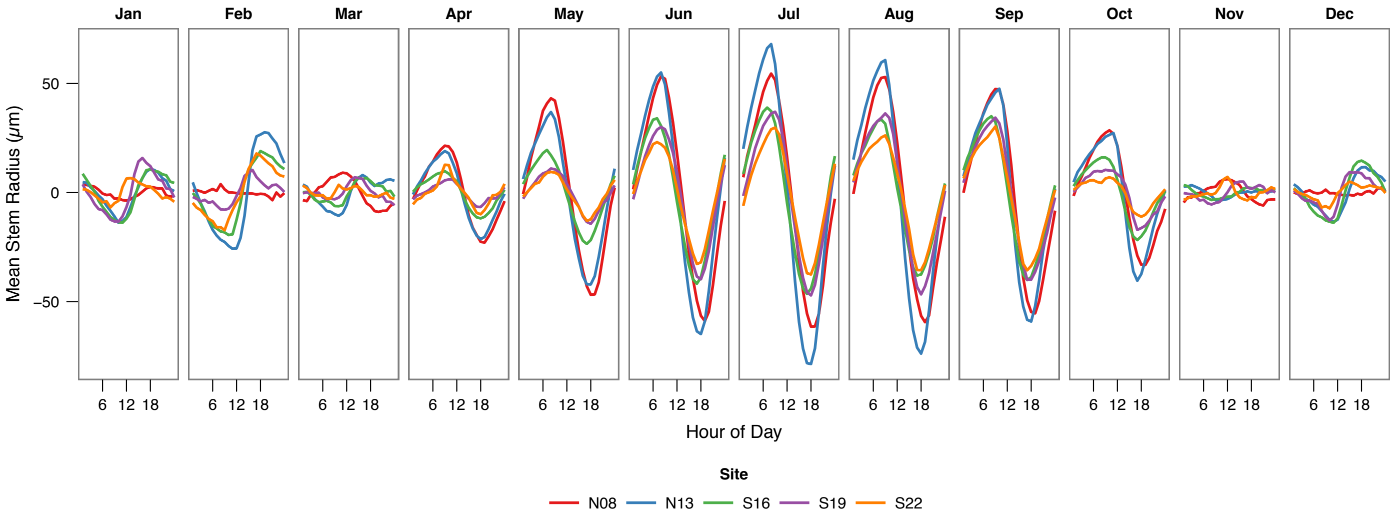


Figure 3.6: Monthly mean circadian cycle for five sites forming a 1400-m altitudinal gradient, shown for point dendrometers installed on larch trees. Data are composited over four complete growing seasons, from January 2007 to November 2010.

approximate 48% increase in amplitude for an average larch (compared to a 30% increase for spruce) during the month of June.

3.2. Climatic influence on diurnal stem cycles

To obtain further insights as to the influence of climatic factors on the diurnal cycle during the growing season (April-October), data were classified according to levels of precipitation, temperature and sunshine (Figure 3.8 and Table 3.2). Amplitude of the daily cycle was inversely related to precipitation amounts (Figure 3.8a). During the growing season, days without precipitation reveal mean amplitudes of 95.8 ± 34.5 μm, compared to 10.6 ± 4.5 μm on days with >10 mm of precipitation. The time of the local maximum stem radius (Rmax) on these days was also delayed by about two hours (8.14 ± 0.2 to 10.25 ± 0.3 hours). Increases in stem radius over a full day were generally observed starting with the 1-5 mm precipitation category, with a tendency toward monotonic increases in stem size during larger precipitation events. Conversely, an increase in the daily cycle amplitude is observed with a higher average daily temperature (Figure 3.8b). Days with a mean temperature from 5 to 10°C show daily amplitudes about 40% smaller than days with mean temperatures between 15 and 20°C. Despite days with higher temperature leading to general decreases in stem radius, no monotonic decreases were observed. Similarly, a greater amount of sunshine results in a greater amplitude of the diurnal cycle (Figure 3.8c). On cloudy days (0 – 20% sunshine) the average amplitude is 11.4 ± 8.6 μm, compared with sunny days (> 60% sunshine) when the average amplitude is 106.2 ± 35.2 μm. Additionally, T_{max} occurs about 4 hours earlier in the day as the sunshine percentage increases from 0% to >60%. These amplitude increases suggest a depletion of stem water reserves in response to greater evaporative demand driven by warmer temperatures and increased sunshine.

While the data show a clear response of diurnal cycles to climatic variables, attributing this response is challenged by the correlation among parameters (e.g., sunny days also tend to be warm days). To help address this, we calculated average daily cycles for a weather index grouping days according to combined radiation (i.e., greater than or less than 40% sunshine) and presence of precipitation (Figure 3.8d). The amplitudes during sunny days are minimally affected by the presence (104.6 ± 30.8 μm) or absence (100.0 ± 33.8 μm) of rain. However, cloudy days (independent of precipitation) have about 50% lower amplitude than sunny days and large differences in amplitude with (28.9 ± 14.2 μm) or without (72.4 ± 27.3 μm) precipitation. These patterns suggest that radiation has a greater influence on the diurnal cycles than precipitation, whose influence is moderated by the amount of radiation. These results support the observation that transient atmospheric conditions are coupled with daily water movement within tree stems.

Table 3.1: Descriptive statistics of the monthly mean diurnal cycles presented in Figure 3.5. Here, all five sites are combined to obtain monthly characteristics. Data are composited over four complete growing seasons (2007-2010).

Month	T_{max} (hour of day)	T_{min} (hour of day)	Amplitude (μm)
January	16.7 ± 0.5	10.2 ± 0.3	19.61 ± 9.42
February	16.7 ± 1.0	10.0 ± 1.0	30.51 ± 17.98
March	13.2 ± 0.6	8.7 ± 1.1	13.24 ± 5.24
April	9.2 ± 0.1	18.2 ± 0.1	28.31 ± 13.46
May	7.8 ± 0.1	17.6 ± 0.1	51.93 ± 30.98
June	7.6 ± 0.1	17.8 ± 0.2	86.67 ± 27.84
July	8.2 ± 0.2	17.8 ± 0.1	99.84 ± 31.47
August	8.8 ± 0.1	18.0 ± 0.2	92.56 ± 30.16
September	9.0 ± 0.2	17.6 ± 0.2	84.92 ± 18.46
October	9.8 ± 0.2	17.6 ± 0.2	42.56 ± 21.5
November	13.9 ± 1.2	10.6 ± 1.5	9.23 ± 2.68
December	17.8 ± 0.8	11.4 ± 0.6	18.35 ± 10.17

In addition to the responses due to short-term atmosphere-tree coupling, the diurnal cycle characteristics can also supply information about plant responses to the more slowly changing soil conditions. In the Lötschental, soil water reserves during the growing season are primarily recharged by precipitation; we therefore investigate diurnal cycle modulation as a function of the time since the last rain event (Figure 3.9). As the length of the rain-free period increases, a clear tendency for decreasing amplitude is observed in both species, although overall, spruce show a 40% greater average amplitude of the diurnal cycle (96.7 ± 18.8 μm) compared with larch (57.6 ± 15.2 μm). Our data appear to reveal species-specific responses to short-term water stress, with spruce maintaining similar daily amplitudes up to 5-6 days following the last rain event before a precipitous 40% decrease of amplitude after 9-10 days. Larch shows a more constant decline in amplitude over time. Although larch has a greater decrease of its amplitude fraction (50% after 9-10 days) than spruce, they do not show any sudden decreases of stem water movement. These observations lead us to infer that the monitored species have different physiological mechanisms for dealing with short-term water stress.

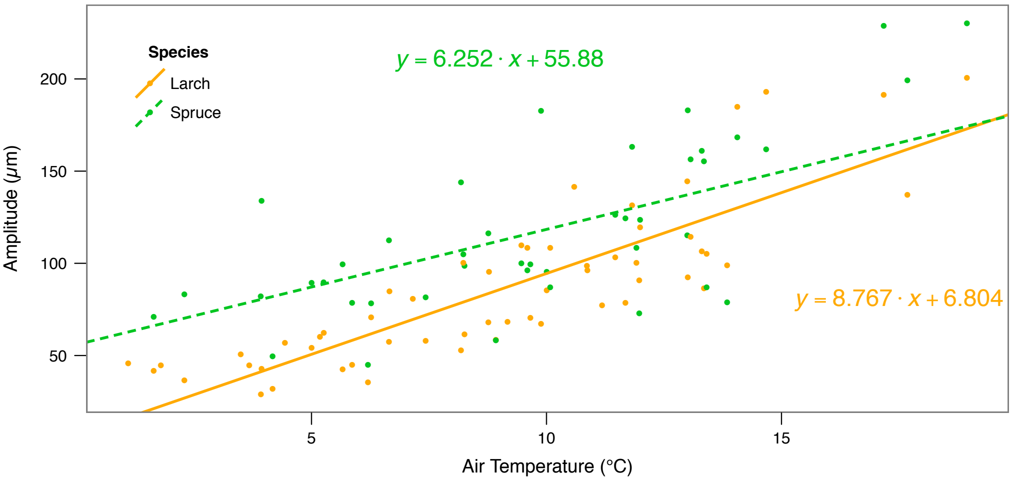
4. Discussion and Conclusions

4.1. Mechanisms

Our high-resolution measurements collected over four years allow us to improve our physiological understanding of diurnal cycle mechanisms: transpiration and water uptake during the growing season and winter freeze-thaw. Both mechanisms are fundamentally linked with water movement and storage in the elastic stem tissues (Zweifel et al., 2001).

During the growing season, the daily cycles (Figure 3.3) reflect a delicate balance between canopy water loss and soil water absorption (Wronski et al., 1985; Kozlowski et al., 1997; Devine and Harrington, 2011). We observed stem contraction generally from mid-morning until early evening when canopy water demand exceeds water absorption via the roots (Herzog et al., 1995; Kozlowski et al., 1997). Conversely, expansion of the stem, when water uptake is greater than losses to the atmosphere (Kozlowski and Winget, 1964), starts in the evening and continues to the following morning. Absorption by the root system occurs more slowly than water losses from transpiration resulting in a more negative water potential within the sapwood (Herzog et al., 1998). This decreased potential initiates a transfer of water from surrounding elastic tissues (e.g., enlarging xylem cells, cambial cells, phloem, and bark parenchyma) towards the xylem resulting in a decrease of stem size (Zweifel et al., 2000; Zweifel and Häsler, 2001; Sevanto et al., 2011). Beginning in the evening, the water potential in the xylem increases as the

Figure 3.7: Relations between mean monthly diurnal cycle amplitude and mean monthly air temperature, grouped by species. Regression lines were calculated based on a linear mixed-effects model and were calculated for each species individually. Data are limited to days with no precipitation during the growing season (April-October) over the four growing seasons (2007-2010).



stem is refilled by the uptake of soil water. These changes in stem size, quantified by the amplitude in our study, are signals integrating physiological responses to environmental variability. The timing and magnitude of daily stem contractions provide insights as to the relative changes in transpiration and/or soil water status over time.

In comparison to the growing season, during winter, a phase shift in the daily cycles is observed with maximum stem size in the late afternoon (Figure 3.6). In our study, the similarity of stem size changes between deciduous larch and evergreen spruce (not shown) demonstrates that transpiration is no longer the primary driver of winter circadian stem cycles. These observations agree with Sevanto et al. (2006), who studied stem diameter variation of Scots pine in southern Finland, and concluded that although photosynthesis was observed during the winter, air temperature is the dominant factor determining stem size fluctuations. The winter cycles have been attributed to osmotic water movement due to temperature changes around the freezing point of the sap (Zweifel and Hasler, 2000; Mayr et al., 2006). When temperatures sink below approximately -5°C, extra-cellular water begins to freeze inducing the osmotic withdrawal of intra-cellular water and thus cell and ultimately stem shrinkage (Zweifel et al., 2000; Pearce, 2001; Strimbeck et al., 2008). When temperatures rise sufficiently during the day the process is reversed and water begins to flow back into the cells, leading to stem expansion and a daily maximum (Turcotte et al., 2009). The daily amplitudes that result from these freeze-thaw processes are much smaller than the transpiration driven signals observed during the growing season. However, the same physiological process can result in sudden large decreases in stem size, as exemplified in Figure 3.5, which correspond to events of sudden, large temperature decreases below the freezing point of sap. We hypothesize that a sudden temperature decrease results in larger stem size reductions because a greater volume of water is frozen. Additional evidence for temperature as the driver of these winter freeze-thaw cycles from our study is the relative absence of freeze-thaw events at N08, the warmest site on the altitudinal transect. A comparison of the air temperatures between N08 and all other sites reveal a difference of ~5°C during the winter, large enough that temperatures rarely descend below the sap-freezing point. Additionally, the mean daily cycle at N08 does not show a clear phase shift during the winter months concurrent with the other seven sites. We conclude that there is a distinct winter diurnal cycle and that is driven by ambient temperature changes. Although not occurring during the growing season, the diurnal cycles captured by dendrometers represent an opportunity to further investigate the impact of freezing and thawing on tree growth.

4.2. Variation of daily cycles over seasons, elevation and between species

The magnitude and duration of annual cycles from our sites are within the range of those described from other gymnosperms at both alpine and boreal locations (Tardif et al., 2001; Zweifel and Häsler, 2001; Biondi et al., 2005; Duchesne and Houle, 2011). Our observation of phase shifts of the diurnal cycle when comparing cycles in both winter and summer broadly agrees with Turcotte et al. (2009), who divide the year into three periods: winter shrinkage, spring rehydration and summer transpiration based on the timing of daily stem contraction and

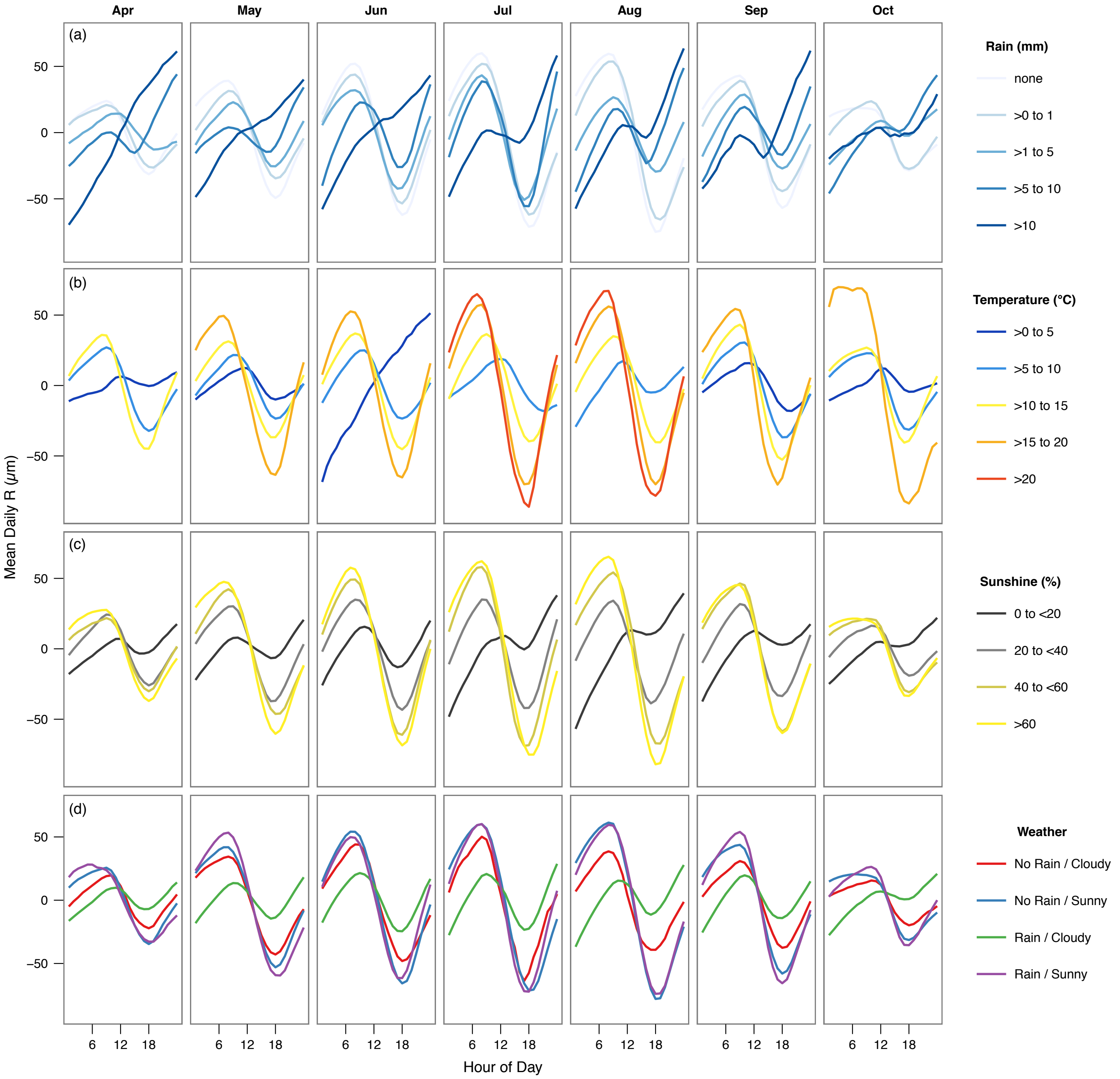


Figure 3.8: Monthly mean circadian cycle during the growing season (April – October) for primary meteorological drivers: (a) the daily amount of rain received; (b) the mean daily temperatures; (c) the amount of sunshine received during a given day adjusted for number of daylight hours; and (d) according to overall weather conditions. Data consist of all point dendrometers averaged across all species and sites and considers four growing seasons (2007-2010). All weather drivers were calculated from the network of five meteorological stations (see Figure 3.1).

expansion as well as the temperature during these phases. However, we did not consistently identify a true spring rehydration period, defined as the period when plant tissues rehydrate in preparation for cell division (Turcotte et al., 2011). Instead, our observation of low diurnal amplitudes in March is a function of the shift between winter and summer and the destructive interference between their respective waveforms, which are either temperature driven or a function of active water movement in the stem. This conclusion is supported by the amplitude minimum observed in November at the end of the growing season and start of winter dominated cycles.

Table 3.2: Descriptive statistics of the mean circadian cycle during the growing season (April – October) for primary meteorological drivers as presented in Figure 3.7. Data consist of all point dendrometers averaged across all species and sites and includes four complete growing seasons (2007-2010).

Environmental Variable		T_{max} (hour of day)	T_{min} (hour of day)	Amplitude (ΔR; µm)	Amplitude Fraction (%)[a]
Rain (mm)	None	8.1 ± 0.2	18.0 ± 0.0	95.8 ± 34.51	1
	>0 to 1	8.7 ± 0.2	18.3 ± 0.1	82.62 ± 29.01	0.86
	>1 to 5	9.6 ± 0.4	17.9 ± 0.2	52.45 ± 27.65	0.55
	>5 to 10	9.7 ± 0.4	16.7 ± 0.3	36.76 ± 29.97	0.38
	>10	10.2 ± 0.3	15.5 ± 0.2	10.61 ± 4.5	0.11
Temperature (°C)	>0 to 5	11.5 ± 0.1	18.5 ± 0.2	19.97 ± 11.36	0.13
	>5 to 10	10 ± 0.3	18.3 ± 0.3	47.68 ± 14.9	0.32
	>10 to 15	8.6 ± 0.1	17.9 ± 0.2	77.99 ± 9.7	0.53
	>15 to 20	6.9 ± 0.5	17.7 ± 0.1	127.14 ± 14.12	0.86
	>20	7.5 ± 0.1	18 ± 0	148.22 ± 3.89	1
Sunshine (%)	0 to <20	11.7 ± 0.3	16.7 ± 0.2	11.42 ± 8.65	0.11
	20 to <40	8.9 ± 0.2	17.6 ± 0.1	63.82 ± 15.62	0.6
	40 to <60	8.6 ± 0.1	18 ± 0.1	93.76 ± 31.04	0.88
	>60	7.9 ± 0.2	18 ± 0	106.21 ± 35.16	1
Weather	No Rain / Cloudy	8.7 ± 0.2	17.9 ± 0.1	72.43 ± 27.36	0.69
	No Rain / Sunny	7.9 ± 0.3	18.0 ± 0.0	99.96 ± 33.79	0.96
	Rain / Cloudy	10.0 ± 0.3	17.3 ± 0.1	28.87 ± 14.21	0.28
	Rain / Sunny	7.9 ± 0.4	18.3 ± 0.1	104.66 ± 30.77	1

[a] Calculated as the fraction of the largest amplitude for each environmental variable

Our study is unique in its ability to assess how seasonal transitions take place across an altitudinal transect, providing a natural ecological experiment with a steep environmental gradient driven by temperature (Körner, 2007). We observe that summer diurnal cycles occur earlier at sites located at lower elevations and similarly, the transition to winter cycles takes place later in the autumn. Based upon our altitudinal gradient, we propose that the primary factor driving transitions between cycles is temperature. Temperature thresholds for the beginning of xylogenesis and more general vegetative biological activity are suggested to occur when daily temperatures exceed 4-6°C (Körner, 2006; Rossi et al., 2008). Sites located at higher elevations (1900 and 2200 m a.s.l.) require more time to surpass these temperatures, inferring that summer diurnal cycles of trees at these locations would start later than trees at 1300 or 800 m a.s.l. This conclusion is supported by the work of Moser et al. (2010), who investigated the timing of xylem formation in *Larix decidua* across this altitudinal gradient during the 2007 growing season. They found that needle budburst and each phase of xylogenesis started later at higher elevations (2.9 - 3.7 days/100 m). These delays were associated with temperature declines as altitude increased and a lengthening of the overall growing season of ~7 days/°C, suggesting that temperature is the primary control for growth onset.

Investigations of the relation between air temperature and daily cycle amplitude at our sites showed clear increases of amplitude with increased temperature, which we relate to increased canopy transpiration rates and water demands. Comparison of the diurnal cycles and regression equations for the monitored species (*Picea abies* and *Larix decidua*) reveals similar timing for the beginning of stem water use, but greater amplitudes in spruce. This implies that during a diurnal period, *Picea abies* exploit their internal stem water reserves more quickly than *Larix decidua*. One possible explanation for this pattern is that European larch have a greater conductivity area and therefore a greater capacity for water reserves compared to Norway spruce (Schulze et al., 1985; Gower and Richards, 1990; Bryukhanova and Fonti, in review). An alternative and perhaps more simple hypothesis relates to the difference in the root systems between the two species. As a pioneer species, larch have deeper root systems than spruce and this may allow larch to access additional soil water resources and limit the use of internal

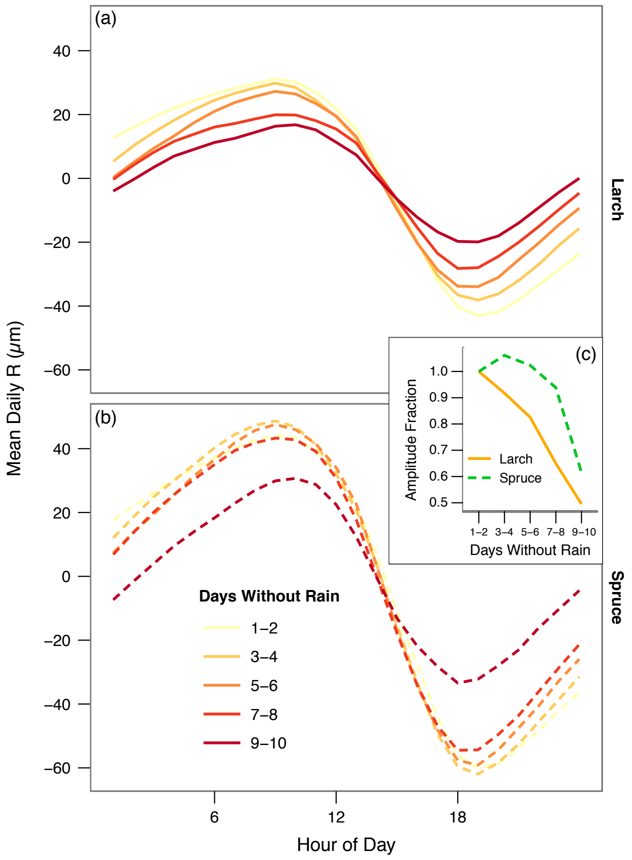


Figure 3.9: Mean circadian cycles during the growing season (April - October) grouped by the number of days since the last precipitation event. Data are divided by species (a) larch, and (b) spruce. Panel (c) shows the amplitude decrease as time since rain event increases for both species.

reserves (Gower and Richards, 1990; Tjoelker et al., 2007). However, with projected warming, we expect larch will experience greater stem contraction with increasing temperatures and at the warmest sites, perhaps eventually exceed the daily amplitude of spruce. These results are in agreement with recent research from lower-elevation sites in the Swiss Alps that has found European larch growth had the most-negative response to soil-water limitations (Eilmann and Rigling, 2012). These high rates of water-loss on already dry sites could severely limit growth and may even lead to tree death.

4.3. Climatic drivers

In addition to examining the spatial and temporal aspects of the diurnal stem cycle, we also investigated the influence of different meteorological variables on circadian rhythms. Similar to our findings, investigations performed with high-resolution measurements of stem size consistently find responses to changes in the short-term weather conditions (Fritts and Fritts, 1955; Kozlowski and Winget, 1964; Herzog et al., 1995; Devine and Harrington, 2011). However, with our methods, we are also able to examine daily stem size variations on an annual scale and find that maximum amplitudes of the daily cycles were found in June and July, coinciding with the highest temperatures at the study sites. Additionally, intensification of the daily amplitude was found with increasing daily mean air temperatures and greater amounts of sunshine. Both of these variables act to increase the amount and rate of daily transpiration (Jarvis and McNaughton, 1986; McKenney and Rosenberg, 1993). Increased transpiration leads to elevated water demand and due to time lags with the root system, individuals must fulfill any difference in water requirements with water stored in internal tissues (Herzog et al., 1998; Zweifel and Häsler, 2001). If trees do not utilize stem water, it may be necessary to close stomata, which can result in carbon starvation and negative consequences for tree growth (McDowell et al., 2008; Eilmann et al., 2009).

We see indications of these patterns when evaluating species diurnal cycle response to periods without rain. Spruce experiences similar stem cycle amplitudes for approximately six days before undergoing a large decrease of stem size oscillations. This behavior is consistent with a more negative xylem water potential and stomatal closure to prevent large water losses when soil reserves are incapable of fully replacing stem water. In contrast, larch experiences a slow, steady amplitude decline indicative of greater water use to buffer increased demand. These results agree with observations from the European Alps, which found that trees reduce their rates of transpiration slowly with decreasing availability of water resources from the soil, and only show large reductions in water use after specific thresholds are exceeded (Anfodillo et al., 1998; Clausnitzer et al., 2011). These observations indicate that the internal storage of water and the mobilization of these sources on a daily basis are essential to tree productivity.

Our data also indicate that days with more precipitation were shown to have smaller and delayed stem contractions. Precipitation events lead to an increase in available soil water, but there is also evidence that rain intercepted by needles and branches can be directly absorbed and used for transpiration demands from the canopy (Katz et al., 1989). However, the delay of stem contraction observed on rainy days may also suggest that instead of changing water sources, the internal reserves of the tree are still utilized, but are refilled nearly at depletion rate (Zweifel and Häsler, 2001; Steppe et al., 2006; Deslauriers et al., 2007). Regardless of the path, availability of water from alternative sources reduces demand on internal water reservoirs. In addition, our weather index revealed that the amount of sunshine moderated the influence of precipitation on the daily stem amplitude. Increased radiation results in greater transpiration rates, leading to increased stem water mobilization and thus greater stem contraction (increased amplitude). These conditions appear to counteract any reduction of stem contraction based on the presence of precipitation. However, during cloudy conditions and presumably decreased transpiration rates, increased water availability from precipitation has a clear offset on the amount of stem contraction.

The plant water system is a continuum between the atmosphere, needles, stems and the soil. Therefore, soil water availability may play a role in modulating the diurnal stem cycle. Kozlowski and Winget (1964) proposed that diurnal stem shrinkage is primarily driven by the depletion of soil water resources. This notion is not supported by our data. Although available soil water certainly plays a role in the dynamics of tree water movement (and specifically short-term water stress), the seasonal course of soil water at our sites decreases over time, from a maximum in spring to a minimum in the fall. These trends do not coincide with the greatest amplitudes at our sites in June and July. A comparison among sites is also unsupportive of the proposal of Kozlowski and Winget (1964). While similar soil water availability exists across the transect (Laubscher, 2009), temperatures show a definite decrease from highest to lowest elevations, again suggesting reduced stem contraction with increasing altitude is more closely linked to temperatures.

4.4. Implications and Opportunities

As dendrometers are broadly regarded to record tree radial growth, many long-term monitoring programs sites rely upon dendrometer measurements (Jolly et al., 2005). However, it is essential to understand how processes unrelated to growth may bias these estimates. Failing to take into account the variability contained within the annual cycle and intra-seasonal variation in weather and tree water status when deciding upon measurement times and intervals can lead to over or under-estimates of growth. Such biases would have important implications on forest models, land-use management, and carbon accounting schemes.

Dendrometers have also been increasingly used to calculate the growth response to climatic variables. The most common approach for analysis of continuous dendrometer data is the stem-cycle method (Downes et al., 1999; Deslauriers et al., 2003). This method splits the diurnal cycle into phases of expansion, contraction and stem radius increment (SRI). Once these have been calculated, each phase is commonly correlated with meteorological variables (Deslauriers et al., 2003, 2007; Bräuning et al., 2009; Duchesne and Houle, 2011). Most studies consider the cumulative SRI to be analogous to radial growth and conclude that water based-measures, such as relative humidity and precipitation are the most important factors influencing growth (Oberhuber and Gruber, 2010; Krepkowski et al., 2011; Volland-Voigt et al., 2011). Our set of methods removes the seasonal growth trend from the dendrometer data, but still reveals a clear increase of stem size on days with precipitation. In the stem-cycle method days with precipitation would be over-represented as days with a positive SRI, resulting in a greater overall contribution to the cumulative SRI. We suggest that this results in artificially inflated importance of moisture related meteorological variables (e.g. precipitation, VPD) during calculation of statistical relations. Although we acknowledge that turgor is required for cell growth (Verbelen and Vissenberg, 2007), there is no indication that greater amounts of growth take place on days with precipitation, as trees always attempt to maintain turgor through self-regulation of internal movement of water (Kroeger et al., 2011). Our analyses suggest that a clear understanding of possible biases and sources of statistical error are essential to working with dendrometers. Our data indicate that factors such as temperature and radiation play a greater role in fine-scale stem radius dynamics and overall tree growth (as calculated from dendrometer records) than widely acknowledged.

Intra-annual dendrometer measurements are primarily composed of two different signals, water movement within the stem and growth (Herzog et al., 1995). The analyses presented herein have provided a valuable step towards improving our understanding of the factors that influence short-term changes in stem radius and have revealed possible biases in attributing the climatic drivers of radial growth. These novel methods also contribute to the assessment of the relative amounts of transpiration (and photosynthetic activity) between individuals, over time and as a function of climate. Additional investigations linking observations of xylem development to dendrometer records will contribute to quantifying how well irreversible radial growth is also captured. This study has clearly shown strong climatic controls on reversible stem size fluctuations.

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Chapter 4

Convergence of leaf and cambial growth phenology sensitivity to temperature

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In Preparation for *Frontiers in Ecology and the Environment*

Introduction

Simulations from the most recent generation of climate models continue to project a high likelihood of increasing temperatures over the next decades to centuries (IPCC, 2013). These increases are projected to perpetuate if not accelerate the warming trends of the past decades to centuries (Brohan et al., 2006; Diffenbaugh & Field, 2013). Understanding the possible impacts of climate change on ecosystems is a priority in order to mitigate or adapt to its most severe negative consequences. In this regard, the impacts of climate variation and extremes on plants, which are the basis of food webs and play key roles in carbon, nutrient and water cycling, are especially important to understand. Despite many observations regarding ecosystem responses to recent warming (Serreze et al., 2000; Parmesan, 2006; Bellard et al., 2012), there is still considerable uncertainty surrounding species' response to both past and projected climatic changes (Jump & Peñuelas, 2005; Chen et al., 2011).

Insights into plant responses to climate have been obtained using several common approaches: long-term observations and reconstructions, small-scale experiments and large-scale remotely sensed observations. Monitoring phenology of plants from natural settings provides insight into inter-annual responses, and in the cases of longer records, even for several centuries (Lauscher, 1978; Sparks & Carey, 1995; Rutishauser et al., 2008). Most phenological records from the past century show a shift of life cycle events (e.g., flowering, budburst) to earlier in the season, the same direction as would be expected in response to global warming (Menzel et al., 2006; Morisette et al., 2008). Long-term observations can also be obtained from tree-ring reconstructions, which can extend back thousands of years (Büntgen et al., 2005). Importantly, these types of studies do not explicitly control the environment, but rather collect observations, perhaps from multiple species (Fitter et al., 1995; Morin et al., 2009), in an attempt to capture the complexity of an ecosystem given natural variability in weather conditions. *In-situ* field-based studies experimentally manipulate small-scale plots, such as by artificially increasing temperatures in one set of plots and comparing the resulting growth with control plots (Harte & Shaw, 1995; Walker et al., 2006). These studies attempt to extrapolate to projected future conditions through isolation of ecosystem drivers. Complementing local observations and experimental manipulations, satellite data provides landscape-scale, spatially explicit monitoring of vegetation dynamics. The normalized difference vegetation index (NDVI) and the Moderate-resolution imaging spectroradiometer (MODIS) datasets are now long enough to compare records against climate, infer possible linkages between warming temperatures and phenology (Ivits et al., 2012), and observe changes in growing season length (Myneni et al., 1997).

However, recent comparisons have shown that these methods provide an inconsistent quantification of plant response to climate change. Wolkovich et al. (2012) compared an extensive database of observational and experimental studies and found that warming experiments underestimate the sensitivity of phenological variation up to 8.5-fold. Although long-term observational datasets are excellent measures of response to climate change (Rosenzweig et al., 2008), complex interaction effects of co-varying environmental parameters may prevent a full understanding of the dynamics, with observed responses unable to be accurately replicated in experiments. Furthermore, these observation methods are only applicable to selected plant functions (i.e. leafing or flowering), and reveal little about carbon assimilation processes such as cellular division and maintenance or radial growth and accumulation of stem and branch biomass (Rossi et al., 2009, 2011). However, recent advances have led to improved investigation of the timing of these secondary growth processes (Rossi et al., 2006; Gricar et al., 2011). Finally, it is not clear whether inter-annual sensitivities derived from observational records correspond to responses that would be found given long-term warming trends.

Improvement of the quantification of warming impacts on plants can be nicely explored using elevational gradients. Gradients enjoy the advantage of observing natural ecosystems, and isolating the temperature differences between elevations as a powerful "natural experiment" (Körner, 2007) whereby increases in temperature are considered analogues for future climate conditions. The close geographical distances of sites along an elevational gradient offer particular benefits in contrast to latitudinal and/or global scale analysis from satellites. Namely, the variability of

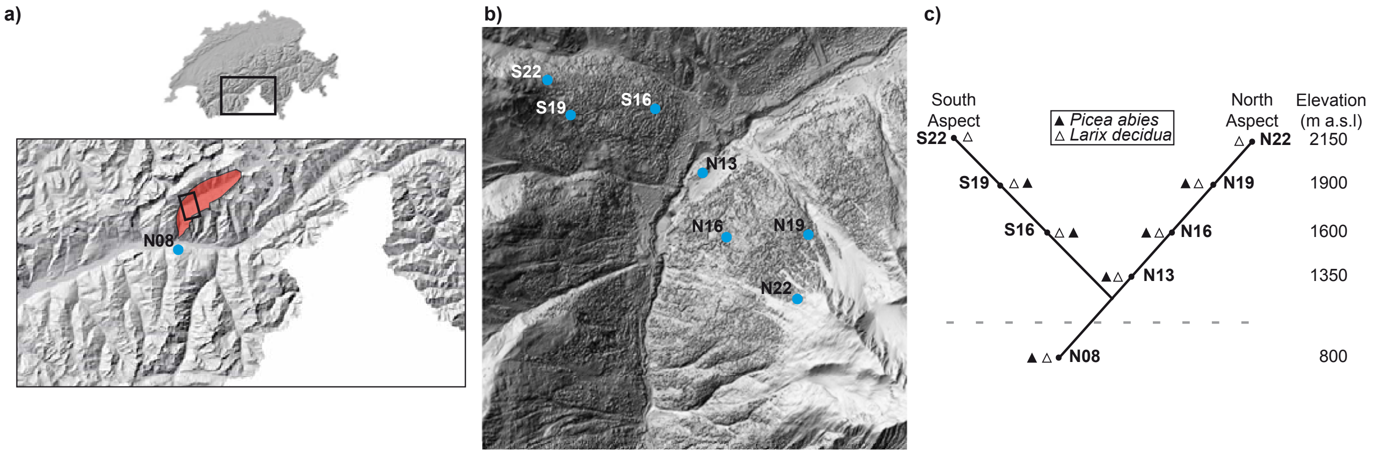


Figure 4.1. Study location. (a) Regional map showing the Lötschental (shaded red polygon). (b) Digital elevation model (DEM) of the primary sampling area showing the sites (blue circles); the 800 m a.s.l. site is located in (a). (c) Schematic diagram of the transect showing site elevations, codes and sampled species.

weather conditions (e.g. rainfall events) are very similar across the elevational bands and additionally the short distances may contribute to high gene flow that counters confounding genetic influences (King et al., 2013).

A pilot study of thermal impacts on the timing and duration of cellular maturation and thus radial growth was presented by Moser et al. (2010) for European larch from a 4°C temperature gradient in the Swiss Alps. This study observed shifts of nearly 7 days / °C but was not able to address species differences, if the data were characteristic of responses from other years (e.g., 2007 was the warmest spring during the past 500 years; Luterbacher et al. 2007), or if climate sensitivities along the elevational gradient are in any way comparable to responses inferred from inter-annual observations.

Here, we present the results from a study of cell phenology across a 1,400-meter elevational transect in the central Swiss Alps. We monitored wood formation of two conifer species (*Picea abies* and *Larix decidua*), over a four-year period (2007-2010), and with an extended temperature gradient corresponding to a 6.5°C warming from the uppermost to lowermost site. In this study, the developmental stages of xylem cells (>130,000 individual cells on 6,500 microsections) were tracked weekly, permitting understanding of xylogenesis onset, duration and cessation. We place our results within the context of both phenology observations and warming experiments to provide crucial quantification of the climate sensitivity of wood formation.

Methods

Study Area and Transect Description

The study area was centered within the Lötschental, an inner-alpine valley located in the Swiss Alps (46°23δ40δN, 7°45δ35δE; Figure 4.1). Sampling sites were established on the forested slopes around the village of Ferden, and consisted of mixed evergreen Norway spruce [*Picea abies (L.)* Karst.] and deciduous European larch [*Larix decidua* Mill.] trees that extend from ~1300 m to ~2300 m (all elevations are a.s.l.). The valley soils are formed from calcareous-free substrate contributing to acidic soils characterized by coarse stone content and low amounts of clay.

In late 2006, seven sites were located along a 900-m elevational transect: a single site at the valley bottom (~1300 m) and three sites at ~300-m elevation intervals along both the north and south slope aspects to ~2200 m, just below the upper forest limit. To further extend the elevational gradient, an additional site was established in early 2008 on a north-facing slope at an elevation of ~800 m in the lower Rhône valley. Codes for aspect and elevation (in hundreds of meters) were assigned to each site (e.g. S16 for 1600 m on the south facing slope).

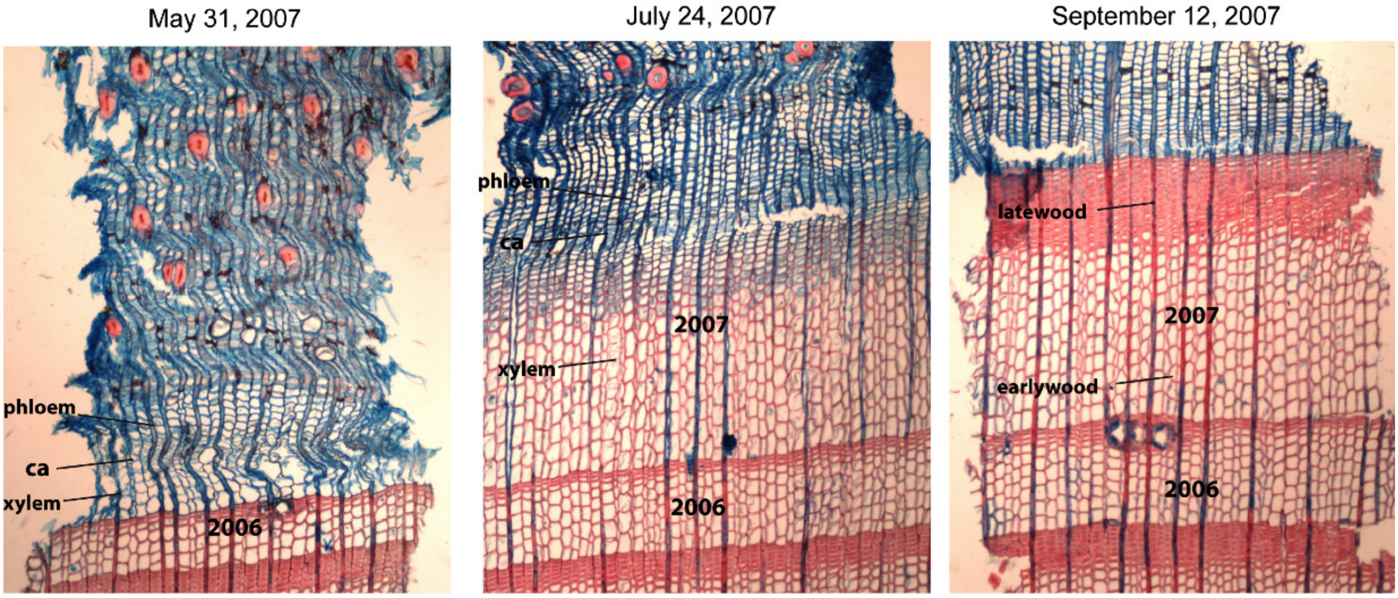


Figure 4.2. Photographs of stained thin-sections prepared from microcores collected during the 2007 growing season. The cambium (ca) and forming xylem and phloem are identified. The final image shows the differentiation between earlywood and latewood.

Based on the records from Blatten a village located in the upper part of the valley, the Lötschental has a mean annual temperature of 5°C, with monthly averages ranging from -3°C (January) to 15°C (July) and an annual mean of ~800 mm precipitation falls more or less regularly throughout the year (1987-2006). Conditions during the study (2007-2010) were broadly similar to these long-term averages, except for a slightly warmer spring (attributable to a very warm 2007), above average late spring – summer precipitation, and lower precipitation in early autumn. Mean temperatures of this region are strongly dependent upon elevation, with the established transect providing a 6.5°C growing season gradient from the lowest (N08) site to the upper treeline sites (N22 & S22). This difference is similar in magnitude to the projected increases in central Europe summer temperatures to the year 2100 under the A1B scenario (CH2011, 2011; IPCC, 2013).

Monitoring of Environmental Conditions

Environmental conditions were monitored *in-situ* at each sampling site. From 2007 onward, stem temperatures were measured every 15 minutes with a logger (Onset, Tidbit) installed on a single tree ~1.6 m above the ground and protected from direct solar radiation. Beginning in spring 2008, air temperatures were recorded at 15-min resolution using a radiation shield-covered sensor (Onset, U23 Pro) on a central tower (~ 2.5 m above the ground) beneath the canopy. Soil temperatures were recorded at an hourly resolution with a logger (Onset, Tidbit) buried at a depth of 15-20 cm. Soil moisture was recorded using 10 sensors (Decagon, EC-5) installed at two depths (5 sensors at 10 cm and 5 sensors at 70 cm) for each site and measured every hour. Site temperatures presented herein are an appended record of the external stem (2007) and air temperatures (2008 onwards) for each site to ensure a full record length. Temperature differences along the gradient were calculated using monthly averages over a five-year period (2007-2011). Annual growing degree days (GDD) were calculated for both air and soil temperatures from each site along the elevational transect. Soil temperatures used a baseline of 0°C and air temperatures used a baseline of 5°C.

Microcore Collection and Sample Preparation

At each site four mature, dominant and healthy trees of each species (*Picea abies* and *Larix decidua*) were selected for observation of radial growth. At N22 and S22, which are above the local altitudinal range for spruce, only larch trees were monitored. Sampled trees have average heights of 27 m and diameters of 44 cm, and were similar for

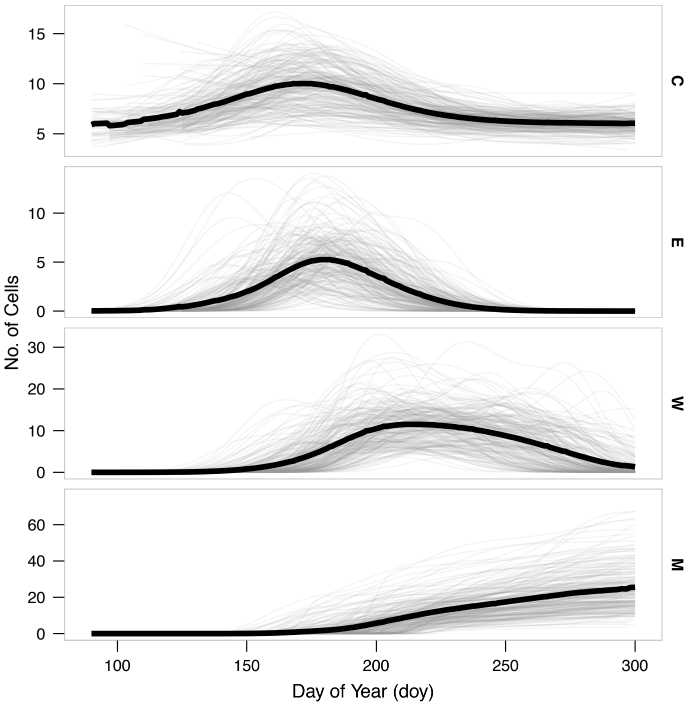


Figure 4.3. Spaghetti plots of general additive model (GAM) curve fits from every tree and year for all cell phases (C = cambial, E = enlarging, W = wall-thickening, and M = mature). Black lines represent the mean curve for each cell phase. Note differences in the y-axis scales for each cell phase.

both species. Trees are generally older at higher elevations (e.g. mean of 251 years at 2200 m and 116 at 1300 m), with larch and spruce exhibiting similar ages at the same site. From 2007 until 2010 two microcores per tree (15 mm in length and 2 mm in diameter, including the phloem, cambial zone and both the last complete ring and the forming one) were collected weekly between April and October using a Trephor (Rossi et al. 2006b). Punches were collected perpendicular to the slope direction to avoid reaction wood. To minimize wound reaction, sampling was conducted along an oblique line (starting from ~50 cm below to ~50 cm above breast height), with all samples positioned approximately 3-5 cm apart. Different sets of trees, located in close proximity at individual sites, were used during the course of the study. The first set, sampled in 2007, contained 48 trees sampled on both sides of the tree and then averaged together, the second set contained 56 trees sampled in 2008 and 2009 (with samples collected on one side of each tree during each year), and a third set of 56 trees was sampled in 2010.

Following collection, samples were immediately placed in a dilution of acetic acid and ethanol to preserve forming cells from degradation. They were kept in this solution for 24 hours and then stored in a 70% alcohol solution. Samples were prepared for cellular analysis by cutting 15–25-µm-thick transversal microsections with a sliding microtome. Microsections were stained with a combination of safranin and astrablue and permanently fixed to microscope slides with Canada balsam.

Leaf phenology data were collected weekly from the sampled trees by observing the percent of a tree that had undergone bud burst in the spring, and for *Larix decidua*, autumnal colour change. To interpolate daily values from weekly observations, we fit a general additive model (GAM). Data represent the date when 50% of a tree crown had reached the identified phenological stage (Brügger & Vassella, 2003).

Assessment of Xylogenesis

Each xylem cell passes through four notable phases of cell division and differentiation over the growing season.

First, tracheids are produced via cell divisions in the cambial tissue. Subsequently, cell enlargement occurs, when thin primary cell walls expand and cell size increases. Cellulose and lignin deposition occurs during the secondary wall-thickening phase. At the end of differentiation, maturation is completed via protoplast autolysis and cells become physiologically capable of conducting water to the needles (Gričar et al., 2005). Each of these phases can be observed using the stained microsections and a light microscope.

Xylem development in each microsection was analyzed at a magnification of 400× – 600× and the number of tracheids in each cell development phase (i.e. cambial (C), enlargement (E), wall-thickening (W) and mature (M)) was counted along three to five radial files (Figure 4.2). The dormant cambium is composed of closely spaced, flat cells with no secondary walls. At the onset of cambial activity, the cambial zone begins to widen rapidly with cell division. Enlarging xylem cells were distinguished when their radial diameter reached at least twice that of a cambial cell. Directional cellulose fibers laid down during formation of the secondary cell wall reflected polarized light, permitting discrimination between enlarging and wall-thickening tracheids. Mature cells were recognized by completely lignified secondary walls (stained red) and empty cell bodies. Average values of the radial file counts for each microsection were used for subsequent analyses.

Data Analyses

Growth Onset, Cessation, Duration and Maximum Rate

Several definitions of secondary growth, the process of stem and root thickening, have been proposed in the literature. Following Rossi et al. (2006), we consider xylem cell development to begin with the first appearance of cells in the enlargement phase and to be complete when cells finished deposition of the secondary cell wall. The rates of cellular differentiation and duration for each phase were calculated based on the cumulative cell counts from i) enlarging, wall-thickening and mature phases (EWM), ii) wall-thickening and mature phases (WM), and iii) only the mature counts (M) (Figure 4.3).

To ensure individual tree signals could be compared to assess variability, we fit a generalized additive model (GAM) to the observed counts for each individual tree and year using R-packages mgcv and scam (Wood, 2006; Pya, 2012), building upon methods proposed by Cuny et al. (2013). A quasi-poisson distribution with a log-link function was found to appropriately model the rapid transition from dormancy to active growth at the beginning of the growing season. To mitigate end effects due to fluctuations in total number of cells after growth had stopped, the last four non-missing values were repeated prior to model fitting (Mann, 2004), with the final model truncated to match the timespan covered by the observed data. Monotonic constraints were applied for the mature (M), and combined (EW and EWM) cell counts to ensure appropriate biological meaning between the empirical fitting and the cumulative cell numbers throughout the growing season. The degree of smoothing in the model was limited to 10 knots in the mgcv and scam functions. This was considered a compromise between the number of observations and the signal to noise ratio, with 5 knots being too stiff to faithfully capture the beginning of the growing season and plateaus in the total number of cells during the earlywood to latewood transition (Cuny et al., 2013) and 20 knots being too sensitive to weekly variability in the number of cells or not showing a significant improvement over 10 knots (Supp. Figure 4.1). As a solution for high within-tree variability of cell numbers at the end of the season, we ensured model convergence by scaling each of the fits to the final number of mature cells. The day of year (doy) for the onset and completion of each cell phase (excluding the cambial cells, which cannot be assigned a true start or cessation) was found by determining when the 5% and 95% completion points for each curve were reached. A first difference series of each curve was also calculated to identify the rates of entry for each cell phase and the respective maximum rate (Supp. Figure 4.2).

Table 4.1. Maximum number (mean) and date of occurrence of the maximum number (calendar dates (DOY)) of cells in the cambial (C), enlargement (E), wall thickening (W) and maturation (M) zones of wood formation grouped by elevation, species and observation years. Values computed from the fitting of a monotonic generalized additive model (GAM).

Cell Phase		Elev					Species		Year			
		800	1300	1600	1900	2200	Larch	Spruce	2007	2008	2009	2010
Cambial (C)	Peak Value	7.5	9.7	10.6	11.0	10.3	9.8	10.6	16.4	10.7	9.6	10.5
	Time of Max	25 May (145)	17 Jun (168)	17 Jun (168)	23 Jun (175)	25 Jun (177)	23 Jun (175)	16 Jun (167)	13 Apr (103)	23 Jun (175)	17 Jun (168)	24 Jun (175)
Enlarging (E)	Peak Value	4.0	5.4	6.3	5.9	5.3	4.7	6.3	5.0	5.7	5.3	5.2
	Time of Max	30 May (150)	23 Jun (175)	25 Jun (177)	5 Jul (185)	4 Jul (184)	3 Jul (182)	25 Jun (177)	27 Jun (178)	3 Jul (182)	27 Jun (178)	30 Jun (181)
Wall-Thickening (W)	Peak Value	8.3	11.2	12.4	12.2	11.5	10.4	13.6	11.0	11.6	11.3	12.5
	Time of Max	7 Aug (220)	21 Aug (233)	22 Jul (203)	3 Aug (216)	4 Aug (217)	7 Aug (220)	22 Jul (203)	8 Aug (220)	12 Aug (225)	30 Jul (211)	27 Jul (208)
Maturation (M)	Peak Value	27.8	29.2	38.7	27.2	22.7	24.2	40.9	25.1	32.9	29.1	27.2
	Time of Max	4 Nov (308)	10 Nov (314)	21 Nov (326)	17 Nov (322)	5 Nov (309)	21 Nov (326)	10 Nov (314)	31 Oct (304)	21 Nov (326)	4 Nov (308)	26 Nov (330)

Investigating drivers of growth

Relationships between growth and possible explanatory variables involved simple linear regression models between onset of growth and air temperature variables.

Based on studies that have highlighted the importance of spring temperatures for the onset of tree growth in species that have low winter chilling requirements (Menzel et al., 2006; Morin et al., 2009), we applied simple linear regressions between air temperatures from a number of likely seasonal windows and the onset of cell phases for all trees grouped by year. From these we identified a single predictor, average April to June air temperatures, as the best predictor for leaf and cambial phenology.

Results

Cambial Phenology and Xylogenesis

The GAM fits of cellular count data reveal a clear seasonal progression of the four main xylem development stages (Figure 4.3, Table 4.1). As raw material for differentiation, cambial zone production commences early in the season. It expands from a minimum number of cells during dormancy (~ 5-6 cells) to a peak around mid-June (doy 168; mean of 10.0 cells), then decreased to dormancy levels by early August. Once divided from the cambium, xylem cells began to enlarge and reach maximum numbers around day 180 (late June/early July: mean of 5.4 cells) before slowly decreasing over the remainder of the growing season. Similarly, the seasonal wall-thickening phase followed the enlargement phase and reached a peak (doy 215; mean of 11.4 cells) approximately a month later. The end of wall-thickening signaled the end of xylem cell development and completion of biomass allocation to the tree stem. Mature cells began to appear in late June and reached a maximum (31.8 cells) in early November.

Although both species follow similar developmental trajectories, spruce reaches the maximum number of cells

Table 4.2. Maximum rate of entry (cells/day) and date of occurrence of the maximum rate (calendar dates (DOY)) for cells entering the enlargement (E), wall thickening (W) and maturation (M) zones of wood formation grouped by elevation, species and observation years. Values computed from the fitting of a monotonic generalized additive model (GAM).

Cell Phase		Elev					Species		Year			
		800	1300	1600	1900	2200	Larch	Spruce	2007	2008	2009	2010
Enlarging (E)	Max Rate	0.47	0.48	0.55	0.51	0.44	0.40	0.65	0.38	0.49	0.54	0.59
	Time of Max	23 May (143)	19 Jun (170)	27 Jun (178)	1 Jul (182)	26 Jun (177)	27 Jun (178)	18 Jun (169)	23 Jun (174)	27 Jun (177)	21 Jun (171)	22 Jun (172)
Wall-Thickening (W)	Max Rate	0.59	0.63	0.67	0.66	0.56	0.55	0.74	0.47	0.63	0.67	0.74
	Time of Max	6 Jun (157)	29 Jun (180)	8 Jul (189)	10 Jul (191)	13 Jul (194)	8 Jul (189)	29 Jun (180)	6 Jul (187)	9 Jul (190)	29 Jun (179)	5 Jul (186)
Maturation (M)	Max Rate	0.61	0.76	0.74	0.65	0.54	0.61	0.75	0.50	0.67	0.64	0.85
	Time of Max	19 Jun (170)	15 Jul (196)	22 Jul (203)	1 Aug (213)	2 Aug (214)	25 Jul (206)	17 Jul (198)	24 Jul (205)	27 Jul (208)	15 Jul (196)	23 Jul (203)

in all phases 10-12 days earlier than larch despite spruce producing 30-50% more cells than larch. Across the elevational gradient, peaks in cambial and enlarging phases occur later at higher elevations. However, this pattern does not hold for wall-thickening, as sites at 1600 m peak earliest (doy 203, an average of 18.5 days earlier than other sites) and have a greater number of cells in that phase (12.4 cells). Considering the total number of cells produced (total mature cells), we also see 1600 m has the most cells produced an average of 12 more cells than other sites. Inter-annual comparisons reveal similarities for the enlarging and wall-thickening phase, but that 2007 showed distinctly different patterns with a much larger peak in cambial cells that occurred almost 2 months earlier than other years. However, 2007 also had a lower total number of cells produced at the end of maturation.

Rates of entry into the three phases were also calculated (Table 4.2) and reveal differences with the peaks of each cell phase discussed above. Cell enlargement has a maximum rate of 0.5 cells/day on doy 174 (late June), while secondary wall thickening shows a slightly higher maximum rate of 0.63 cells/day in early July (doy 185). Cells becoming mature have a maximum rate of 0.67 cells/day and show that peak in late July (doy 203). Splitting these trends up by species show that spruce have higher maximum rates of cell production for each phase, and also reach those peaks approximately a week earlier than larch. Maximum rates of entry into each phase peaked at mid-elevations (1300 or 1600 m) but were also delayed as elevation increased. Inter-annually there was little difference of when the maximum rate took place, but each subsequent year showed increasing rates.

Contrasting Leaf and Cambial Growth Phenology

Comparisons of leaf and cambial growth phenology between larch and spruce reveal that leaf phenology does not appear dependent upon cambial activation (Figure 4.4; Table 4.3). *Larix decidua* showed average leaf development in the middle of May (doy 135.0±11.4), earlier than the beginning of xylem formation, which started on average at the end of May (doy 149.3±11.6). Conversely, *Picea abies* revealed leaf development in early June (doy 155.1±7.4) after the beginning of stem growth at the end of May (doy 142.8±12.8). This also shows that on average, stem growth begins a week earlier in spruce than larch. As stem growth for both species is completed in early September (larch = 252.8±18.3; spruce 251.8±20.2), overall durations are comparable for both species (larch =103.4±16.1 days; spruce =108.9±15.8 days). Larch needle colouration, the first step towards leaf senescence, was monitored (from 2008-2010) and occurred on average in late October (doy 293±7.6). Using this leaf phenology completion

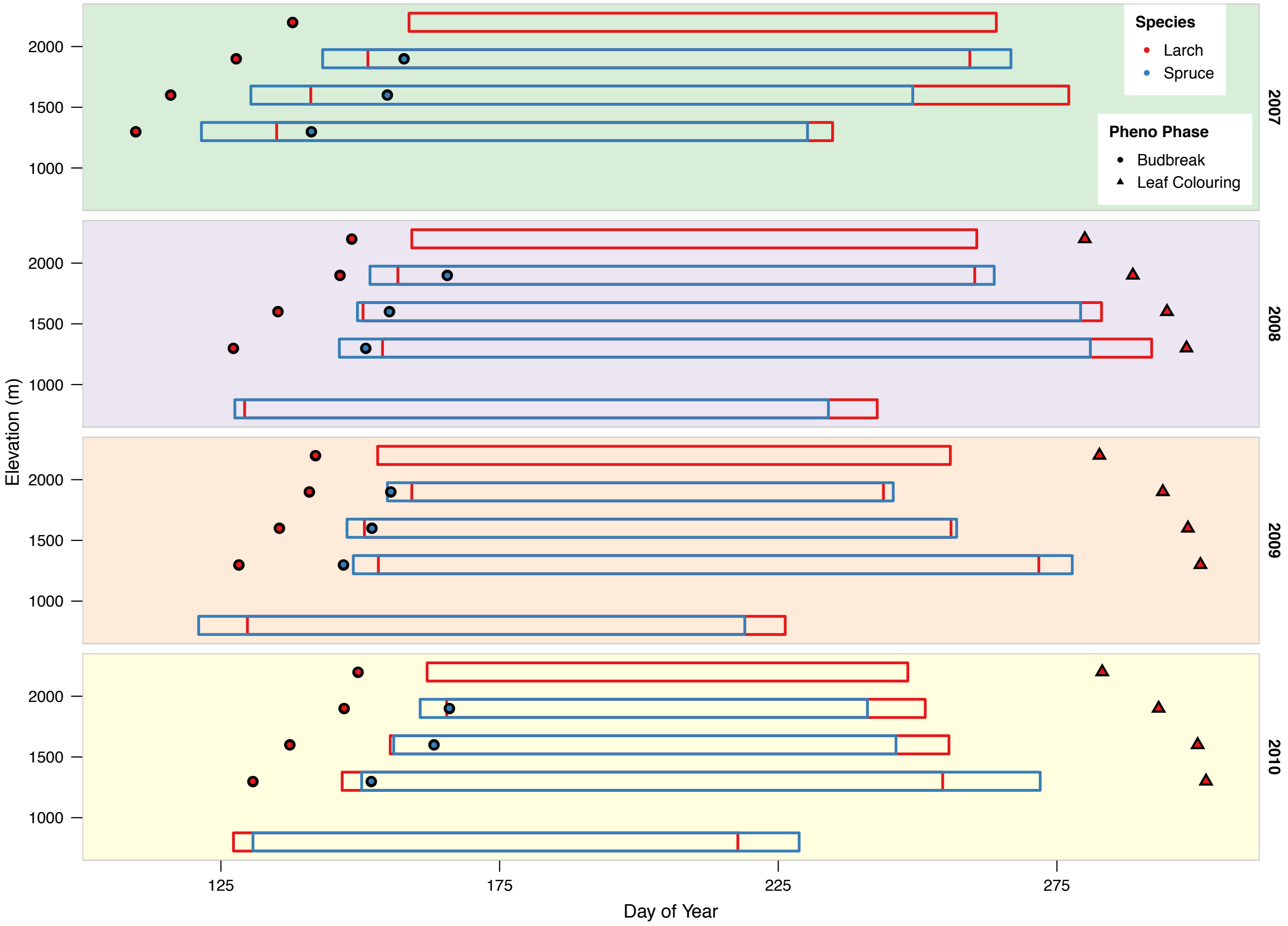


Figure 4.4. Inter-annual dynamics of leaf and cambial growth of larch (red) and spruce (blue) trees along the elevational transect. Points represent needle emergence (circles) and colour change (triangles), while rectangles indicate the extent of radial growth. All data are mean elevation values with each facet showing a different year from 2007-2010.

date, a season duration from leaf out to leaf fall for larch is on average 154.0±14.9 days, about 50 days longer than active stem growth.

Across the elevational gradient leaf phenology patterns are similar between species, budbreak is delayed as elevation increases (mean of 2.36 days/100 m for larch and 2.24 days/100 m for spruce). Radial growth onset also shows delays as site elevation increases (larch = 2.26 days/100 m; spruce = 2.88 days/100 m), although without as much consistency as phenology. However, cessation of growth does not follow the expected inversion of this pattern, as predicted by theory. Completion of xylem development often occurs latest in the middle of our sampled range (1300 or 1600 m), resulting in an approximate parabolic shape across the gradient. Similarly, leaf colour change occurs earlier at higher elevations, although differences between elevations decrease lower on the slope suggesting a similar parabolic pattern.

Inter-annually, two distinct patterns emerge, one concerning the onset of both leaf and cambial phenology, and the second dealing with the completion of stem and leaf processes (Figure 4.4). Comparing onset, all years show relatively similar response with the exception of 2007. Compared to the average from 2008-2010 (doy 139, May 19), larch bud break shifted 17 days earlier and a similar pattern emerged for secondary growth in spruce (14 days earlier than doy 145, May 25). This translated into earlier stem growth in larch across most elevations, but did not materialize at the highest elevation (2200 m), while spruce bud burst remained consistent with other observation years. Cessation of growth is more variable among years, with completion showing 5 times greater variability than growth onset (4.8 days compared to 24.6 days). Both larch and spruce showed the earliest completion of growth

Table 4.3. Timing of primary (budbreak and colour change) and secondary growth (onset, cessation and duration) for larch and spruce. Values represent means for elevations along the gradient and years of observation (means ± SDs).

Timing		Elev					Year			
		800	1300	1600	1900	2200	2007	2008	2009	2010
Budbreak	Larch		124.0 ± 8.5	131.0 ± 9.0	140.5 ± 8.7	144.5 ± 5.7	124.7 ± 11.4	141.1 ± 8.0	137.9 ± 5.0	142.7 ± 7.4
	Spruce		147.8 ± 4.5	156.4 ± 4.7	161.2 ± 5.2		153.4 ± 6.6	158.6 ± 6.3	152.4 ± 3.5	162.1 ± 5.9
Onset	Larch	128.8 ± 2.9	147.3 ± 8.3	149.4 ± 6.7	158.2 ± 8.9	158.3 ± 7.0	148.2 ± 10.6	152.0 ± 11.1	151.2 ± 10.1	155.0 ± 13.1
	Spruce	126.4 ± 8.7	141.7 ± 12.7	145.9 ± 10.5	152.7 ± 8.3		133.8 ± 9.5	146.0 ± 9.8	145.8 ± 13.3	152.4 ± 11.6
Cessation	Larch	228.9 ± 34.6	263.3 ± 33.9	267.9 ± 27.8	253.7 ± 23.2	257.2 ± 19.4	262.3 ± 25.7	267.8 ± 31.6	251.2 ± 25.9	247.8 ± 24.9
	Spruce	227.3 ± 28.4	265.3 ± 30.0	257.9 ± 26.7	254.3 ± 23.4		252.4 ± 22.2	266.8 ± 27.5	250.3 ± 29.5	245.8 ± 29.3
Duration	Larch	100.2 ± 34.0	116 ± 28.8	118. 5 ± 29.0	95. 5 ± 23.8	98.9 ± 22.5	114.1 ± 26.4	115.8 ± 30.6	100.0 ± 25.3	92.9 ± 23.6
	Spruce	100.8 ± 27.9	123.6 ± 24.6	112.0 ± 27.8	101.6 ± 27.8		118.7 ± 18.4	120.8 ± 24.4	104.5 ± 28.4	93.4 ± 30.7
Colour Change	Larch		300.2 ± 2.7	297.8 ± 4.3	292.0 ± 5.1	281.9 ± 6.1		289.3 ± 8.5	293.0 ± 8.1	293.6 ± 8.4

in 2010. Conversely, leaf colour change is the most consistent phase with a standard deviation over 3 years of only 8 days.

Environmental Drivers of Cambial Phenology

Spring air temperatures (April-June) have a clear link with growth onset (Figure 4.5). All years of observation show R2 values of greater than 0.6 with 2010 showing the highest value at 0.8. The regression coefficients of the linear models reveal the sensitivity of growth onset to spring air temperatures. Negative coefficients indicate earlier onset associated with warmer temperatures. The gradient revealed almost double the sensitivity in 2007 (-6.8 days/°C) compared to the following years (2008-2010: -3.6 days/°C; Figure 4.5). To ensure that these sensitivities were not biased by influence from the most extreme single site (N08), we constructed models excluding these observations. Comparisons between full and reduced datasets showed no significant differences in coefficients (Supp. Figure 4.3).

We expanded this analysis of regression coefficients to isolate the onset of each xylem development phase, investigate species differences and compare sensitivities across the elevation gradient which represent longer-term climate trends to inter-annual fluctuations (Figure 4.6). Across all phases we find a mean response (mean ± SD) of -5.2 ± 7.6 days/°C, with maturation showing slightly less sensitivity. Comparisons between species suggest larch has lower (-3.3 ± 7.3 days/°C) sensitivity of onset to spring air temperatures than spruce (-7.3 ± 7.4 days/°C). Regression coefficients across elevation revealed temperature sensitivity ranging from -3 days/°C to -9 days/°C, with a mean of -4.9 ± 1.4 days/°C while inter-annual temperature sensitivity revealed a wider range of sensitivities ranging from -16.3 days/°C and 2.2 days/°C and a mean of -7.4 ± 4.9 days/°C. Despite much more variability inter-annually, the peak likelihood sensitivity of both gradient and inter-annual converges at approximately 5 to 7 days earlier per °C (Figure 4.6).

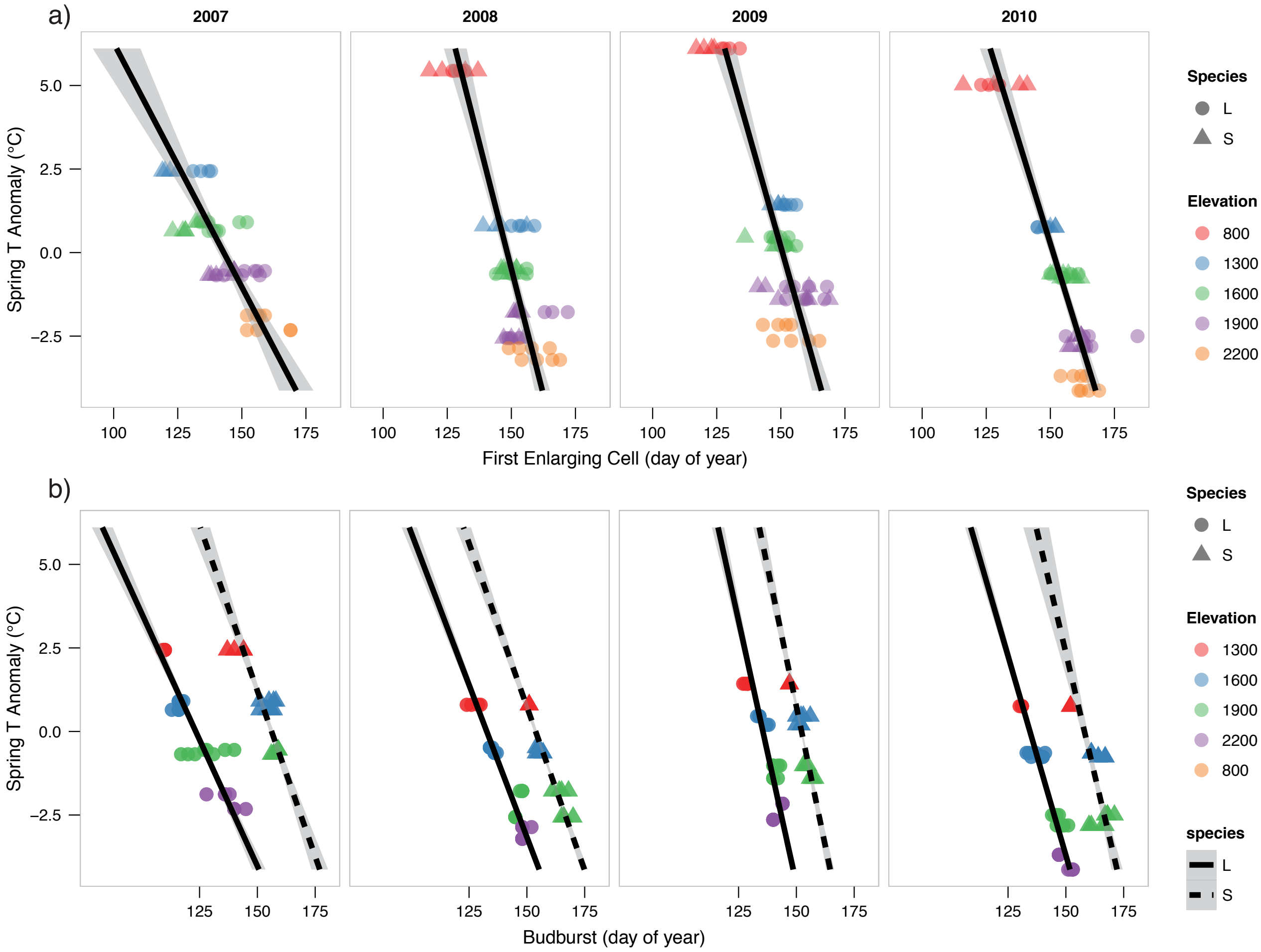


Figure 4.5. Stem growth onset (first enlarging cell; A) and leaf phenology (B) compared against spring (AprJun) air temperature anomalies. Solid black lines are least-squares linear regressions fit along the elevational gradient with panels showing results for each year with grey bands representing a single standard error. Triangles represent spruce and circles represent larch. Leaf phenology fits are done separately for each species.

Discussion

Currently, most ecosystem carbon models incorporate knowledge of growing season length from leaf phenology data, but the generally modest covariation between phenology and growth data in temperate sites (e.g., Jolly et al. 2005) and the generally poor model representation of leaf phenological measurements (Richardson et al., 2013) raise the question if we need to rethink the current approaches and datasets guiding model parameterization. Our study advanced quantification of the links between external observations of growing season (typically based upon budbreak, flowering, or leaf senescence) and cambial phenology. Although we see a convergence between the temperature sensitivity of needle and cambial phenology (5-7 days earlier/°C) we also find that these observations reveal notable differences in defining the growing season and thus its timing, thermal thresholds, and duration. For example, if we define a growing season for larch based on leaf phenology from budburst to leaf senescence, it is 50% longer than a growing season defined by active cambial dynamics. Our data indicate this length is more driven by the end of season yellowing, which is consistent between years, whereas stem growth which is more variable and may have a greater impact on year to year carbon storage. Although photosynthesis in leaves produces carbohydrates, cambial phenology and associated radial growth represents a decades to centennial carbon sink, significant even in older trees (Carey et al., 2001; Luyssaert et al., 2008).

Distinct morphological and physiological strategies were revealed between conifer species as *Larix decidua* produces needles prior to stem growth, while *Picea abies* produces leaves slightly after or simultaneously with radial growth

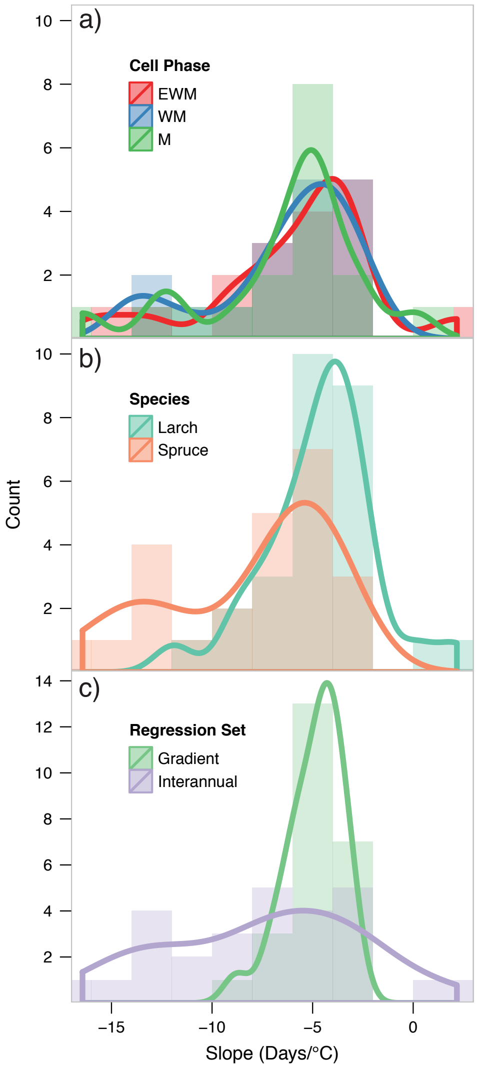


Figure 4.6. Histograms and scaled density estimates of the regression coefficients (days/°C) from linear regressions between the onset of cell phases and spring (AprJun) air temperatures split by (a) cell phases: enlargement (EWM), wall-thickening (WM) and maturation (M); (b) species: larch and spruce; and (c) grouping: across the elevational gradient or inter-annual.

(Figure 4.4). In contrast to evergreen Norway spruce, which retains needles and photosynthetic capability over the winter, deciduous larch must establish a crown each growing season prior to synthesizing new carbohydrates for radial growth. Our results significantly contribute to the growing body of evidence that individual species or plant functional types temporally prioritize radial growth over leaf development, while others first produce leaves and only then begin xylogenesis. Similar observations for budburst preceding xylogenesis in larch were found at the Italian alpine treeline (Rossi et al. 2009), yet these patterns are also not generalizable to the evergreen versus deciduous traits. For example, oak with its prominent ring-porous wood anatomical structure, has been shown to produce earlywood vessels about 1 week prior to budburst (Sass-Klaassen et al., 2011). We furthermore observed that the difference in timing between leaf and cambial phenology is not constant along the elevational gradient. Delays between leaf-out and cambial activation in larch decrease with elevation. This suggests trees at higher elevations can begin radial growth at colder temperatures, although they produce less cells at lower rates. Our findings have important implications for remote-sensed based observations of growing season duration. Namely, leaf phonological processes are not representative of or linearly related with growth and carbon fixation in tree stems.

Studying only larch along the elevational gradient, Moser et al. (2010) calculated a temperature response for the onset of growth of ~7 days earlier/°C. With these additional data we can now suggest that results from Moser et al. (2010) represent an exceptional forest response due to an extremely warm 2007. Both temperature reconstructions and long-term phenological records from across Europe indicate the winter and spring of 2007 were one of the warmest over the last 500 years (Luterbacher et al., 2007; Rutishauser et al., 2008). However, this exceptional response which is also revealed by the timing and large peak of cells in the cambial zone in 2007, appears dampened across elevation. Although extremely warm March & April temperatures promoted growth at lower sites, higher elevation sites remained too cold for growth initiation. The limitation may have involved root activation, because if temperatures do not melt the snow cover, substantive soil warming may inhibit direct response to air temperature cues (Vaganov et al., 1999). Among years we observe that although larch budburst occurred extremely early in 2007, timing of cambial division and cell enlargement showed less difference from subsequent years. Similarly, secondary growth in Norway spruce began much earlier in 2007, but needle budburst was more similar to other years. These trends are less pronounced at lower elevations, suggesting that both species have an initial biological process with greater sensitivity (i.e. larch needle emergence and spruce cambial phenology).

Most of the above discussion and phenological literature in general focuses on the initiation of growth and leaf phenology, yet our results have suggested that most of the variation in cambial growing season duration is attributable to the termination of these processes (Michelot et al., 2012). We also observe greater differences between leaf and cambial phenology at the end of the growing season in comparison to the spring.

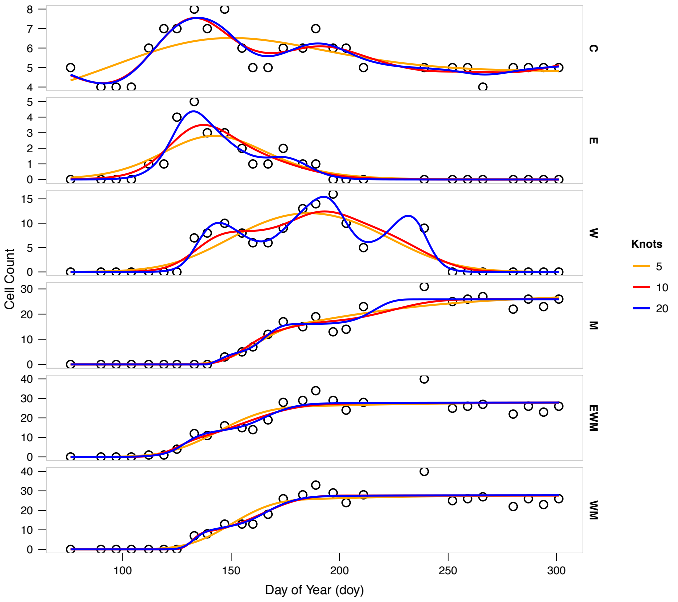
Despite well-reported effects of warmer spring air temperatures on earlier budburst of trees (Menzel & Fabian, 1999; Cleland et al., 2007), end of season processes such as needle colouration are less well understood. Previous investigations on deciduous tree species reveal links between fall temperatures and leaf colouration (Estrella & Menzel, 2006; Busetto et al., 2010; Archetti et al., 2013). However as temperatures in late summer and early fall are not a reliable cue of frost events, which can physically damage trees that have not initiated dormancy, day length signals for growth cessation have also been advocated (Wodzicki, 1964; Heide, 1974; Way, 2011). Our data show that both growth cessation and leaf colouration processes occur at different times along the elevational gradient, suggesting that photoperiod, which is considered constant across such small horizontal distances, indicate little influence of photoperiod and a much more prominent influence of temperature, such as evidence that warmer fall temperatures lead to delayed leaf colouration (Supp. Figure 4.4).

Prior observations of xylem formation tend to prominently report linkages with onset, but either do not report on environmental influences of cessation (Deslauriers et al., 2003; Rossi et al., 2006; Gričar & Čufar, 2008) or indicate there were no links found (Moser et al., 2010). The tight linear links between spring climate and the onset of both leaf and cambial phenology, were not found for the completion of these processes. Instead, across a long elevational gradient, we find indications that radial growth cessation (completion of wall thickening phase) appears to follow a parabolic shape with earlier completion at both higher and lower elevations (i.e. 2200, 1900, 800 m), and later at mid-range elevations (i.e. 1300, 1600 m). We propose this response reflects a climatic optimum for growth and species distribution. Ecological theory proposes that each population exists across a spatial gradient, with stable and resilient individuals at its center (i.e. greatest growth), and increased pressure at the limits of a range (Chapin et al., 2004). Many trees exhibit altitudinal or latitudinal upper limits with temperature generally considered to be the primary constraint (Körner & Paulsen, 2004) while lower limits are more often shaped by moisture limitations (Fritts, 1976; Zweifel et al., 2009; Babst et al., 2013; Lévesque et al., 2013). Consistent with the phenological observations for optimal conditions towards the center of the elevational transect, we found there were more cells produced on average at mid-elevation sites along the transect for both species (Table 4.1) and wider annual rings (King et al., 2013). Warming temperatures have already strongly influenced species response at range margins both in latitude and altitude (Tryjanowski et al., 2005; Hickling et al., 2006) and further movement may be expected, although it will be slower for trees with long-generation times. As a species' climate envelope warms, changes in optimum elevation for growth will likely take place, although there is disagreement if this movement will result in an increase mediated solely by temperature (Lenoir et al., 2008) or if water availability may exert a downward pressure on the optima (Crimmins et al., 2011). Trees at mid-elevations producing the most cells is linked with these elevations also having the longest duration of growth due to greater time requirements for wall-thickening and lignification of a greater number of cells (Rossi et al., 2012).

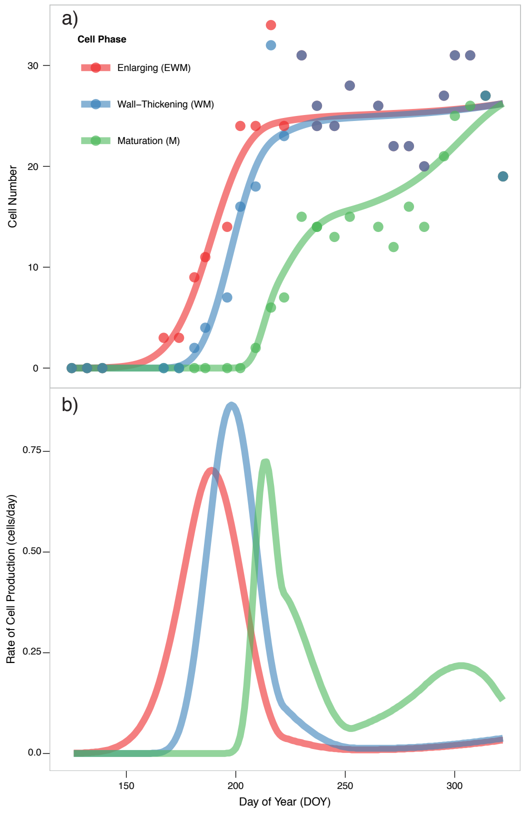
Conclusions & Implications

Our study serves as an ideal platform to characterize the impacts of natural plant growth to projected warming, especially as experiments appear to underestimate sensitivity to temperature warming (Wolkovich et al., 2012) and leaf phenological observations are only applicable to selected apical plant functions (i.e. leafing or flowering), and likely reveal little about long-term carbon assimilation processes. Here, using a natural experiment we provide precise quantification of the developmental stages of >10,000 xylem and cambial cells in mature trees growing along an elevational gradient and offer novel insights into the response of forests and physiological processes to climate change. Our study revealed that trees in Central Europe are actively producing wood from May to September, with maximum cellular development rates during June. The initiation of wood formation and thus processes responsible for the majority of living biomass in our study region was found to advance by 4-6 days/°C warming. Remarkably this temperature sensitivity was found to be consistent for both inter-annual variations in spring weather and also long-term mean climatology. We show that about 60% of the variability in growing season duration was attributed to the completion of xylem cell maturation. And in contrast to inferences derived from leaf phonological observations, we find evidence from the duration of xylogenesis, the quantity of cell division, and radial growth for optimal growing season conditions towards the mid-transect elevations (i.e., 1300 – 1600 m a.s.l.) with mean annual temperatures of 5.5°C.

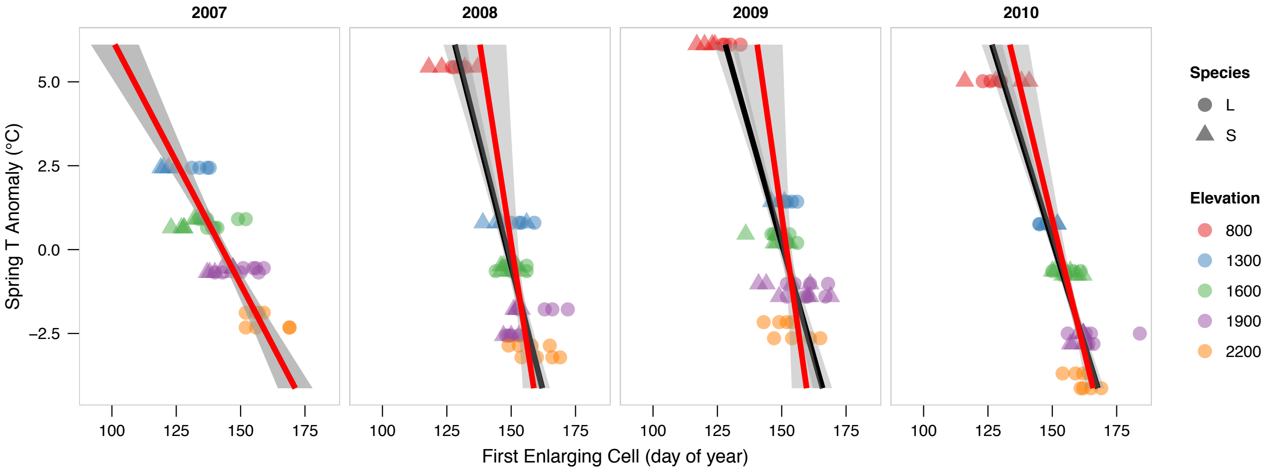
Supplemental Material to Chapter 4



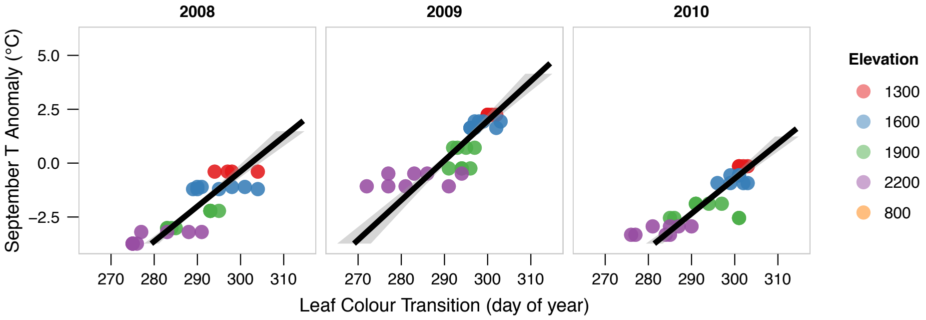
Supplementary Figure 4.1. Comparison of different knot numbers in the general additive models for each of the different cell phases (independent and combined).



Supplementary Figure 4.2. Example of (a) generalized additive model (GAM) fits for cell count data from site N19 in 2010 and (b) the calculated rate of cell production for each of the three phases. Points in (a) represent observed cell counts and lines represent predicted cell counts using a monotonic GAM.



Supplementary Figure 4.3. Linear models showing the difference between including N08 (black lines) and excluding N08 (red lines). Shaded areas represent a single standard error. Data is the exact same as shown in Figure 4.6.



Supplementary Figure 4.4. Larch leaf colouration timing compared against September air temperature anaomalies. Positive slopes indicate delayed colouration with warmer September temperatures.

Chapter 5

Intra-annual dynamics of non-structural carbohydrates in the cambium of mature conifer trees reflect radial growth demands

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Introduction

Tree growth and survival depends not only on their capacity to produce and use carbohydrates, but also on efficient carbohydrate storage and remobilization. Carbohydrates are supplied by fixing atmospheric carbon (C) during photosynthesis and are vital in almost all plant physiological processes including the maintenance of existing tissue, the formation and enlargement of organs, and all associated metabolic processes. Investigating ecosystem productivity under changing climatic conditions requires understanding how various physiological and environmental factors constrain plant growth. In this regard, studies of non-structural carbohydrates (NSC; defined here as soluble sugars + starch) have been widely used to assess the source-sink balance of trees (Barbaroux and Bréda 2002; Damesin and Lelarge 2003; Fischer and Holl 1992; Gruber et al. 2012; Michelot et al. 2012; Oberhuber et al. 2011; Woodruff and Meinzer 2011). Stored NSCs can be viewed as reservoirs refilled when C demand for growth and maintenance is low and called upon during periods of high C requirements. Under this functional interpretation of NSCs, accumulation of large C pools is inconsistent with the hypothesis of carbon supply limiting growth, and rather suggests “sink” limitations (Hoch and Körner 2012; Körner 2003; Millard et al. 2007). Large pools of stored C have been observed in mature coniferous and deciduous trees with, for example, C reserves in deciduous trees sufficient to replace the entire canopy several times (Hoch et al. 2003). Trees growing under conditions of high environmental stress such as towards their cold thermal limits of growth (Fajardo et al. 2013; Hoch and Körner 2003; Hoch and Körner 2012; Hoch et al. 2003), following severe water stress (Breda et al. 2006; Gruber et al. 2012), or defoliation (Hoch 2005; Palacio et al. 2008), also showed high levels of stored NSCs associated with, or despite, reduced growth.

However, in the context of on-going climate change and concurrent increases in tree mortality, the role of carbon allocation has been revisited with several studies concluding that significant amounts of mobile carbohydrates in various tree tissues may not be simply interpreted as a sink-limitation (McDowell and Sevanto 2010; Millard and Grelet 2010; Ryan 2011; Sala et al. 2010; Wiley and Helliker 2012). Data obtained by interrupting the supply of new photosynthates via phloem girdling (BhupinderpalSingh et al. 2003), or following girdling and defoliation (Hoch 2005), has revealed significant NSC pools that remain unused by trees. This may reflect a condition intermediate to the “source” and “sink” limitations. Namely, trees appear physiologically capable of incorporating stored photosynthates into permanent tissues, yet do not do so. It is still unclear to what extent these data (i) indicate the inability of trees to supply the growing tissues with carbohydrates, (ii) reflect a prioritization of resources as a safety margin in the face of environmental stochasticity, or (iii) some other underlying mechanism. Regardless of the cause, active carbon storage competing with growth has been evidenced in different species (Chapin et al. 1990; Genet et al. 2010; Silpi et al. 2007). Consequently, if a significant fraction of the C pool is actively stored (thus competing with growth) or sequestered (i.e., unavailable for any further physiological processes), then the observed overabundance of C in trees is not a useful indicator for a sink limitation (Millard and Grelet 2010). Therefore, under a long-term perspective, competing requirements for NSCs (e.g. respiration, defense and export, maintenance of hydraulic integrity) might cause a source limitation (Epron et al. 2012; Hartmann et al. 2013; Sala et al. 2012; Wiley and Helliker 2012).

Despite abundant literature on NSCs, the processes and pathways related to NSC allocation and storage within trees remain poorly understood (Wiley and Helliker 2012). Progress is hampered by the scarcity of field data necessary for model testing, with studies in natural mature forests particularly needed (Barbaroux & Breda, 2002; Hoch et al., 2003; Gough et al., 2009, Richardson et al. 2013). NSC concentrations have been measured in diverse tree organs including stems, branches, foliage and roots (Barbaroux and Bréda 2002; Damesin and Lelarge 2003; Fischer and Holl 1992; Gruber et al. 2012; Michelot et al. 2012; Oberhuber et al. 2011; Uggla et al. 2001; Woodruff and Meinzer 2011). In these studies, a general conclusion was that high variation in intra-annual NSC content was observed nearer to sites of active growth (e.g., apical and root meristems), while low variation was recorded within the storage tissues (e.g., sapwood, coarse roots, ray parenchyma). Investigations at the primary sink location would be expected to yield better insights into the seasonal carbohydrate supply and demand in trees.

Due to technical challenges associated with the sampling and isolation of NSC in the cambial zone, very few studies have reported on carbon stock measurements where secondary growth occurs (Deslauriers et al. 2009; Giovannelli et al. 2011; Sundberg et al. 1993; Uggla et al. 2001). The cambial zone in tree stems is composed of a thin layer of meristematic cells and only recently has a procedure been developed to solve the challenge of separating this tissue for NSC extraction (Giovannelli et al. 2011).

Here we use this new procedure to contribute to the debate on carbon dynamics in trees by supplying a detailed description of the intra-annual carbon fluctuation (soluble NSCs and starch content) in the cambial zone of mature conifer trees. We aim at elucidating the mechanisms controlling growth and at better understanding the effective sink strength of the cambium and its variability over time. Therefore we perform measurements in a deciduous and an evergreen conifer species at specific phenophases of wood formation, as well as at two sites at contrasting elevations. In addition, we quantify the individual sugars such as glucose, fructose, sucrose, raffinose, pinitol, and starch to better assign a functional meaning to the seasonal variations in NSC concentrations. This design allows us to address specific questions related to growth limitation and carbon demand, such as: (i) Is secondary growth limited by the availability of carbon or constrained by environmental conditions acting upon the sink function? (ii) How does carbon supply in the cambium respond to the carbon demand for stem growth and concurrent foliage production? (iii) Is there a species- and/or site-specific strategy in the production, storage and supply of NSCs/individual sugars in terms of carbon allocation to growth, management of reserves, and protection against environmental stress?

Material and Methods

Study sites and field activities

Our study was performed in the Lötschental (46°23'40"N, 7°45'35"E), a southwest-northeast oriented inner-alpine valley in the central Swiss Alps. The valley bottom is surrounded by steep forested slopes primarily composed of mixed, evergreen Norway spruce (*Picea abies* Karst.) and deciduous European larch (*Larix decidua* Mill.). The climate of the region is cool and relatively dry, with a mean annual temperature of 6°C, ranging from -3°C (January) to 15°C (July) and a mean annual precipitation exceeding 800 mm (data from MeteoSwiss for the period 1987-2006).

Field activities were conducted in 2010 at two sites, about 1km apart from each other, with contrasting elevations. The high elevation site is located at the upper tree line at ~2200 m a.s.l., on a south-facing slope and consists solely of larch (this site is abbreviated S22L to reflect the aspect (South), elevation (2200 m a.s.l.), and species (Larch)). The low elevation site is near the valley bottom close to the north-facing slope at 1300 m a.s.l. and is a mixture of larch and spruce (similarly abbreviated N13L and N13S). The mean temperature difference between sites, as monitored from April to October 2010, was 3.5°C (average maximum and minimum temperature difference of 6 and 2.5°C, respectively). Hydrological conditions are generally dryer at the low elevation site due to a combination of less precipitation and higher evaporative demand. The soils of both sites are ~60 cm deep podzolic cambisols characterized by significant coarse stone content and low clay amounts. Field activities involved i) collecting stem samples to follow NSC dynamics in cambial tissue, and ii) weekly monitoring of foliar and wood formation to document the progress of growth at the time of cambial sampling.

NSC sampling and biochemical analysis

NSC sampling was performed on five different dates on 45 mature trees in total, with 15 trees per species and site (S22L, N13L, and N13S). Sampling dates were selected to target five relevant phases of annual ring formation, i.e., 1) when cambial division / and earlywood cell enlargement are highly active, 2) when earlywood cells are in both phases, enlargement and wall thickening, 3) after cellular division has stopped but enlargement and wall

Stage	Period	N13	S22
		DOY (Date)	DOY (Date)
Highly active cambial division/EW cell enlargement	1	155 (31.05.2010)	174 (23.06.2010)
EW cell enlargement and wall thickening	2	186 (05.07.2010)	196 (15.07.2010)
No cell division but active wall thickening processes	3	216 (04.08.2010)	216 (04.08.2010)
LW cell wall thickening	4	258 (15.09.2010)	258 (15.09.2010)
Dormancy	5	68 (09.03.2011)	39 (08.02.2011)

EW = earlywood, LW = latewood

Table 5.1: Days of the year (DOY) and corresponding dates of NSC sampling for each related phenological stage and site.

thickening phases continue, 4) when only latewood cells are conducting secondary wall thickening and, 5) during dormancy of cambium (Table 5.1). The sampling dates were estimated based upon data from 2007-2009 (King et al. in. prep.; Moser et al. 2010).

Two samples per tree and sampling date were taken at about 50 cm height using 37 mm diameter metal punchers. The samples, comprising phloem, cambium and xylem, were kept on ice during fieldwork, stored at -22°C once in the laboratory, and freeze-dried. Subsequently the samples were prepared for biochemical analysis according to the protocol described in Giovannelli et al. (2011). Accordingly, samples were split along the tangential plane in the cambial zone and then the differentiating phloem and cambial cells from the phloem side sample were gently scraped with a razor blade to obtain a powder from the cambial tissue. Due to the low amount of cambium powder per sample, blocks of five trees per site and species were pooled to obtain enough material for sugar extraction. After pooling and homogenizing an equal amount of cambium powder per tree, a 40 mg subsample was used for sugar extraction. NSCs were extracted from the cambial powder using chemical procedures described in Giovannelli et al. (2011). The sugar content was determined by High-Performance Liquid Chromatography (HPLC) analysis equipped with a SHODEX SUGAR Series SC 1011 8x300mm column (Showa Denko, Germany) preceded by a pre-column Guard Pak Insert Sugar Pak II (Waters). The mobile phase was water, Milli Q grade, at 0.5 ml min-1. Soluble carbohydrate identification was verified using carbohydrate standards (Sigma, USA) and quantified by means of an internal standard. Concentrations of Glucose, fructose, sucrose, pinitol and raffinose were thus obtained. The remaining pellet after soluble NSC extraction was used for starch quantification. The starch content was measured after an extraction procedure: the residual pellet was suspended in 1.5 ml acetate buffer (pH 5), brought to boil at 100°C for 1h in a sand bath, and then cooled at room temperature. After incubation at 55°C for 16h with 150 µl amyloglucosidase from Aspergillus niger (Fluka), samples were diluted with distilled water to 5 ml and three 0.25 ml aliquots of each sample were assayed colorimetrically using glucose oxydase (Sigma-Aldrich, Italy).

Seasonal changes in soluble NSCs and starch content were compared between elevation and species using repeated-measures analysis of variance (ANOVAr) (Gumpertz and Brownie 1993; von Ende 1993). For the within-subject analysis, a Huynh-Feldt corrected probability was used to overcome the sphericity assumption in the case of univariate repeated-measures analysis (von Ende 1993). Differences were considered significant at $P<0.05$. When significant effects were found, mean comparisons by sampling date using ANOVA were performed to identify when the differences occurred. All ANOVA analyses were performed using the JMP® 8.0 software (SAS Institute Inc.).

Elevation (m)	Species	Onset of xylem production (DOY)	End of xylem differentiation (DOY)	Duration of xylogenesis (days)	Budburst (DOY)
1300	Picea	143 (0.0)	305 (6.9)	162 (6.9)	149 (3.5)
1300	Larix	138 (3.0)	311 (0.0)	173 (3.0)	135 (3.5)
2200	Larix	159 (3.5)	302 (6.0)	143 (7.9)	149 (3.5)

Table 5.2: Onset and duration of the growing season, and budburst for larch growing at the valley bottom and the treeline (1300 m a.s.l. and 2200 m a.s.l., respectively) and spruce growing at the valley bottom. Numbers in parenthesis are the standard deviation of the mean (n=4).

Monitoring of foliar and wood formation

Foliar and cambial phenology were monitored on a weekly basis over the entire growing season 2010. For this purpose, and to prevent any potential influence of the sugar sampling on growth, 12 additional trees were selected: four spruce and larch trees at N13 and four larch trees at S22. The date of budburst for each tree was defined when 50% of its buds were broken (Brügger and Vassella 2003). Exact dates were estimated by linear interpolation, with these values averaged per site and species for a site date.

Cambial phenology was quantified weekly on the same trees. The forming annual ring was monitored for each tree by analysing tracheid formation from microcores collected weekly between April and November after Moser et al. (2010). Microcores were collected from the stem at 1-2 m height using a Trephor (Rossi et al. 2006a) preferentially perpendicular to the slope direction to avoid reaction wood. Sampling was conducted along an oblique line and 3–5 cm apart to minimize wound reactions caused by earlier samplings (Forster et al. 2000). Microcores were placed for 24 h in a dilution of acetic acid and ethanol to preserve forming cells from degradation, and then stored in a 70% alcohol solution. Samples were prepared for cellular analysis by cutting 20–30 µm thick transversal microsections using a sliding microtome. Microsections were stained with safranin and astrablue and fixed to microscope slides with Canada balsam. Ring formation was analysed at a magnification of 400–600x and the number of tracheids in the different phases of cell development (i.e. enlargement, wall thickening and maturity) assessed by averaging the counting along three radial files of each microsection. Enlarging cells were characterized by thin primary cell walls with radial diameter roughly two or more times larger than that of dividing cambial cells. Polarized light was used to discriminate between enlarging and wall thickening tracheids. Mature cells were recognized by completely lignified secondary walls and empty cell bodies (Rossi et al. 2006b; Rossi et al. 2007). Dates representing critical phenological stages of wood formation were calculated for each site and species based upon the cell counts.

Results

Growth dynamics and timing of NSC sampling

At the valley bottom, larch budburst occurred on DOY 135 ± 3.5 (mean ± standard deviation) and xylem formation (defined in this work as the timing of the first observed enlarging xylem cells) on DOY 138 ± 3. Budburst and xylem formation in spruce occurred later, i.e. on DOY 149 ± 3.5 and DOY 143 ± 0, respectively. Budburst in larch occurred before or close to the first observations of xylem cells entering the enlargement phase. In contrast, spruce initiated growth of new xylem tracheids before the emergence of current-year needles. These observations indicate that spruce and larch adopt different sequential arrangements in the timing of foliar and xylem growth resumption. At S22L, in comparison to larch growing 900m lower or at a 3.5°C warmer site, budburst was delayed by about two weeks (DOY 149 ± 3.5) and xylem formation onset by about three weeks (DOY 159 ± 3.5) (Figure 5.1, Table 5.2). The timing of growth resumption significantly differed (Table 5.3) between species (~6 days; P=0.01) and elevation (~22 days; P<.0001).

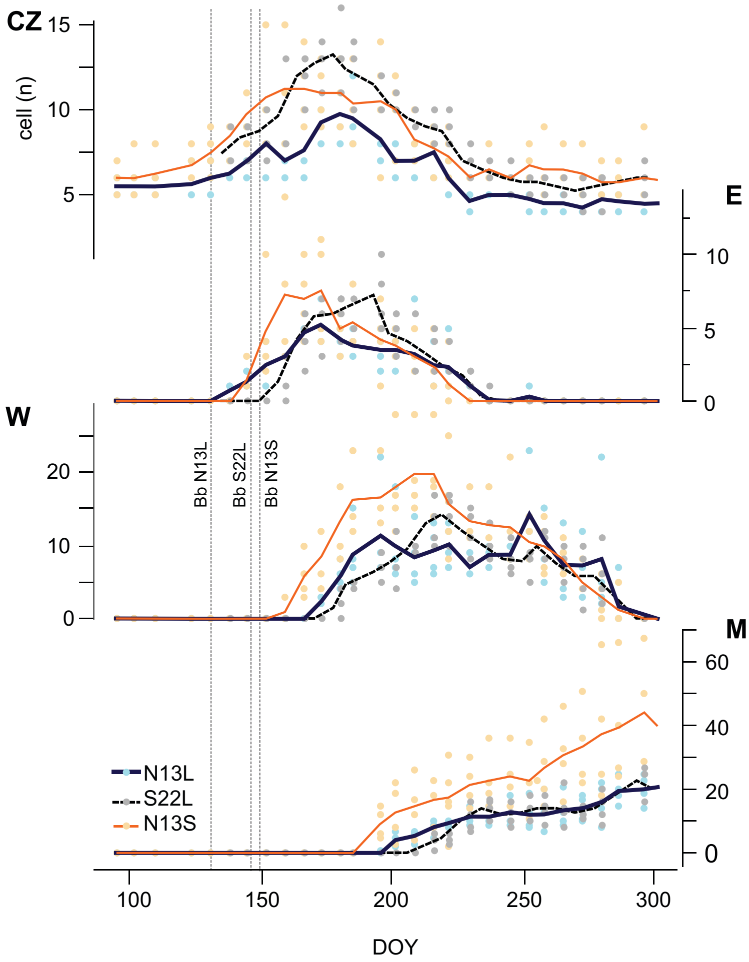

Figure 5.1: Number of cells in the cambial zone (CZ), enlarging phase (E), secondary wall formation and lignification phase (W) and mature xylem cells (M) of individual larch (blue - 1300 m; grey - 2200 m; n=4) and spruce (orange -1300 m; n=4) at both elevations in the Lötschental as counted on a weekly basis during the growing season 2010. The average number of cells during the season is shown. The date of budburst (Bb) for both species at both sites is also indicated.

The onset of wall thickening, the formation of fully mature cells, as well as the maximum number of cells observed in the phase of enlargement and wall thickening occurred earlier at N13S than N13L (Figure 5.1). The first wall thickening and mature cells were both observed 8 days later for spruce. Despite their delay in the onset of wall thickening (9 days) and mature cells (12 days), S22L soon reached similar levels of cell production as found at N13L. The total number of xylem cells in the 2010 ring varied among larch (ranging from 10 to 30) and spruce (from 25 to 70), but was in general higher for spruce.

The differences between both species and elevation decreased toward the end of the growing season. Significant differences in the end of xylogenesis (DOY 308 in average) between N13L and N13S disappeared, although larch at the treeline stopped xylogenesis a few days earlier. Thus, the shorter total duration of xylogenesis at S22L compared to N13L (18 days, *P*=0.0004) and for N13S compared to N13L (8 days, *P*=0.02) was mainly due to a difference in the onset of xylem differentiation (Table 5.3).

Non-structural carbohydrate concentrations

Significant seasonal variations of NSCs in the cambial zone were observed (Figure 5.2). In general, the soluble

	Onset of xylem differentiation		End of xylem differentiation		Duration of xylogenesis		Budburst	
Source	F	P-value	F	P-value	F	P-value	F	P-value
Species	13.44	*	3.00	ns	9.28	*	409.9	***
Elevation	85.00	***	9.00	*	51.37	**	630.1	***

** P<.05, ** P<.001, *** P<.0001, ns = non significant*

Table 5.3: ANOVA (F- and P-values) of the onset, end and duration of xylem differentiation, and budburst, for differences between species and elevation. Significance at ($P < 0.05$).

fraction peaked between July and August, corresponding to the times of high rates of cell division and enlargement (period 2) and when many cells were in the wall thickening phase (period 3; Table 5.1). Total soluble NSC concentrations increased by more than 50% between the onset of the growing season and the period of maximum cell division. In September, during latewood cell wall thickening (period 4) the soluble carbohydrate concentrations decreased, and increased again, during the subsequent dormant season (period 5). Larch and spruce displayed similar seasonal variations, with particularly high NSC concentrations during periods 2 and 3, but the NSC concentrations in spruce peaked during the dormant season. S22L and N13L showed similar seasonal patterns.

The starch concentrations for all sites and species were high towards the end of the growing season (periods 3 and 4) and decreased dramatically during dormancy. However, lower starch concentrations were measured at N13L during the early growing season (period 1) .

On average, cambium sugars consist of around 40% glucose, 35% fructose, 10% starch, 10% pinitol, 5% sucrose and less than 1% raffinose (Figure 5.2). However, these proportions slightly vary in time, between species and elevations. Total NSC concentrations closely follow those of glucose and fructose together, showing similar patterns and accounting for nearly up to 80% of the growing season soluble NSCs (Figure 5.2) and leading to high hexose (glucose + fructose) to sucrose ratios (in average from 15 to 30 during the growing season). A fructose to glucose ratio of approximately 1 was also observed throughout the whole growing season.

Glucose, fructose and sucrose concentrations for spruce tend to be lower during the growing season and higher during the dormant season, however fructose concentration was not found to be as species dependant as the other sugars (Table 5.4). Notably, both raffinose and pinitol displayed unique intra-seasonal changes in concentrations. For all species and elevations raffinose was only found during the dormant season (period 5), whereas pinitol, in contrast to all other sugars did not show an increase during the dormant season. In addition, pinitol was the only sugar with higher concentrations in spruce during the active growing seasons (periods 2-3).

Highly significant relationships between species and the phases of cambial phenology were observed for starch, glucose, sucrose, and raffinose, while elevation was not a significant factor contributing to NSC concentration variations in the cambial zone (Table 5.4).

Discussion and Conclusions

Our intra-annual NSC measurements sampled directly in the stem cambial zone of mature trees growing in the subalpine zone allow us to the improve understanding of carbohydrate variation during a complete annual cycle. The dynamics of the different mobile carbohydrates observed at both elevations and for the two species reflect the changing requirements for storage, mobilisation and use of C resources needed to sustain growth as well as protecting vital tissue from harsh (e.g. winter freezing, summer drought) environmental conditions.

The annual cycle

In temperate regions with a distinct seasonal cycle and a dormant period for vegetation in winter, the onset of wood

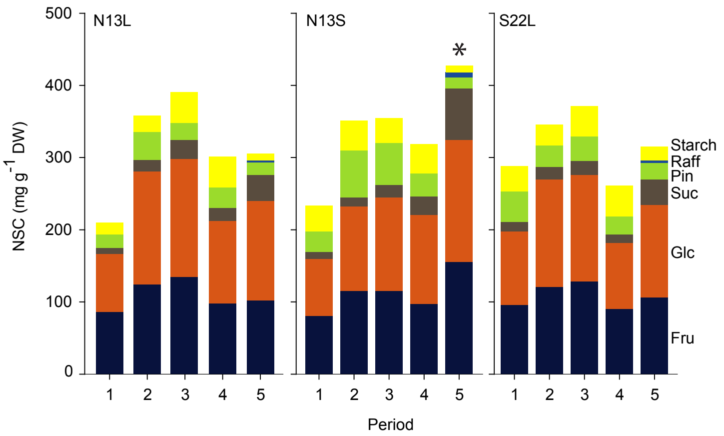

Figure 5.2: Concentration of non-structural carbohydrates (Fru=fructose, Glc=glucose, Suc=sucrose, Pin=pinitol, Raff=raffinose, Starch; mg g-1 DW) in the cambial zone of larch and spruce at both elevation in the Lötschental during the growing season 2010. Mean values are presented (n=3; each representing 5 pooled trees). DW = dry weight. Stars indicate significant differences between larch and spruce at 1300 m.

formation is usually temperature driven (Moser et al. 2010). Photoperiod may also provide secondary control for the growth onset, at least for the primary meristem (Chuine et al. 2010; Körner and Basler 2010). Wood formation is terminated in late fall when the chain of maturation processes is completed (Rossi et al. 2012). These notions of the annual cycle are broadly reflected in the cellular developmental stages (Figure 5.1) and concentrations of the different types of NSCs (Figure 5.3) for both larch and spruce.

The general paralleling of the NSC compounds in the cambium and the annual dynamics of wood formation has also been observed in the other few studies with comparable approaches, i.e. for poplars (Deslauriers et al. 2009; Giovannelli et al. 2011), Scots pines (Sundberg et al. 1993; Uggla et al. 2001) and eucalyptus (Stewart et al. 1973). Collectively, these studies suggest that the dynamics of NSC concentrations in the cambium of different species, habitats, and angiosperm versus gymnosperm lineages follow a similar seasonal pattern.

During the growing season, NSCs sustain the metabolic processes involved in the formation of new cells within the cambium. We observed very high amounts of soluble sugars (primarily glucose and fructose, collectively named hexose) in the cambial zone of both larch and spruce. While lower at the beginning and the end of the growing season, soluble sugar concentration increased rapidly and peaked when the resource demand was highest, i.e. when a greater number of cells were both in the enlargement and cell wall thickening phases. Such high concentrations of hexose are unusually high compared to what is normally observed in other tissues such as stem wood (Damesin and Lelarge (2003), Gruber et al. (2011), Streit et al. (2013)). In our study of larch and spruce, we found similar concentrations of fructose and glucose within the cambial zones. Glucose to fructose ratios approximately equal to unity were similarly reported for Scots pine by Uggla et al. (2001), who also observed strongly decreasing sucrose concentration gradients (yet, strongly increasing glucose and fructose levels) from functional phloem to developing xylem. Relative to sucrose, high levels of hexose within the cambial zone are consistent with the high metabolic activity of the dividing and rapidly growing cells.

In addition to serving as the building blocks for growth itself, sugars play an important role as signalling molecules and/or as global regulators of gene expression (Eveland and Jackson (2011), Koch (2004)). Glucose and fructose in spruce and larch likely originated from the cleavage of sucrose as suggested by high levels of sucrose cleaving enzymes such as AI (acid invertase), and to a lesser extent Susy (sucrose synthase), in the cambial zone of Scots pine (Uggla et al. 2001). The relative ratio of hexose to sucrose concentrations are maintained by these various enzymes which collectively coordinate and fine-tune growth during key phases of development (Eveland and Jackson 2011;

Carbohydrates	Source	*F*	*P*-value	Source	*F*	*P*-value
Glucose	S	5.85	ns	E	9.27	ns
	P	29.03	***	P	17.33	***
	S*P	7.64	**	E*P	2.81	ns
Fructose	S	0.24	ns	E	0.04	ns
	P	4.25	*	P	3.57	*
	S*P	2.57	ns	E*P	3.56	ns
Sucrose	S	2.76	ns	E	0.28	ns
	P	60.53	***	P	11.38	**
	S*P	14.71	***	E*P	1.38	ns
Raffinose	S	100.76	**	E	0.24	ns
	P	412.71	***	P	59.73	**
	S*P	100.76	***	E*P	0.24	ns
Pinitol	S	40.82	*	E	7.48	ns
	P	8.37	**	P	1.78	ns
	S*P	2.64	ns	E*P	2.9	ns
NSC total	S	4.39	ns	E	0.53	ns
	P	15.40	***	P	9.74	**
	S*P	3.47	*	E*P	2.49	ns
Starch	S	2.11	ns	E	6.4	ns
	P	20.37	***	P	15.22	**
	S*P	5.41	**	E*P	0.77	ns

** P<.05, ** P<.001, *** P<.0001, ns = non significant*

Table 5.4: ANOVA (F- and P-values) for testing the differences in the carbohydrates compounds. Tests include differences between species (S), period (P) and their interaction (S*P); and for elevation (E), period (P) and their interaction (E*P). P-values for repeated-measures ANOVA are presented with Huynh-Feldt corrected probabilities. Significance at ($P < 0.05$).

Koch 2004). Hexose is regarded to have a greater signalling potential in promoting growth and cell proliferation whereas sucrose is typically associated with differentiation and maturation (Eveland and Jackson 2011; Koch 2004). While we did not measure enzyme concentrations, we found that particularly the hexose to sucrose ratio in the cambium zone of spruce and larch generally decreased during the growing season, as the cambial phenology shifted from rapidly dividing cells peaking around DOY 165 (10.06.2010) to the wall-thickening phases peaking approximately 2 months later (Figure 5.1).

Starch concentration commonly shows considerable seasonal variation in the stems and branches of temperate zone trees. Lower reserves at budburst, a late summer maximum as growth slows down, and starch hydrolysis to sugar in autumn when days are short and nights cold are all patterns previously reported (Gruber et al. 2011; Kozlowski 1992). Decreasing levels of starch during the summer in tree stem, branches and cambial zone have also been observed (Deslauriers et al. 2009; Hoch et al. 2003; Sundberg et al. 1993). In comparison to reports from poplar (Deslauriers et al. 2009) or Scots pine (Sundberg et al. 1993) cambial zones, we found starch levels in the cambial zone of spruce and larch at all three sites remained relatively constant during the growing season (Figure 5.3). Similarly, Geisler-Lee et al. (2006) found little expression of genes related to starch metabolism in comparison to carbohydrate-related enzymes (e.g. Susy, cellulose synthase) in the cambium of poplar during xylogenesis.

Large amounts of starch are consumed during cambial reactivation, with reserves replenished only sometime after the onset of xylem differentiation (Begum et al. 2013). The concentrations we observed tended to be lower than those reported for Scots pine (Sundberg et al. 1993) and higher than those from poplar (Deslauriers et al. 2009). Our first sampling campaign (DOY 155 - N13, DOY 178 - S22) was possibly too late to catch the minimum starch level as cambium reactivation, xylem differentiation, and budburst had already occurred. During reactivation of

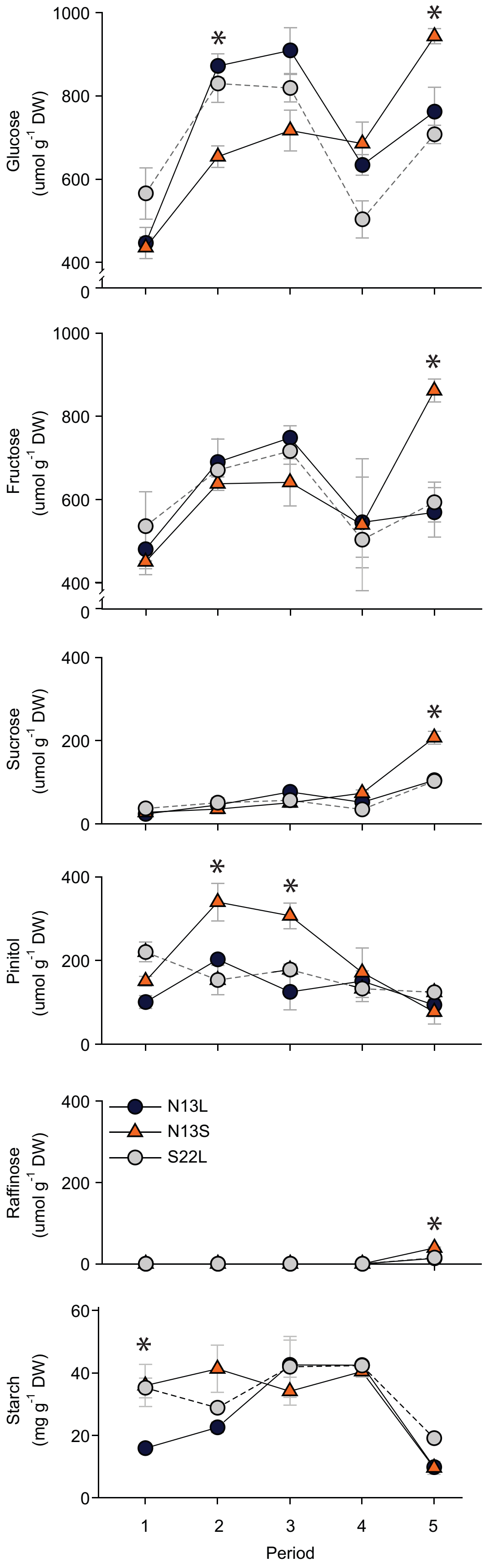

Figure 5.3: Individual non-structural carbohydrates (glucose, fructose, sucrose, pinitol, raffinose: µmol g-1 DW; starch: mg g-1 DW) concentrations in the cambial zone of larch (blue circles-full line - 1300 m; grey circles-dotted line - 2200 m) and spruce (orange triangles-full line) at both elevations in the Lötschental during the growing season 2010. Values are mean ± 1 SE (n=3; each representing 5 pooled trees). Note the scale break for glucose and fructose. DW = dry weight. Stars indicate significant differences between larch and spruce at 1300 m.

the cambium, starch is used as the main source of energy, but later on, the continuation of cambial activity seems to require a continuous supply of sucrose (Oribe et al. 2003) for cell wall biosynthesis. The low variation of starch during the growing season suggests a constant supply of fresh assimilates to the cambium of larch and spruce at our sites.

For all of the tree groups investigated in our study, we found starch breakdown during the cold season, and synthesis from soluble sugars in late winter-springtime (Figure 5.3). Our findings support previous observations of α-amylase activation and starch synthase genes in dormant poplar tissues during cold periods supporting starch breakdown for cryoprotection purposes (Geisler-Lee et al. 2006). Similarly, resynthesis of starch in late winter (Kozlowski 1992), was documented in needles (Bansal and Germino 2009; Chen et al. 2012; Hansen and Beck 1994; Hoch et al. 2003) and in the trunk (Fischer and Holl 1992; Hansen and Beck 1994; Hoch et al. 2003; Michelot et al. 2012) of various tree species. Our results demonstrate that these starch dynamics also apply to the cambial region.

Raffinose and pinitol are both compatible solutes (i.e., osmotically active compounds) that help cells survive osmotic stress (Bachmann et al. 1994; Bohnert and Shen 1998). While increased concentrations of raffinose and pinitol can decrease the osmotic potential of cells to maintain the water balance, their main function might be to stabilize proteins, protein complexes, or membranes by scavenging radical oxygen species (ROS) that build up during environmental stress (e.g. cold, drought, high salinity) (Bohnert and Shen 1998; Orthen et al. 1994). Levels of raffinose increased during winter (Figure 5.3), thereby presumably protecting cell membranes from damage during frost-induced dehydration by detoxifying ROS that accumulate at low temperatures (Nishizawa et al. 2008). Pinitol was present year-round in both species, however, concentrations peaked in the cambial zone of spruce when growth processes were most active. Streit et al. (2013) observed higher pinitol concentrations in branch bark and branch wood of larch growing at another tree line location in Switzerland in comparison to larch from lowland (500 m a.s.l.) and interpreted this difference in terms of long-term adaptation to high levels of ROS in response to low temperature. Similar pinitol concentrations to Streit et al. (2013) in larch growing both at the valley bottom (1300 m a.s.l.) and the tree line (2200 m a.s.l.) suggest that long-term adaptation responses to high levels of ROS already occur at 1300 m a.s.l. and that pinitol concentrations may not increase linearly with elevation. The conclusions for greater environmental stress towards the upper elevations and the valley bottom are similarly supported by analyses of the climatic sensitivity of radial growth variations along an ~900-meter elevational transect (King et al. 2013b).

Species and climatic controls on NSC

The general trends in the dynamics of NSC concentrations in this study were similar between the species and elevations. Differences relate primarily to the absolute concentrations: the protective sugars raffinose (during dormancy) and pinitol (during the warmest period corresponding to July/August), as well as glucose, fructose and sucrose (during dormancy) differed in spruce and larch. The species-specific concentrations are potentially related to the differing climatic sensitivities of larch and spruce, also observed in an Europe-wide multi-species tree-ring network (Babst et al. 2013) to cold (in winter) and drought (in summer). Hinesley et al. (1992) observed links between the raffinose content in the foliage of different conifers and their level of cold hardiness. A similar relationship was also found for the presence of other NSCs (glucose, fructose, sucrose and raffinose) in the shoots of shrubs and in tree stems (Lee et al. 2012; Morin et al. 2007). Trees with thinner bark like spruce are more sensitive to frost damage in the cambium zone (Gurskaya and Shiyatov 2006) and might thus need a different strategy for additional protection. Higher levels of soluble NSCs in the cambial zone of spruce, in comparison to larch, in a period where the risk of freezing injuries is high would meet this requirement. The lower level of raffinose in particular, and total soluble NSCs in general, we observed in larch might also reflect a higher degree of spring de-hardening compared to spruce in early March (sampling period 5). This hypothesis is further supported by the earlier bud break and onset of xylem differentiation of larch.

During June and July, pinitol concentrations were found to be higher in spruce compared to larch (on average 1.5 to 2.5-fold) at the valley bottom, while glucose levels were lower. Glucose has been described as one of the precursors of pinitol synthesis (Obendorf et al. 2008) and therefore the lower levels of glucose relative to pinitol in spruce suggest that some glucose was directed towards pinitol synthesis. However, why the pinitol levels significantly increased in the cambial zone of spruce during the summer months, while remaining constant (and at similar levels) in larch at both the valley and tree line sites, is uncertain. These patterns might reflect a greater need to maintain turgor potential (Aranjuelo et al. 2011) within the cambial zone of spruce to sustain cell enlargement, or may result from a higher sensitivity of spruce to environmental stress leading to enhanced generation of ROS (Orthen et al. 1994). A recent investigation showed that spruce growing at our study site exploit their internal stem water reserves more quickly during drier conditions compared to larch (King et al. 2013a).

While total NSC concentrations remained similar throughout the growing season in our study, higher concentrations of the soluble sugar fraction (except pinitol) during dormancy were observed in spruce compared to larch. While winter respiration of living tissue of the dormant deciduous trees depend exclusively on reserves (starch + soluble sugars), the higher levels in our evergreen species might reflect photosynthesis (Kozlowski 1992) and phloem transport (Blechschmidt-Schneider 1990) during mild winter days.

Despite a temperature difference of 3.5°C and differences in the length of the growing season of 30 days between the treeline and valley bottom larch sites, we found similar seasonal variations in the NSC concentrations. This similarity likely has to do with our sampling keyed into expected cambial activity rather than only calendar dates. Notable differences between elevations occurred only for starch concentrations in the early growing season (period 1) although no statistically significant differences were found (Table 5.4). Starch concentrations were already near their maximum in early summer at the treeline whereas at the valley bottom they only peaked at the beginning of July. Attributing these differences to particular mechanisms remains speculative. However, higher respiration losses due to warmer temperatures at the valley bottom in addition to active growth of competing sinks while needles are still not fully photosynthetically functional (Kozlowski 1992) would be consistent with our intra-seasonal NSC data.

Is secondary growth allocation C or sink limited?

Our data, although focused to one growing cycle and limited to only the cambial zone, contribute to a better understanding of the potential functions on NSC allocation and storage within trees. Notable for our investigation are the direct observations of NSC concentrations at the sites of secondary growth, and the ability to link these changes with cambial zone phenology. The reduced growth (i.e. number of tracheids produced) observed for larch in comparison to spruce growing at the same site and for S22L compared to N13L, cannot be explained by temporary limitation in NSC supply in the cambium. The high similarities in the NSC concentrations during the growing season of trees at different elevations, together with the delayed start of cambial activity and xylem cell production at the tree line, support the hypothesis of Körner (2003) that tree line trees are not carbon limited. Factors affecting carbohydrate conversion to new tissues, rather than carbohydrate availability, seem to control cambial activity (Kozlowski 1992; Sundberg et al. 1993). Similarly, Hoch et al. (2002) observed higher levels of mobile carbon pools in needles, branches, stems and roots of trees with increasing elevation and concluded that sink activity of trees growing at high elevations was limited by low temperature rather than carbon availability. Similar conclusions were reached by Fajardo et al. (2013) who compared deciduous and evergreen treeline species across elevational gradients. Although no significant increase of NSC concentration in the cambium zone was observed with elevation in our study, we did observe a higher contribution of starch to the total NSC pool in larch at the tree line (P=0.03, data not shown). Our data thus support growth limitation at the tree line in response to sink activity rather than the inability of trees to supply growing tissues with carbohydrates.

In conclusion, our first study of intra-seasonal NSC dynamics at the cambial level in subalpine forests indicates that carbohydrate fluctuations at the sink level are closely linked to cambial activity (carbon sink demand) and the metabolic needs associated with cell formation, rather than to species or climate (elevation). Variation in the concentration of NSC with more specific functions such as raffinose or pinitol seems to be, however, species dependant. Observations on larch growing across an elevational gradient of nearly 1000 m suggest that temperature limits the cambial activity of trees and not the availability of carbohydrates within the cambium.

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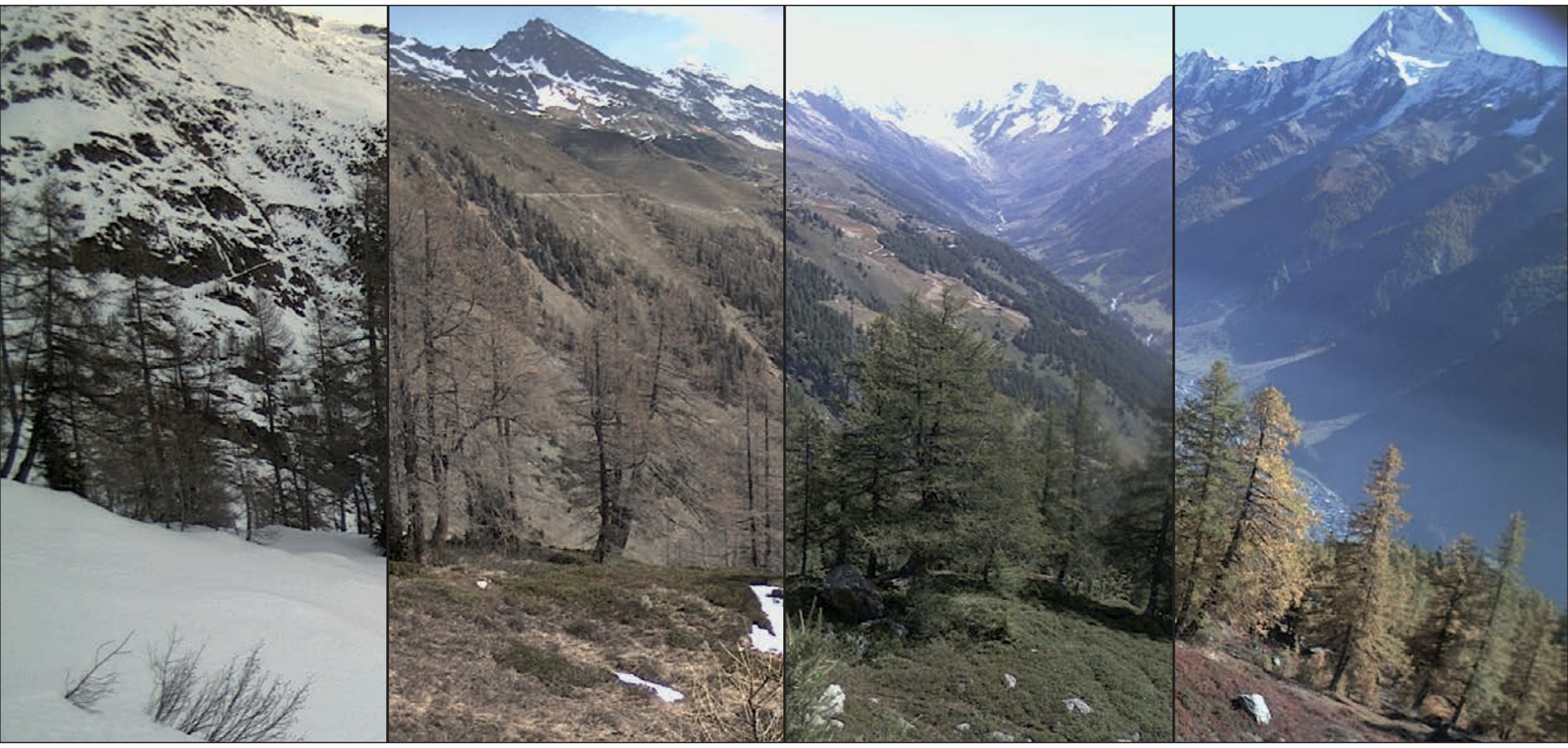

Chapter 6

Synthesis

Synthesis

Tree rings provide annually resolved, climatic reconstructions over the past several thousand years. However, these proxies of environmental variability represent an integrated record of a complete growing season, often overlooking essential information contained within the building blocks of a tree ring, the xylem cells. Knowledge concerning wood formation at seasonal and finer timescales presents an opportunity to bridge gaps between the physiological understanding of tree growth and tree-ring studies, vastly improving both.

Each of the previous chapters contributes to the primary goal of this dissertation; assessing tree and forest response to a warming climate across several scales of investigation. Traditionally, several major techniques have been used to gather perspective on this topic including long-term observations (Cook et al., 2012), experimental manipulations (Danby & Hik, 2007), satellite observations (Zhao & Running, 2010), and gradients across latitude (Thuiller et al., 2005; Lloyd et al., 2011). Each method has advantages and disadvantages (Wolkovich et al., 2012). This dissertation demonstrated that elevational transects offer an exceptionally strong platform to monitor natural populations across a confined spatial scale, limiting several possible confounding variables. CHAPTER II showed that the growing season temperature differences along our 1400-m transect (~6°C) capture the variability of regional temperature increases over the past century (~5°C) and are representative of climate model projections for the Alpine region in the year 2100 (~5°C). As locally adapted (i.e. genetically differentiated) populations may confound inter-site comparison, an understanding of the genetic landscape is essential. We demonstrated high gene flow between all populations within the Lötschental, likely counteracting local adaptation, and validating our use of sites along the transect as analogues of future tree climate conditions. We compared the climate sensitivities within this genetically confined population with those from across the Alps (Babst et al., 2013), and found that genetic control over the climate response signal within tree rings is likely small in comparison to regional climate influences. Further dendrochronology studies investigating climatic influences on tree-ring width under a measured genetic landscape will both significantly improve those studies and also contribute to the concept of trait plasticity and what role genetic adaptation will play for forests under projected warming.

Response to climate change is a complex topic and the application of diverse techniques across multiple scales is essential to provide answers. In this dissertation we worked on temporal scales ranging from hourly (CHAPTER III), to intra-seasonal (CHAPTER IV, CHAPTER V), to multi-decadal (CHAPTER II). Our results show that each of these scales of tree response is sensitive to climate, including coherent sensitivity between inter-annual and longer-term climatology with respect to the onset of stem and leaf growth in the spring. I also apply a range of scientific fields, including dendroclimatology (CHAPTER II), tree physiology (CHAPTER III, CHAPTER IV) and population genetics (CHAPTER II), revealing that interactions across these fields are essential to understand larger patterns. Multiple perspectives, both scientific disciplines and timescales, contribute to a greater understanding of how forests are responding to climatic warming and how future responses may shift or have feedback effects on the ecosystem. Further integration of these time-scales, such as assembling xylem cell chronologies over several centuries would provide greater context for the capacity of physiological response under global change (Fonti et al., 2010).

Two different methods, one indirect and one direct were used to investigate high-resolution tree growth response across a temperature gradient. Dendrometers provide an indirect, continuous measurement of tree stem radial dynamics. Although most studies attempt to isolate a growth signal, here, I use a novel methodology to investigate individual diurnal cycles and what they tell us about tree water use (CHAPTER III). Results provide quantification of how daily stem water movements are influenced by short-term meteorological variables, and how warming trends are likely to influence future stem-water movement. Direct observation of wood formation through weekly collection of micro-cores along with weekly observations of leaf phenology (CHAPTER IV) established that the phenological onset of both leaf and cambial growth occur about 5 days earlier/°C of late winter and early spring air warming. Considering both sets of results, dendrometers and microcores provide insight into the growing season onset, cessation, duration and dynamics as well as the sensitivity of these processes to external drivers. Future investigations further uniting microcoring and dendrometers are needed, as outcomes will contribute to differentiating irreversible radial growth signals compared to stem water signals and provide greater context for both.

It is generally accepted that species will respond uniquely to climate change on the basis of different capabilities to track or adapt to warming (Guisan & Zimmermann, 2000; Thuiller et al., 2008). I show different responses when comparing two common European conifer species with distinct morphological and physiological characteristics. Across the elevational gradient both the evergreen *Picea abies* and deciduous *Larix decidua* exist in mixed species sites, permitting isolation of climatic driven responses. For both species we measured sub-hourly stem water movement using dendrometers to show that spruce had greater daily water movement and under longer periods of drought were more likely than larch to reveal large decreases in stem water movement (CHAPTER III). We also assessed annual productivity using tree-ring widths and found that larger spruce ring-widths (i.e. increased productivity) are associated with greater participation, whereas larch responds more positively to warmer temperatures (CHAPTER II). Combined, these findings suggest greater water limitation in spruce which exploit internal water reservoirs more quickly and have a shallow, opportunistic root system. Conversely larch generally has a larger stem conductive area and a deeper root system.

Weekly observations show that leaf budburst precedes cambial activation in larch and follows or occurs simultaneously in spruce, while at the end of the season larch completes cell growth, including cell wall thickening, prior to leaf colouration and senescence (CHAPTER IV). These differences are related to canopy morphology. *Larix* lacks the capability to produce required structural carbohydrates for stem growth until leaf emergence in the spring, whereas, under favourable conditions, evergreens such as *Picea* are able to conduct photosynthesis all year and commence growth prior to new needle formation (Sevanto et al., 2006). The inconsistent timing between leaf emergence and onset of radial stem growth, the major sink for produced carbon, means that ecosystem vegetation models parameterized solely on leaf phenology are likely biased. Recent investigations into model limitations found that improved growing season length parameters resulted in large improvements of model accuracy (Tan et al., submitted).

Establishment of a large gradient allowed an assessment of *Larix decidua* intra-annual growth across an almost complete local elevational range. This unique opportunity revealed that cessation of both stem cell development and leaf senescence appear to follow a parabolic pattern, with earlier completion (i.e. shorter growing season duration) and leaf colouration at both the upper and lower elevations. I propose these observations are driven by limiting factors at the edges of a species range including temperature at treeline (Körner & Paulsen, 2004) and drought/competition at lower elevations (Eilmann et al., 2009). Supporting this hypothesis, CHAPTER II revealed that correlations between radial growth and temperature for larch were strongest at the highest elevation and response to precipitation was strongest at the lowest elevation. This complete reversal of limiting factors across the range is indicative of a limiting response at extremes and may suggest future range contraction under continued warming.

Tree rings represent one of the most frequently used proxies to reconstruct global climate trends over the past millennia. However, greater understanding of the underlying seasonality captured and the abiotic and biotic factors acting on the cambium during ring formation can improve results and constrain uncertainty. Further collection of intra-annual data such as that in CHAPTERS III and IV, as well as uniting the data into organized hemispheric and global networks will contribute to the development and improved parameterization of mechanistic models of cambium dynamics (Anchukaitis et al., 2006; Breitenmoser et al., 2013). In turn, model simulations can be compared amongst themselves and against collected chronologies to investigate forcing on inter-annual to longer-term scale response. Improved models represent one of the best ways to project how tree response may change under future conditions.

Extensive intra-annual growth analyses has often been limited by time constraints, and although these have begun to be overcome or simply accepted, further assessment of possible data processing biases are also needed. Currently, most treatments of dendrometer data rely on the stem cycle method to specifically isolate a tree growth signal and identify environmental drivers. It was demonstrated in CHAPTER III that these methods inflate the importance of several environmental variables. Thus, we propose that researchers carefully consider if their results may be biased by the link between increased stem water movement and precipitation. It is also essential for the field to not focus solely on growth at the expense of other signals, such as stem water movement (CHAPTER III). Application of signal processing approaches or use of statistical methods such as single spectrum analyses (SSA) or mixed effect models would present a major step forward, as superior analysis will improve our understanding of the data and the signals they contain. Similarly, direct cambial observation often relies on relatively inflexible sigmoid models such as the Gompertz. In this dissertation I utilize general additive models (GAM) which provide flexible splines to improve model fit and the ability to resolve differences between early and late season growth processes (Cuny et al., 2013, CHAPTER IV). Little research has examined the drivers behind the transition between earlywood and latewood (Griffin et al., 2013) and the flexible splines used in this dissertation can improve the resolution of this progression and provide improved understanding of the underlying processes of this transition and its impact on larger-scale forest dynamics.

As presented in CHAPTER V, linking cambial dynamics with additional perspectives of tree growth (e.g. mobile carboyhdrates, growth hormones, etc.) is an essential step to shrink the gap between fine-scale physiological and broader-scale ecological understanding. Currently, projects building on this dissertation are underway along the same elevational gradient investigating high-resolution intra-seasonal isotopic signatures in the soil-tree water continuum. These results will complement my findings and contribute to a greater understanding of how natural tree populations are likely to respond to continued warming temperature.

The combination of research approaches should be viewed as a successful strategy for future studies that aim to gather an ecosystem perspective and integrate information from across multiple spatial and temporal scales. The results from this dissertation offer improved understanding and quantification of forest response to climatic warming that is unprecedented in terms of magnitude and rate.

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Erklärung

gemäss Art. 28 Abs. 2 RSL 05

Name/Vorname: King, Gregory M

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Studiengang: Climate Sciences

Bachelor ☐ Master ☐ Dissertation ☑

Titel der Arbeit: Intra-annual tree growth response to warming along an elevational gradient

LeiterIn der Arbeit: Prof. Dr. Fortunat Joos

Dr. David C. Frank

Ich erkläre hiermit, dass ich diese Arbeit selbständig verfasst und keine anderen als die angegebenen Quellen benutzt habe. Alle Stellen, die wörtlich oder sinngemäss aus Quellen entnommen wurden, habe ich als solche gekennzeichnet. Mir ist bekannt, dass andernfalls der Senat gemäss Artikel 36 Absatz 1 Buchstabe o des Gesetztes vom 5. September 1996 über die Universität zum Entzug des auf Grund dieser Arbeit verliehenen Titels berechtigt ist.

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Ort/Datum

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Unterschrift

Curriculum Vitae

PERSONAL

Born 28 February, 1984 in Victoria, British Columbia, Canada

EDUCATION

2009-2014 Ph.D. Climate Sciences
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2002-2006 B.Sc. (Hons.) Environmental Science with a minor in Biology
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PROFESSIONAL EXPERIENCE

2013 - 2014 Part-Time Faculty
Mount Allison University, Sackville, NB
Taught biogeography, weather and climate and contemporary environmental studies

2007 NSERC Northern Internship
Northwest Territories Department of Natural Resources, Forestry Division, Inuvik, NT, Canada

2005 - 2008 Teaching Assistant
Carleton University, Ottawa, ON

PUBLICATIONS

Peer-Reviewed Journal Articles

King, G.M., Gugerli, F., Fonti, F., Frank, D.C. 2013. Tree growth response along an elevational gradient: climate or genetics? Oecologia. doi: 10.1007/s00442-013-2696-6

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Monographs

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King, G.M. 2009. Factors influencing the growth of white spruce (*Picea glauca*) in the Mackenzie Delta, Northwest Territories. M.Sc. Thesis, Carleton University, Ottawa, Ontario, Canada. 146 pp.

CONFERENCE CONTRIBUTIONS

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King, G.M., Fonti, P., Gugerli, F., Joos, F., Frank, D.C. Influences on and inferences from the circadian cycles of radial growth in two conifer species across a 900-m altitudinal gradient in the Swiss Alps. European Dendrochronology Conference. Engelberg, CH. September 2011. POSTER.

King, G.M., Fonti, P., Frank, D.C. Circadian stem size dynamics in larch and spruce along a 900-meter elevational gradient. World Dendrochronological Congress. Rovaniemi, Finland. June 2010. ORAL.

Fonti, P., Franzen, J., **King, G.M.**, Frank, D. Cufar, K., Gricar, J. Seasonal dynamics of ring formation in *Larix decidua* and *Picea abies* growing along an altitudinal gradient in Switzerland. World Dendrochronological Congress. Rovaniemi, Finland. June 2010. POSTER.

Fonti, P., Moser, L., Franzen, J., **King, G.M.**, Nievergelt, D., Buentgen, U., Esper, J., Luterbacher, J., Frank, D.C. Temperature-induced differences in timing of intra-annual growth of subalpine *Larix decidua* and *Picea abies*. European Geophysical Union (EGU). Vienna, Austria. May 2010. POSTER.

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King, G.M., Pisaric, M.F.J. and Kokelj, S.V. Factors influencing the growth of white spruce (*Picea glauca*), Mackenzie Delta, Northwest Territories, Canada. TRACE: Tree Rings in Archaeology, Climatology and Ecology. Otočec, Slovenia. April 2009. ORAL.

REVIEW AND EDITORIAL WORK

Canadian Journal of Forestry, Annals of Forestry, Journal of Climate Research, Theoretical and Applied Climatology, Journal of Geophysical Research, Global Change Biology

RESEARCH INTERESTS

Climatic change and ecosystem impacts, Dendroclimatology and intra-annual tree ring analysis, Phenology, Alpine and Boreal forest ecology, Urban forests, Sustainable development