Department of Environmental Sciences
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Environmental and climatic dependences of stable isotope ratios in tree rings on different temporal scales

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ACADEMIC DISSERTATION

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Contents

Publications

This thesis is based on the following papers, and are referred to in the text by their Roman numerals I – IV.

I   **Hilasvuori E.**, Kolari P., Oinonen M., Berninger F., Hari P. Diurnal patterns of carbon isotope fractionation in Scots pine. Manuscript.

II   Susiluoto S., **Hilasvuori E**., Berninger F. 2010. Testing the growth limitation hypothesis for subarctic Scots pine. Journal of Ecology, 98, 1186–1195.

III   **Hilasvuori E**., Berninger F., Sonninen E., Tuomenvirta H., Jungner H. 2009. Stability of climate signal in carbon and oxygen isotope records and ring width from Scots pine (*Pinus sylvestris* L*.*) in Finland. Journal of Quaternary Science 24, 469–480.

IV   **Hilasvuori E.**, Berninger F. 2010. Dependence of tree ring stable isotope abundances and ring width on climate in Finnish oak. Tree Physiology 30, 636–647.

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Contribution

Paper I   Study design and methods: P.H. and E.H.
Material collection: E.H., P.K., E.S.
Data analysis: E.H., P.H. and P.K.
Manuscript preparation: E.H. was responsible; P.K. and P.H. participated and M.O. and F.B. commented on the text.

Paper II   Study design and methods: S.S. and F.B.
Material collection: S.S.
Laboratory analysis: S.S., E.H. and E.S.
Data analysis: S.S. and F.B.
Manuscript preparation: S.S. was responsible; E.H. was responsible of the isotope part and. F.B contributed

Paper III   Study design and methods: defined by the EU project ISONET
Material collection: E.H., H.J. and E.S.
Laboratory analysis: E.H. (ring width measurements and chronology construction) E.S. (isotope data)
Data analysis: E.H.
Manuscript preparation: E.H. was responsible; E.S., H.T., H.J. and F.B. participated.

Paper IV   Study design and methods: defined by the EU project ISONET
Material collection: E.H., H.J. and E.S.
Laboratory analysis: E.H. (ring width measurements and chronology construction) E.S. (isotope data)
Data analysis: E.H.
Manuscript preparation: E.H. was responsible and F.B. participated.

Abstract

This work examines stable isotope ratios of carbon ($\delta^{13}C$), oxygen ($\delta^{18}O$) and hydrogen (δ^2H) in annual growth rings of trees. Isotopic composition in wood cellulose extracted from tree rings is used as a tool to study past climate. The method benefits from the accurate and precise dating provided by dendrochronology. In this study the origin, nature and the strength of climatic correlations are studied on different temporal scales and at different sites in Finland.

The origin of carbon isotopic signal is in photosynthetic fractionation. The basic physical and chemical fractionations involved are reasonably well understood. This was confirmed by measuring instantaneous photosynthetic discrimination on Scots pine (*Pinus sylvestris* L.). However, the internal conductance of CO_2 was recognized to have a significant impact on the observed fractionation, and further investigations are suggested to quantify its role in controlling the isotopic signal of photosynthates. Isotopic composition of the produced biomass can potentially be affected by variety of external factors that induce physiological changes in trees. Response of carbon isotopic signal in tree ring cellulose to changes in resource availability was assessed in a manipulation experiment. It showed that the signal was relatively stable despite of changes in water and nitrogen availability to the tree.

Palaeoclimatic reconstructions are typically based on functions describing empirical relationship between isotopic and climatic parameters. These empirical relationships may change depending on the site conditions, species and timeframe studied. Annual variation in Scots pine tree ring carbon and oxygen isotopic composition was studied in northern and in central eastern Finland and annual variation in tree ring latewood carbon, oxygen and hydrogen isotopic ratio in Oak (*Quercus robur* L.) was studied in southern Finland. In all of the studied sites at least one of the studied isotope ratios was shown to record climate strongly enough to be used in climatic reconstructions. Using the observed relationships, four-century-long climate reconstructions from living Scots pine were created for northern and central eastern Finland. Also temporal stability of the relationships between three proxy indicators, tree ring growth and carbon and oxygen isotopic composition was studied during the four-hundred-year period.

Isotope ratios measured from tree rings in Finland were shown to be sensitive indicators of climate. Increasing understanding of environmental controls and physiological mechanisms affecting tree ring isotopic composition will make possible more accurate interpretation of isotope data. The knowledge will also help in selecting study sites in order to maximize the potential climate sensitivity within the data. This study also demonstrated that by measuring multiple isotopes and physical proxies from the same tree rings, additional information on tree physiology can be obtained. Thus isotopic ratios measured from tree ring cellulose provide means to improve the reliability of climate reconstructions.

1 Introduction

Throughout the human history climate has played a significant role in determining livelihood, health and welfare of human societies (e.g. Zhang et al. 2007). However, only recently human activity has started to significantly impact climate mainly through the effect of increased greenhouse gas emissions, in particular emissions of CO_2 (IPCC 2007). To characterize and quantify the human induced impact on climate, its natural variability must first be known. This variability can be studied retrospectively from a variety of naturally occurring archives such as ice cores, lake and ocean sediments, corals, speleothems and growth rings of trees. Information can be extracted from these archives by studying the growth rate, physical or chemical properties of the material or their stable isotope composition.

In many respects, trees hold great potential for reconstructing past environmental conditions. They provide an archive that can be exactly dated on an annual scale. Trees are also widespread, so that it is possible to examine geographical variations in the environmental conditions. Furthermore it is possible to create chronologies of several overlapping trees, and analyze the variability within the measurements. Sometimes trees can be found in large amounts in deposits where they have been preserved under favourable conditions (dry, anoxic, frozen in the permafrost) for millennia allowing long continuous chronologies to be built (e.g. Pilcher et al. 1984, Eronen et al. 2002, Grudd et al. 2002, Hantemirov and Shiyatov 2002). Wood components, cellulose and lignin are often well preserved (Loader et al 2003, Poole and van Bergen 2006).

The main constituents of wood, elements of carbon (C), oxygen (O) and hydrogen (H), have all more than one naturally occurring stable, non-radioactive, isotope. Carbon has two stable isotopes: the predominant carbon-12, and the minor carbon-13. Oxygen has three isotopes, predominant oxygen-16, oxygen-17 and oxygen-18. From the minor isotopes oxygen-18 is more abundant and thus more commonly used in isotope studies. Hydrogen has two stable isotopes, the predominant hydrogen-1 and minor hydrogen-2, also called deuterium. The cause of the variations in the abundances of stable isotopes is fractionation, a change in isotopic ratio from source to product, that occur due to small mass-related differences between isotopes of an element in physical, chemical, and biological processes.

By analyzing stable isotopes from tree rings, environmental conditions during the growth period can be studied. Increasing understanding of the underlying mechanisms leading to isotopic fractionation also allows studying the ecophysiological processes affected by changing environmental conditions. This will help to improve the current climate reconstructions and also to predict the ecosystem responses that will occur as the environment will continue to change.

1.1 Stable isotopes in tree rings: history and background

Natural variation in carbon isotopic composition among plant species and organs was first studied in the 1950s (Craig, 1953, 1954a). The early studies already included research on isotopic composition of tree rings (Craig 1954b). Although already Urey (1947) suggested that plants may contain a temperature signal in their carbon isotopic ratios, for some time, the main aim was to use the tree ring archive to reconstruct past changes in the $^{13}C/^{12}C$ ratio of atmospheric carbon dioxide in order to expand the time span covered from direct measures of atmospheric composition (Tans and Mook 1980, Freyer and Belacy 1983, Leavitt and Long 1983, Stuiver et al. 1984). One of the first discoveries towards more precise understanding of isotope fractionation in plant processes was the finding that stable carbon isotope ratios differed between plants with C3 and C4 photosynthetic pathways (Bender 1971, Troughton et al. 1974). Later the theories of carbon isotopic fractionation in plant photosynthesis evolved and related the variation in isotope ratios to the regulation of leaf gas exchange (Vogel 1980, O'Leary 1981, Farquhar et al. 1982). On the basis of those theories, a link to plant water use efficiency was established (Farquhar et al. 1982) and carbon isotopes became a frequently used tool to study plant-water relations in plant ecology. The fractionation theories were explained in the point of view of tree ring research by Francey and Farquhar (1982). This justified the use of annual variations in tree ring carbon isotopes as a proxy for past variations in temperature, light or moisture stress (e.g. Leavitt and Long 1991).

As the oxygen and hydrogen isotopic composition of precipitation was already recognized to depend upon the temperature (Dansgaard 1964), early work on oxygen and hydrogen in plant material was conducted with the aim of using tree rings as the isotopic thermometer (Libby and Pandolfi 1974). Although it first seemed probable that the O in plant material came from CO_2 it was shortly discovered that O exchange between CO_2 and water occurs, thus forming a direct link to the local precipitation (Epstein et al. 1977, DeNiro and Epstein 1979). Burk and Stuiver (1981) showed that oxygen isotopic composition in wood cellulose was possible to model using the known environmental factors that determine the oxygen isotopic signature: isotopic composition of source water, atmospheric humidity, leaf boundary layer dynamics and isotopic composition of atmospheric water vapour. During the following decades the somewhat general models developed into more detailed descriptions of isotope fractionations and exchange processes associated with tree metabolism (Sternberg et al. 1986, Yakir and DeNiro 1990, Roden et al. 2000) and isotopic enrichment of leaf water (Barbour et al. 2000). In addition to the use as humidity, precipitation or temperature proxy (Treydte et al. 2006, Edwards et al. 2008) or proxy for tree water sources (Ehleringer and Dawson 1992) the oxygen stable isotope composition has been shown to provide additional information when combined with carbon isotopes. It can be used to distinguish effects of stomatal conductance from effects of changing photosynthetic capacity on the carbon isotopic composition of organic matter (Saurer et al. 2004, Saurer and Siegwolf 2007).

Hydrogen was expected to behave like oxygen (Luo and Sternberg 1992, Yakir 1992). Strong correlations were reported for samples of wide geographic

distributions (Yapp and Epstein 1982, Gray and Song 1984, Roden and Ehleringer 2000), however, comparisons of tree ring hydrogen isotope time series with annual temperature records have been less successful (Epstein and Yapp 1976, Lipp et al. 1991, Waterhouse et al. 2002). This and perhaps the more difficult measurement technique and more laborious sample preparation procedure have made deuterium the less frequently used isotope in the recent literature.

In combination with improved understanding of isotopic fractionations in plants also analytical advances have promoted the tree-ring isotope research. Development in techniques of isotope measurement (Preston and Owens 1985, Meier-Augenstein 1999) and sample preparation (Leavitt and Danzer 1993, Loader et al. 1997), that advanced especially during late 1980's and 1990's, made the construction of long chronologies with high degree of replication and temporal range possible (eg. Treydte et al. 2009). In the future, development of new laser-ablation techniques may provide even faster means of measuring tree ring isotopes with the possibility to also measure at higher resolution (Schulze et al. 2004).

A long tradition in tree ring research (e.g. Laitakari 1920, Hustich 1945, 1948, Mikola, 1956, Sirén 1961, Lindholm 1996, Helama 2004) and the possibility to construct pine chronologies from subfossil wood (Eronen et al. 1991, 2002) has made a good basis for tree ring isotope studies in Finland. The first investigations on tree ring isotopes were conducted by Sonninen and Jungner (1995, 1996), who studied living and subfossil pines from the Holocene period from Lapland and presented the first comparisons of carbon isotopic ratios to local temperatures. The climate signal in carbon isotope ratio of Finnish pines was further evaluated by studies along a transect in Lapland (McCarroll and Pawellek 1998, 2001), and by comparisons of different potential climate proxy measures recovered from these trees (McCarroll et al. 2003). A study exploring the mechanistic links between carbon isotopes and climate was conducted by Berninger et al. (2000). The first attempt to produce a temperature reconstruction based on carbon isotopes extending back to three and a half centuries was published by Gagen et al. (2007) indicating that the northern pines have high potential for precise temperature reconstruction. Besides Scots pines, carbon isotope ratios in oaks in southern Finland were studied by Robertson et al. (1997) in Turku Ruissalo. These studies all concentrated on carbon isotopes while the first oxygen and hydrogen isotope studies on Finnish trees are included in this thesis.

1.2 Isotopic fractionation in trees

1.2.1 Delta notation

Isotope composition of material or compound is usually expressed using delta (δ) notation as deviations from internationally accepted standard material for which the isotopic ratio is known. δ values are calculated using the equation:

$$\delta\text{‰} = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right) \times 1000 \tag{1}$$

where R is the molar ratio of the heavy to light isotope. Commonly used standards for plant material are Vienna Pee Dee Belemnite (VPDB) for carbon and Vienna Standard Mean Ocean Water (VSMOW) for oxygen and hydrogen.

1.2.2 ^{13}C

Carbon isotope composition of plant tissue is determined by the isotopic composition of CO_2 in the atmosphere and by the extent to which plants discriminate against the heavier isotope. The discrimination occurring in the incorporation of source (CO_2) into product (plant biomass) is reported as discrimination (Δ). The reader must note that Δ is different from isotope composition reported as delta values (δ). The discrimination occurring in plants is defined as:

$$\Delta = \frac{(\delta^{13}C_{\text{air}} - \delta^{13}C_{\text{plant}})}{(1 + \delta^{13}C_{\text{plant}}/1000)} \tag{2}$$

The magnitude of discrimination is dependent on the significance of various physical and biochemical steps during the CO_2 uptake (Figure 1). Of these, the most important are diffusion of CO_2 through stomata, diffusion through mesophyll to the site of carboxylation and enzymatic carbon fixation by the primary carboxylating enzyme, ribulose-1,5-bisphosphate carboxylase (RuBisCo). The simplest and the most often used version of the model of Farquhar et al. (1982) relates the discrimination to leaf internal and ambient CO_2 concentrations, implicitly accounting for internal CO_2 transfer:

$$\Delta_{\text{simple}} = a + (b' - a)\frac{C_i}{C_a} \tag{3}$$

where a is the fractionation due to diffusion of CO_2 into the leaf (-4.4‰, Craig 1954, O'Leary 1981), and b' is the biochemical fractionate (approximately -27‰, Farquhar and Richards 1984) adjusted to take into account leaf internal CO_2 transfer. C_i and C_a are the leaf internal and external CO_2 concentrations. This function can be arranged to calculate the assimilation weighed C_i/C_a ratio of photosynthesizing leaves:

$$\frac{C_i}{C_a} = \frac{\Delta_{\text{simple}} - a}{b' - a} \tag{4}$$

The obtained linear relationship is often utilized to calculate the intrinsic water use efficiency, the ratio of net carbon uptake to stomatal conductance, $W_i = A/g_{sw}$, of plant leaves (Ehleringer and Cerling 1995). Since according to Fick's law: $A = g_{sc}(C_a - C_i)$, where g_{sc} is the stomatal conductance for CO_2 and since stomatal conductances

of CO_2 and water (g_{sw}) are related by a constant factor ($g_{sw} = 1.6g_{sc}$), water use efficiency can be linked to isotope discrimination:

$$W_i = \frac{C_a}{1.6} \left(\frac{b\prime - \Delta_{simple}}{b\prime - a} \right) \tag{5}$$

This approach assumes that environmental factors such as temperature, humidity and light remain stable during the period studied and that the leaf internal conductance to CO_2 does not vary (Seibt et al. 2008).

There is, however, a more comprehensive approach to model carbon isotopic fractionation in photosynthesis. It is based on the biochemical model of photosynthesis (Farquhar and von Caemmerer 1982) and it takes into account also such factors as leaf internal conductance to CO_2 and respiration as components of the isotopic budget of net photosynthesis (Farquhar et al. 1982):

$$\Delta_{comp} = a_b \frac{(C_a - C_s)}{C_a} + a \frac{(C_s - C_i)}{C_a} + a_m \frac{(C_i - C_c)}{C_a} + b \frac{C_c}{C_a} - f \frac{\Gamma^*}{C_a} - e \frac{R_d}{kC_a} \tag{6}$$

where a_b is fractionation factor associated with CO_2 diffusion through the leaf boundary, a_m is the sum of the fractionation factors during internal CO_2 transfer, combining an equilibrium fractionation of CO_2 entering solution (Mook et al. 1974) and a diffusional fractionation of dissolved CO_2 in water (O'Leary 1984). b is the net fractionation attributed to PEP (phosphoenolpyruvate) carboxylase and Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) activity (Roeske and O'Leary 1984). The respiratory fractionation factors are f for photorespiration (Gillon and Griffiths 1997) and e for dark respiration, R_d. C_c is the CO_2 concentration at the sites of carboxylation, k is the carboxylation efficiency and Γ^* is the CO_2 compensation point in the absence of R_d. Rather than being sensitive to C_i/C_a, as the simple form of the model suggests, the comprehensive model suggests that the carbon isotope fractionation is sensitive to the ratio of the chloroplast to ambient CO_2 mole fraction C_c/C_a.

1.2.3 ^{18}O and ^2H

Local precipitation is the main source of both the hydrogen and oxygen atoms in the cellulose of annual growth rings of trees (Figure 1). The isotopic composition of atmospheric precipitation is dependent on environmental factors, to a large degree on the condensation temperature of precipitation (Dansgaard 1964, Welker 2000). When a tree takes up water from the soil with its roots no isotopic fractionation is assumed to happen. So, the isotopic composition of stem water has the same isotopic composition as the soil water (White et al. 1985, Ehleringer and Dawson 1992). However, there are points along the pathway between water uptake and cellulose formation where the isotopic composition of either water or organic molecules may be altered. Some of these alterations are dependent on environmental factors and

thus tree ring isotope composition cannot be used as straight forward indicator of rain water.

The water that has been transported into the leaf enriches with the heavier isotope due to transpiration. The degree of enrichment depends on air humidity conditions since transpiration in leaves is strongly dependent on the leaf-air vapour pressure difference. The isotopic composition of the evaporative pool of leaf water can be calculated using Craig – Gordon -type equation (Craig and Gordon 1965):

$$\delta_{\text{evapor pool}} = \delta_{\text{stem water}} + \varepsilon^* + \varepsilon_k + e_a/e_i\left(\delta_{\text{atmosphere}} - \varepsilon_k - \delta_{\text{stem water}}\right) \quad (7)$$

where δ is isotope ratios, e_a/e_i is the ratio of ambient water vapour pressure to that inside the leaf, and ε* and ε_k, the equilibrium and kinetic isotopic effects respectively. Photosynthetic carbohydrates produced in the leaf will inherit the isotopic signal of the bulk leaf water, the leaf water as a whole, which has been observed to be less enriched than the leaf water fraction subjected to evaporation (Barbour et al. 2000). The following equation can be used to predict the bulk leaf water isotopic composition (e.g. Sternberg 2009):

$$\delta_{\text{leaf water}} = \left((1 - \alpha)\delta_{\text{stem water}}\right) + \left(\alpha\, \delta_{\text{evapourative pool}}\right) \quad (8)$$

where α is the fraction of enriched water in the leaf. α can be adjusted to take into account the so called Péclet effect (Farquhar and Lloyd 1993). The theory suggests that the isotopic composition of bulk leaf water is determined by the balance between two water fluxes in opposite directions: diffusion of enriched water away from the sites of evaporation and convection of source water to those sites (Barbour and Farquhar 2000). The net effect depends on transpiration rate as well as leaf morphologic characteristics.

The oxygen isotopic composition of the synthesized carbohydrates is altered due to carbonyl oxygen exchange with the water in the leaf. The exchange results in an enrichment of the organic oxygen compared to leaf water. A similar exchange of carbonyl oxygen will also take place later, when the translocated carbohydrates are utilized to synthesize cellulose in the stem (Figure 1). In the stem an average of 40% of the oxygen destined for the cellulose molecule will exchange during the synthesis with stem water present (Sternberg et al. 1986, Yakir and DeNiro 1990, Roden and Ehleringer 1999, Sternberg et al. 2006). This can be expressed according to Roden and Ehleringer (1999) as:

$$\delta^{18}O_{\text{cellulose}} = f\left(\delta^{18}O_{\text{stem water}} + \varepsilon_O\right) + (1 - f)\left(\delta^{18}O_{\text{leaf water}} + \varepsilon_O\right) \quad (9)$$

where f is the proportion that undergoes exchange with the water and ε_O is the fractionation factor for both fractionations in leaf and in cellulose synthesis in stem, approximately 27‰ (Sternberg and DeNiro 1983, Sternberg et al. 2006).

Also carbon-bound hydrogen atoms in carbohydrates undergo exchange with water in leaf via enzyme-mediated reactions. And again in stem approximately in average 40% of carbon-bound hydrogen atoms in carbohydrates exchange with

water during cellulose synthesis (Roden and Ehleringer 1999, Waterhouse et al. 2002, Augusti et al. 2006). However, the reactions and fractionations factors are different. This can be expressed as:

$$\delta\,^2H_{cellulose} = f\left(\delta\,^2H_{stem\,water} + \varepsilon_{Hh}\right) + (1 - f)\left(\delta\,^2H_{leaf} + \varepsilon_{Ha}\right) \qquad (10)$$

where f is the fraction that exchanges with the water present, ε_{Ha} and ε_{Hh} are the isotope effects for exchange with water during carbohydrate formation in leaf and during cellulose synthesis. The values for ε_{Ha} and ε_{Hh} are estimated to be approximately +150‰ and -150‰, respectively (Yakir and DeNiro 1990; Luo and Sternberg 1992).

Consequently, the oxygen and hydrogen isotope signals in tree ring cellulose will record leaf level processes controlled to a large extent by humidity and source water signal controlled by temperature. However, since the size of kinetic isotope effect depends on the ratio of masses of the heavy and light isotope, and since that is especially large for ^1H and ^2H, the enzymatic biochemical fractionations that are kinetic in nature, are relatively more important for hydrogen (Augusti et al. 2006, 2008). Thus the ^2H signal from equilibrium fractionations during evaporation of water at the leaf level can be expected to be more efficiently overwritten during later biochemical steps.

1.2.4 Postphotosynthetic fractionation

The isotopic signal that is imprinted on photosynthates is transported through the tree before it is laid down in the tree-ring archive. On the way, metabolic compounds undergo fractionation steps associated with metabolic pathways and transport processes that are referred to as postphotosynthetic fractionation. It has been long known that different chemical compounds like cellulose and lignin (Robertson et al. 2004) as well as different plant organs (Leavitt and Long 1986) have different isotopic compositions. However, the significance of postphotosynthetic processes in potentially uncoupling the relationships between photosynthetic discrimination and isotopic signature in tree ring is still largely unknown (Helle and Schleser 2004, Badeck et al. 2005, Brandes et al. 2006, Cernusak et al. 2009, Gessler et al. 2009). In addition to postphotosynthetic fractionation, isotope signals measured from annual growth rings can be dampened by mixing and carryover from stored carbohydrates (Barbaroux and Bréda 2002, Kagawa et al. 2006). Moreover, those carbohydrates have possibly undergone different biosynthetic pathways from photosynthates to tree ring cellulose which may change the overall fractionation (Helle and Schleser 2004).
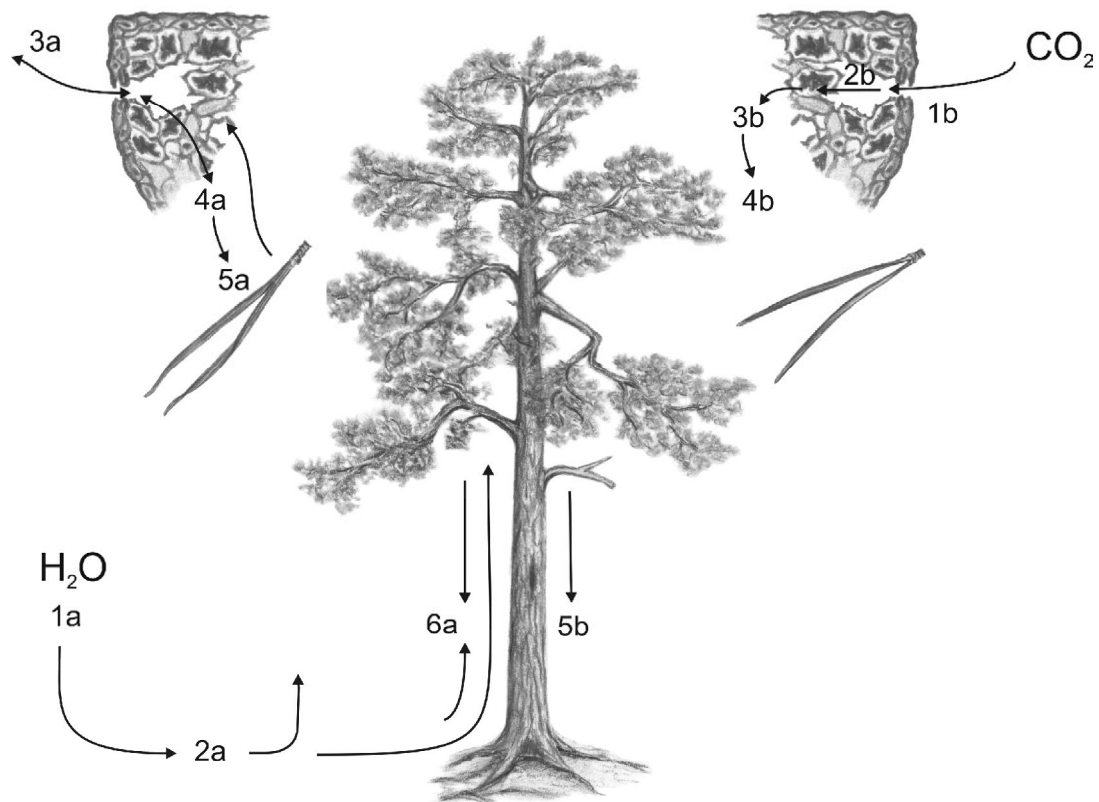
Figure 1 The main processes potentially affecting isotopic fractionation. Water isotopes $^{18}O/^{16}O$ and $^{2}H/^{1}H$: (1a) Evaporation and condensation of rain water, (2a) Evaporation of soil water (3a) Transpiration in leaf, (4a) Péclet effect, (5a) Carbohydrate synthesis and carbonyl exchange with leaf water, (6a) Exchange with xylem water during cellulose synthesis. Carbon $^{13}C/^{12}C$: (1b) Diffusion through leaf boundary layer and through stomata, (2b) Diffusion in mesophyll to chloroplast, (3b) Carboxylation, (4b) Biochemical processes leading to cellulose synthesis, (5b) Cellulose synthesis.

1.3   Aims of this study

In this thesis the isotopic variability in tree ring cellulose in Finland is studied as a potential source of information on past climate and as a source of information on ecophysiological processes affected by these conditions. Carbon isotopes are studied in more detail for the physiological basis through which the isotopic signature in tree rings is connected to environmental variables. Oxygen and hydrogen isotopes are studied as a potential source of climatic and environmental information. The information from isotopes is also compared to and combined with information from more commonly used method to extract climatic information, the ring width analysis. Three out of four papers included in this thesis focus on Scots pine (I, II, III) (*Pinus sylvestris* L.), the most commonly used tree species in climate

reconstructions in northern Fennoscandia. However, pedunculate oak (*Quercus robur* L.) is also studied as a potential source for palaeoclimatic information (IV).

Specific objectives of the thesis were to:

- define the main mechanisms affecting carbon isotope signature in Scots pine in Finland (I, III).

- study how changes in tree resource availability affect carbon isotope discrimination and tree growth in tree line trees (II).

- study how the climate signal in carbon and oxygen isotopic time series vary between northern Finland and central eastern Finland (III).

- study if the isotopic time series measured from oaks growing on the border of their distribution range could provide a proxy for climatic information (IV).

- study if the climatic information in isotopic time series of carbon and oxygen and ring width differ in annual scale and in long term (II, III, IV).

2 Materials and methods

2.1 Study sites and sampling

The pine tree studied in paper I grows in an experimental plot at Hyytiälä SMEAR II field station (Station for Measuring Forest Ecosystem-Atmosphere Relations) located in central Finland (Figure 2). The forest is 45-year-old (in 2007) pine dominated forest. A description of the site can be found in eg. Hari and Kulmala (2005). The gas samples for isotope analysis were taken from a gas exchange chamber placed in the upper canopy. The shoot in the chamber had grown during the previous summer.

The manipulation study in paper II was conducted in Värriö Nature Park in Salla, in north of Finland (Figure 2) close to Värriö Subarctic Research Station. The forest in the area is coniferous and belongs to the north boreal forest zone. On the upper slopes of the fells Scots pine trees grow close to their altitudinal tree line. 25 of these trees that grow on Värriö I fell at 470 m a.s.l on the north facing slope were selected for the manipulation experiment. The vegetation on the site consisted of scattered Scots pine trees and fell field vegetation. Although the selected trees were only 2 to 5 meters tall and only 7.2 cm in diameter in average (SD = 1.9 cm), they were estimated to be approximately 50 years old. The mean annual precipitation in the area is about 600 mm and average mean temperature -0.5 °C (at Värriö research station, altitude 380 m, for period 1981-2010).

For the paper III living trees of approximately 400 years old were sampled from two sites in Finland. Four trees from both sites were selected for isotope analyses, but more trees were cored and used in ring width chronologies. The more northerly site Kessi, in Inari, locates in the vicinity of forest limit in the northern part of Finland on the eastern shore of Lake Inari. The forest is pine forest belonging to the north boreal zone. The mean annual temperature is -1 °C at the nearest weather station, Nellim, and the average yearly precipitation sum is around 480 mm (for period 1971-2000). The diameter at breast height of the four selected trees was on average 49.2 cm (SD = 1.4 cm). The trees located on rather large area 1-2 km from each other. The second site Sivakkovaara, in Ilomantsi, locates in central eastern Finland on the border of middle and southern boreal zones (Figure 2). The forest is coniferous forest with both Scots pine and Norway spruce (*Picea abies* L.) Mean annual temperature at the closest weather station, Ilomantsi, is 2 °C and the average yearly precipitation sum 685 mm (for period 1971-2000). The diameter at breast height of the four selected trees was on average 67.3 cm (SD = 5 cm). The trees were cored with a 12 mm increment corer at breast height from four directions of the stem or the trees were felled and discs cut to match the size of the cores.

The studied oak trees in paper IV grow in Bromarv (Figure 2) in Southern Finland close to the coast of Gulf of Finland. In this area oak grows on the northernmost border of its distribution range. The forest at the site is deciduous and locates in the hemiboreal forest zone. All the sampled trees grow rather close to each other on an east-facing slope. The selected trees are one hundred or more years old.

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They were sampled for isotope and ring width analyses from at least two directions of the stem. The stem diameter of the sampled trees varies between 60 and 85 cm at breast height. 7 trees were cored from which 4 were used in the isotope analysis. The mean annual temperature (for period 1971-2000) measured at the Salo weather station is 5 °C. The average yearly precipitation sum for the same period is 675 mm.
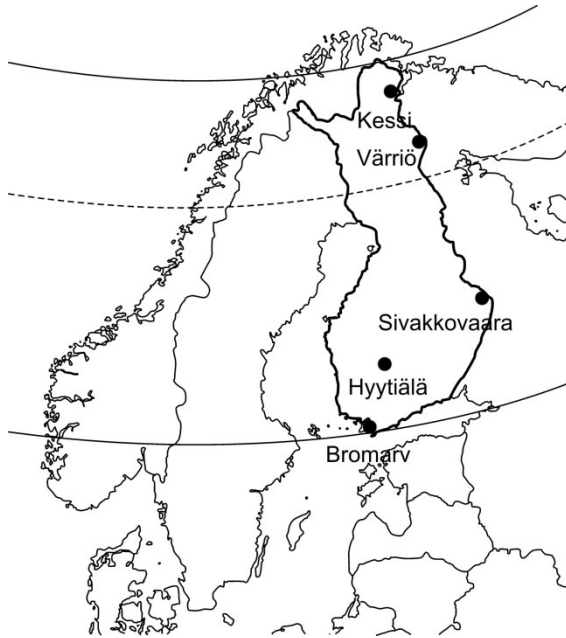

Figure 2 Map showing the study sites.

2.2 Measuring and modelling carbon isotope discrimination during photosynthesis

The isotopic fractionation in photosynthesis can be studied by measuring air passing over lit leaves (Evans et al. 1986). In paper I this was done in conjunction with automated gas exchange measurements at SMEAR II field station. The measuring setup consisted of a chamber, a pneumatic system that opens and closes the chamber, sample tubing and gas analysers (Altimir et al. 2002). The volume of the chamber was 1 dm^3 and it was made of transparent acrylic plastic. The backplate of the chamber was fixed to the tree and the enclosure slid horizontally away from the backplate, exposing the shoot to the ambient conditions when the chamber was open (Figure 3). Samples for isotopic measurements were taken from air around the shoot just before the chamber closed and from air going in and going out of the chamber. The CO_2 concentration was obtained from the automated measurements. Calculation of observed net discrimination against $^{13}CO_2$ was conducted according to (Evans et al. 1986):

$$\Delta_{obs} = \frac{\xi(\delta_o - \delta)}{1 + \delta_o - \xi(\delta_o - \delta)} \qquad (11)$$

where $\xi=C(C–C_o)$ is the ratio of the reference CO_2 concentration in the air entering the chamber (C) relative to its difference to the CO_2 concentration in the sample gas going out from the chamber (C_o), and δ and δ_o are the $\delta^{13}C$ values of the in and out going gases respectively. The observed net change in $\delta^{13}C$ and CO_2 concentration is a result of two different processes, photosynthesis which consumes CO_2 and respiration which produces CO_2.

Isotopic fractionation was predicted with a model that was derived from a photosynthesis model called Opitimal stomatal control model (OSM) (Hari et al. 1986, Hari and Mäkelä 2003, Hari et al. 2008). The model is based on the optimality hypothesis proposed by Cowan and Farquhar (1977). To include isotopes to the model the calculations were done separately for ^{12}C and ^{13}C with different rates of diffusion (g_c) and carboxylation efficiency (β) specified for $^{13}CO_2$ and $^{12}CO_2$:

$$^{12}p = f_{osm}(I,D,T,\,^{12}\beta,\,^{12}g) \tag{12}$$

$$^{13}p = f_{osm}(I,D,T,\,^{13}\beta,\,^{13}g) \tag{13}$$

The discrimination was calculated following Farquhar et al. (1989):

$$\Delta = \frac{^{13}C_a/^{12}C_a}{^{13}p/^{12}p} - 1 \tag{14}$$

where $^{12}C_a$ and $^{13}C_a$ are the $^{12}CO_2$ and $^{13}CO_2$ concentrations of the air around the shoot and ^{12}p and ^{13}p are the photosynthetic rates of $^{12}CO_2$ and $^{13}CO_2$ respectively. To be able to model the isotopic discrimination with the model, mesophyll conductance had to be explicitly included in it. This was done by calculating the overall conductance to CO_2 (g_c) to consist of stomatal conductance g_{sc} and mesophyll conductance g_m as:

$$g_c = g_{sc}g_m/(g_{sc}+g_m) \tag{15}$$

Figure 3 Chamber used at SMEAR II for measuring gas exchange of shoots.

2.3   Manipulation of tree resources

In paper II manipulation experiments in field conditions were used to test growth responses of pines to changes in the availability of resources. The trees were divided into four groups. Three of the groups, six trees per group, were assigned to manipulation treatments and one, with seven trees in it, was left as a control group. Availability of nitrogen was increased by fertilizing trees in the end of May and in August in 2003 and again in May in 2004 before the samples were taken for growth and isotope analysis in the autumn 2004. The availability of carbon was restricted by removing all needles except the ones produced during the previous growing season. This means that at the beginning of the growing season the trees had one needle cohort and at the end two needle cohorts. The availability of carbon for growth was improved by removing part of the buds but leaving the apically dominant shoot intact. The apical buds were left intact since they are known to create hormonal signals that affect growth (Forest et al. 2004). These both treatments were done in spring 2003 and in spring 2004.

Tree responses to the manipulation treatments were studied by measuring growth of shoots, height, tree ring width, carbon isotope ratios in tree ring cellulose, needle nitrogen concentrations and carbohydrate concentrations. The trees were cored with a 5 mm increment corer for tree ring width and isotope analysis from two directions of the stem below the lowest living branches. However from the group from which buds were removed isotope analyses were not done as we did not have a clear hypothesis for the changes due to the treatment.

2.4   Dating tree rings and constructing a chronology

The tree rings can be dated annually to provide exact calendar years for every tree ring in a sample. The practice is called cross-dating and it is based on matching the pattern of wide and narrow rings to demonstrate dating between trees (e.g. Fritts 1976). Dating of tree rings in papers II, III and IV is based on this technique. The dating was carried out starting with two radii from the same tree and then continuing to the site level. The obtained site chronology was then compared to site chronologies from different sites close by. Visual dating of the measured ring widths was checked by statistical comparison using the computer program COFECHA (Holmes 1983).

To be able to extract climate signal from tree ring width series and to compare them to isotope chronologies the individual series have to be detrended and standardized in order to remove age-size related trends in growth (papers III, IV). This is done by fitting a curve through the ring width series. Determining the curve can be done in different ways and the choice of the technique significantly influences the obtained indices. In principle the selected technique can be deterministic, meaning that it follows a predetermined model of tree growth that is based on the idea that adding the same volume of wood on the surface of ever increasing cylinder decreases the width of the ring in time. Or it can be empirical, meaning that the best

fit to a series of data can be chosen through experimentation. In papers III and IV a cubic smoothing spline function was used. That is a polynomial of time that is fitted piecewise to different parts of the time series. In paper III 200 year spline functions and in paper IV 67% spline functions were used with 50% variance cutoff. Once the trend has been identified, indices are usually extracted from the curve by dividing each ring by corresponding value of the curve or subtracting the curve from the ring widths. Calculating indices as ratios is often preferred since it simultaneously stabilizes the trend in variance that might accompany the trend in mean, if the trend in variance is not otherwise removed using techniques such as power transformation (Cook and Peters 1997). In this thesis division was used in paper IV and subtraction with power transformation in paper III.

Ring growth in a certain year depends on growth conditions in current but also on the growth conditions in the preceding years. Thus statistical correlation can be found between ring growth in previous and current year. This can be described mathematically as autoregressive (AR) and as autoregressive-moving average (ARMA) processes (Box and Jenkins 1970). In papers III and IV these models were used to minimize non-climatic influences.

Finally site chronologies are created by averaging individual series of indices. This will average out individual tree variability and enhance the common growth signals characteristic to the site. Confidence of the chronology was studied in papers III and IV with expressed population signal (EPS) that measures the common signal in time series (Wigley et al. 1984, Briffa and Jones 1990) and sets a theoretical level on how many trees are required for building a statistically robust chronology for a particular site and species. All calculations concerning detrending and computing the mean chronology were done using the ARSTAN software (Holmes et al. 1986).

2.5 Isotope analysis

2.5.1 Tree ring cutting and cellulose extraction

The cores or sections of discs chosen for isotope analysis were cut ring by ring. With pine this was done for the whole ring containing early and late wood (papers II and III). With oak, however, earlywood and latewood were separated and only latewood was analyzed for isotopes (paper IV). This is because in deciduous trees the earlywood that is laid down in spring, possibly already before the leaves have emerged, contains isotopic signal from previous year (Robertson et al. 1995, Helle and Schleser 2004). The actual cutting was done under a microscope, using a surgical knife. Once the rings had been cut, samples taken from two directions of the stem were pooled together to average out circumferential variation in trees (Leavitt and Long 1986). For pine chronologies in paper III and for the latter part of the chronology in paper IV, separate trees were also pooled to ensure sample masses large enough to measure all desired isotopes.

Before isotope analysis α-cellulose was extracted from the wood samples to eliminate complications in the record caused by the chemical heterogeneity of the wood. Cellulose extraction in papers II, III and IV was carried out following the method described by Loader et al. (1997). As suggested in Loader et al. (1997), resin extraction was carried out for pine samples (papers II and III). For oak this step is not required and was not done.

The cellulose molecule contains hydrogen atoms that can easily exchange with hydrogen from external sources (atmospheric moisture, etc.). Therefore, before hydrogen isotope analysis these hydrogen atoms must be either replaced with nitro groups by nitration or equilibrated with water of known isotopic composition (Filot et al. 2006, Leuenberger et al. 2009). In paper IV, α-cellulose was nitrated following the method described by Green (1963).

2.5.2 Isotope measurements

All isotope measurements were conducted in Dating Laboratory at the University of Helsinki using continuous-flow isotope ratio mass spectrometry (CF-IRMS). Dried cellulose samples were first weighed into tin capsules for carbon isotope analysis and into silver capsules for oxygen and hydrogen isotope analysis. Oxygen and hydrogen samples were kept in a vacuum oven at 55°C in open capsules to avoid adsorption of humidity, before measurement. The CF-IRMS technique involves on-line combustion, purification and transfer of the sample gas directly to the mass spectrometer in a continuous flow of carrier gas. Samples for carbon isotope ratio measurement were combusted and CO_2 separated in an elemental analyzer (NC 2500) and samples for oxygen and hydrogen analyses were pyrolysed in a high-temperature elemental analyser (Thermofinnigan TC/EA). The gases were then introduced to mass spectrometers via an interface (ConFlo II or III), CO_2 and CO to Delta[plus]Advantage (Thermofinnigan, Bremen, Germany) and H_2 to Delta[plus] XL (Finnigan). The air samples for paper I were collected in the field to exetainers that were flushed with helium gas beforehand to get rid of atmospheric CO_2 in the vials. The gas samples were analyzed in the laboratory with an isotope ratio mass spectrometer Delta[plus] XL.

The obtained results were compared with standard reference gases and samples of known isotopic composition. For cellulose samples always two or more replicates were analyzed and the result calculated as their average. Isotope ratios are expressed using the delta (δ) notation as deviations from the internationally accepted standards, Vienna Pee Dee Belemnite (VPDB) for carbon and Vienna Standard Mean Ocean Water (VSMOW) for oxygen and hydrogen (Eq. 1)
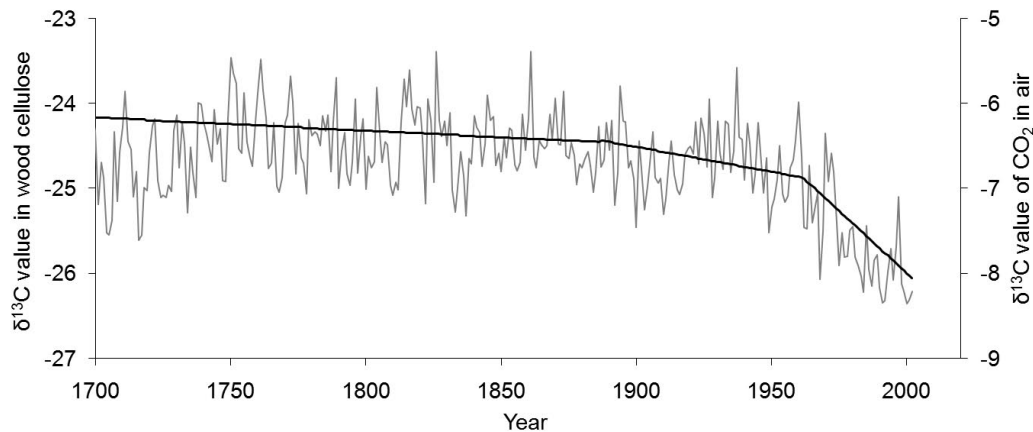
Figure 4 Change in the $\delta^{13}C$ of atmospheric CO_2 according to Leuenberger (2007) and annual $\delta^{13}C$ value of tree ring cellulose from Kessi.

2.6   Corrections to the isotope chronology

In principle there is no need to detrend the isotope series similar to ring width series, since long lasting age related trends have not been discovered (Gagen et al. 2008). However, isotopes might exhibit several other trends that complicate the analysis of climatic and other environmental controls on annual variation.

Carbon isotopes ratios in tree rings have been shown to exhibit so called "juvenile effect" (Craig, 1954b), a tendency for $\delta^{13}C$ to increase in the first decades of a tree's life. Gagen et al. (2007) quantified this period to be fifty years for Scots pine in Northern Finland; however the length of this period may differ according to species and site characteristics (Esper et al. 2010). Several hypotheses have been presented to explain the phenomenon. Shift in source air isotopic composition due to the soil respiration may affect trees growing under canopy (Buchmann et al. 1997). Shading of crown on leaves changes as the tree grows (Francey and Farquhar 1982). Furthermore, water transport to the leaves may be less efficient as trees age or grow larger (McDowell et al. 2002). To eliminate the effect of "juvenile effect" on long chronologies many studies discard the first few decades of growth rings before isotopic analysis. In paper III the juvenile trend has been removed by statistical detrending.

Significant decrease in $\delta^{13}C$ value of atmospheric CO_2 has occurred after 1850 that is caused by changes in land use and increased fossil fuel burning releasing depleted CO_2 to the atmosphere (Epstein and Krishnamurthy 1990, Joos et al. 1999) (Figure 4). The change in isotopic composition of atmospheric CO_2 is reflected in carbon isotope series measured from tree rings. This trend is removed from time series before further analysis using a correction curve that is based on instrumental measurements of atmospheric CO_2 and isotopic composition of CO_2 in ice cores. In papers III and IV correction curve of Leuenberger (2007) was used, however also other correction curves, giving almost identical results, have been suggested and used elsewhere (Saurer et al. 1997, McCarroll and Loader 2004).

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Also the rise in air CO_2 concentration is likely to change isotope ratios in trees by changing their water balance (Berninger et al. 2000). In theory this affects mainly carbon isotopes, but also to minor extent water isotopes via changes in stomatal regulation. However, it has been discovered that different tree species in different environments respond to the changing CO_2 concentration in different ways (McCarroll et al. 2009). There is no simple solution available to resolve this problem, although some corrections have been suggested to compensate for the change in carbon isotope time series (Treydte et al. 2001, McCarroll et al. 2009). In papers III and IV possible trends caused by increased atmospheric CO_2 concentration are not directly addressed.

2.7 Dendroclimatological analysis and climate reconstruction

Unfortunately, owing to the number of variables and complexity of the interactions, the mechanistic models of isotopic fractionation are not capable to run outside the period of instrumental weather observations. Palaeoclimatic reconstructions have thus been restricted to environments where dominance by an individual environmental factor can be assured. The reconstructions are done using statistical techniques adopted from traditional dendroclimatology. Knowledge on mechanisms affecting isotopic fractionation in a tree can, however, help to interpret the isotope signal and to select the climate variable against which to test the isotope time series.

Before the climate reconstruction can be done the climatic signal in isotope time series must first be evaluated. This involves studying climatic response using correlation between a tree ring isotope record and instrumentally recorded climate variable or related techniques. In paper III a response function obtained by Dendroclim (Biondi and Waikul 2004) was used. In paper IV time series were tested against monthly climate data using Pearson correlation coefficients. After studying the climate signal the conducted statistical tests and theoretical constrains are used to guide to choose the appropriate climate variable to reconstruct. The proxy series is then calibrated against instrumental measurements of this climate variable using linear regression after which the empirical relationship is validated. In paper III this is done by withholding half of the instrumental record during calibration and using the regression coefficients to reconstruct the climate variable in the remaining period and comparing the result with the instrumental record (Figure 5). The skill to reconstruct is then assessed with reduction of error (RE), coefficient of efficiency (CE) and mean square error (MSE) statistics (Fritts 1990). The reduction of error statistic compares the skill of the reconstruction with that obtained by using the mean value of the calibration period for every year. This method is useful since it also checks whether a proxy is able to follow the lower frequency changes in climate between the calibration and verification period. For the final reconstruction regression algorithm or the so called transfer function is calculated using the whole instrumental record. This methodology requires two assumptions: linearity of the

relationship between proxy and climate and stationarity of the signal throughout the studied period.
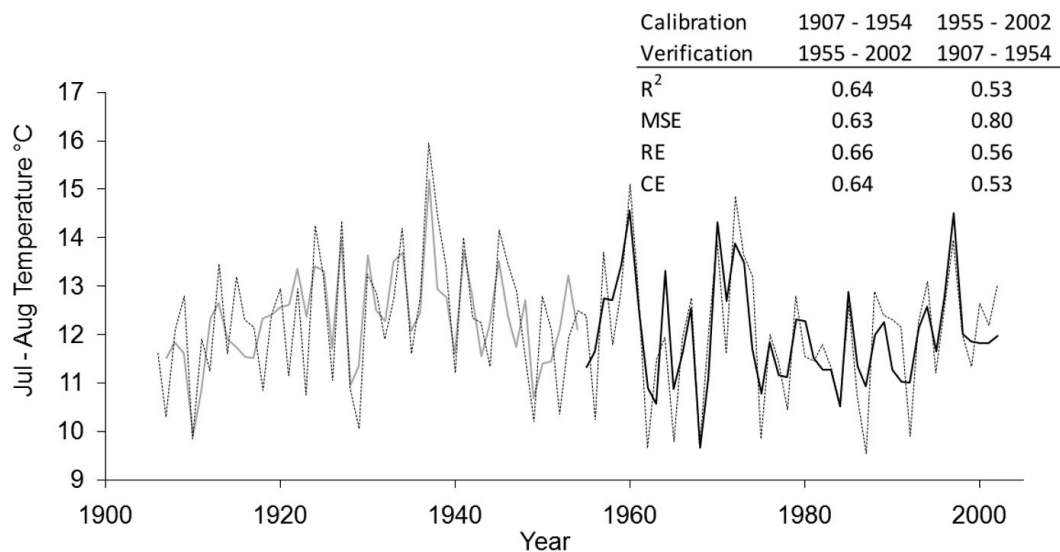

Calibration	1907 - 1954	1955 - 2002
Verification	1955 - 2002	1907 - 1954
R^2	0.64	0.53
MSE	0.63	0.80
RE	0.66	0.56
CE	0.64	0.53

Figure 5 Verification of Northern Finland July–August temperature reconstruction (paper III) and calibration and verification statistics. Black line represents reconstruction created for 1955 – 2002 based on calibration in 1907 – 1954. Gray line represents reconstruction created for 1907 – 1954 based on calibration in 1955 – 2002. Dotted line is instrumental temperature measurements from Inari.

2.8   Other statistical analyses

In paper II we analyzed changes in the growth rates of each tree choosing the pre-treatment growth levels as the reference. To analyze branch extension we used a nonlinear mixed effect model (with treatments as fixed, and trees as random effects). All statistical analyses were performed using R2.5.1 statistical software package (R Development Core Team 2007) and the STAT, NLME (Pinheiro et al. 2007) and multicomp libraries (Hothorn et al. 2008). Analysis of variance was used to test differences between treatments and Dunnett's test was used to test the differences between the treatments and the control. In paper III Pearson correlation coefficients and root mean square error (RMSE) was used to study the relationships between proxies. Changes through time in the relationships were examined by dividing the study period into shorter periods and comparing the r and RMSE values among these periods. In paper IV the relationship between different tree ring isotope records and ring width were compared using Pearson correlation coefficients and principal component analysis (PCA) calculated using program R (version 2.2.1), library Vegan (Oksanen et al. 2010).

2.9   Environmental data

The environmental data used in the modelling experiment (paper I): CO_2 concentration, water vapour, air temperature and PAR (photosynthetically active radiation) were measured on site with an automated system connected to the gas exchange chamber. The daily weather data used for the interpretations of paper II was obtained from the Värriö research station. Monthly weather data used to study the climate signal in tree rings and for calibrating the reconstruction in paper III were obtained from Finnish Meteorological Institute. The weather stations close to the sites were used (Inari and Tohmajärvi for Kessi and Sivakkovaara respectively). However, since it is important to obtain as long and as continues records as possible for the analysis of the climate signal, missing values in weather series were estimated based on data from nearby weather stations using linear interpolation. For paper IV weather data from nearby Salo weather station, provided by the Finnish Meteorological Institute, was used, although this was not the closest one to the site. The weather station was chosen since the record was considerably long and also daily temperatures, including daily minimum and maximum temperatures, daily precipitation sum, cloud cover and relative humidity were available.

3 Results and discussion

The physical, chemical and biological processes that determine the isotopic signal in trees are affected by changing environmental conditions. At the leaf-level the newly formed photosynthates are labelled by the short-term changes in these processes. The photosynthates are then transported through different carbon pools in a tree and during this the isotopic signal gets "dampened" before it reaches the forming tree ring (Ogee et al. 2009). On annual scale the isotope composition of a growth ring is affected by seasonal carbon allocation dynamics and the timing of the photosynthetic production. For longer temporal periods the complexity of the environmental interactions further increases. The environment surrounding the tree may change in time, changing the available resources to the tree affecting the fractionating processes. In scale of centuries and decades forest growth dynamics and human induced changes in the environment must be taken into account. Further, in different climate regimes and in geographic locations the significance of the different fractionating processes in determining the isotope signal may differ. Depending on these factors and time scales, how the isotope signal in tree ring represents variations in the average weather conditions varies.

In this chapter the results and their interpretations are presented starting from the principle mechanisms of instantaneous carbon isotope fractionation and continued to longer temporal scales and comparisons of the isotopic signals between different sites and species. At the end it is discussed how these studies can contribute to the current palaeoclimate research.

3.1 The controls of carbon isotope discrimination on short term

Carbon isotope discrimination in a plant leaf varies according to the balance between the supply of CO_2 by diffusion to the chloroplast and the demand of CO_2 in the chloroplast and is thus related directly to CO_2 concentrations on chloroplast. CO_2 diffuses into the leaf mainly via stomata. Stomata has two functions, they control the entry of CO_2 into leaves and the exit of water vapour from them. Plants control stomatal conductance by adjusting aperture of the stomatal pore by two guard cells surrounding a pore. The degree of stomatal opening is often described to respond to water vapour concentration difference between air and leaf, to CO_2 concentration within the leaf (Ball et al. 1987, Leuning 1995) and to be effected by soil water content during drought (Duursma et al. 2008). The demand of CO_2 in the chlorophyll depends in short term on light intensity and in longer term on the efficiency of photochemistry that may vary over season (Kolari et al. 2007). Since environmental conditions change throughout a day and a growing season, the balance between processes controlling the CO_2 assimilation in the leaf changes and consequently changes the observed isotope discrimination. The instantaneous photosynthetic discrimination (Δ) measured from a pine shoot (paper I) in Hyytiälä during one day varied between 14.7‰ and 28.7‰. The range is similar to those reported in other

studies despite of the ecosystem or species (Harwood et al. 1998, Wingate et al. 2007, Bickford et al. 2009). High discrimination values occurred in dawn and dusk when also respiration contributed to the result and lowest values occurred in the middle of the day in full sunlight when the rate of photosynthesis was high.

Variation in isotopic discrimination was explained with Opitimal stomatal control model (OSM) that uses environmental variables to calculate stomatal conductance and photosynthesis (Hari et al. 2008). After introducing the isotopes to the photosynthesis model we discovered that the model version that does not take into account leaf internal conductance to CO_2, was not able to explain the full range of measured discrimination. This result suggests that the pine leaves have low internal conductance to CO_2 movement through the mesophyll cells and, as a result, the CO_2 concentration at the site of carboxylation becomes significantly lower than in the internal airspaces in the leaf (von Caemmerer and Evans 1991). The CO_2 movement in the mesophyll contains several processes. To enter the mesophyll CO_2 molecules first diffuse through intercellular airspaces and then dissolve in the aqueous layer at the mesophyll cell surface and diffuse to the chloroplast. Although the results indicate that including the mesophyll conductance in the model is important, we were able to determine the value of this parameter only to a certain range. That is because there are numerous factors affecting the estimation of the parameter. It is dependent on the estimation of several other parameters which can together affect the value up to some tens of percent (paper I). These are especially the fractionation factor for carboxylation and to a lesser extent discrimination in respiration. To better estimate these parameters, larger temporal coverage of measurements from different environmental conditions would be required. Further, the study was conducted on one shoot without spatial replication. Thus it must be noted before generalizing the result, that fractionating processes can vary in a tree from shoot to shoot in a vertical gradient due to functional differences in leaves and differences in e.g. light availability (e.g. Duursma and Marshall 2006). Also, while our approach assumes that the mesophyll conductance is constant throughout the day, it has been shown that it can vary over different time scales (Niinemets et al. 2009). Due to short temporal coverage of measurements in our experiment, we were not able to determine the possible environmentally dependent variations in this parameter.

Environmental controls that most strongly affect instantaneous photosynthetic carbon isotope discrimination are solar irradiance and water vapour deficit in air. Temperature is expected to increase the diffusion of CO_2 in air and in cytoplasm, increase the rate of biochemical reactions and to slow down the process of CO_2 dissolving in the water film on the mesophyll cell walls. However, instantaneous temperature response of CO_2 assimilation in Scots pine has been found to be relatively weak (Aalto 1998, Kolari et al. 2009). Also, from the photosynthesis model that was used here the short term temperature response of biochemical reactions was omitted due to the weak temperature response and to keep the model relatively simple (e.g. Hari and Mäkelä 2003, Mäkelä et al. 2004). Temperature may, however, affect the overall discrimination in the model by increasing respiration. Tests of the model with environmental data from different days showed that the

predicted pattern of photosynthetic discrimation during a cloudier day was closely associated with measured PAR (photosynthetically active radiation) values. On a sunny day, when vapour pressure deficit was high, carbon discrimination stayed relatively stable once it had reached a certain level. Yet, typically short-term variations in light, temperature and water vapour deficit in the air are strongly intercorrelated and differentiating between their effects is difficult in field studies.

3.2 Influence of local environmental conditions on the isotopic signals in pine tree ring

There are several factors that potentially influence the isotopic signature in tree rings. Which of these factors dominate, is determined by the environmental conditions in which the tree grows: the species in question, climatic regime and local conditions. In paper III the isotopic composition in pine tree rings and response of carbon and oxygen isotope ratios and ring width to temperature and precipitation on inter-annual scale in the 20th century were investigated at two sites in Finland.

The two carbon chronologies measured in paper III showed a constant offset of approximately 1.2‰ between sites in northern Finland ($\delta^{13}C$ in average -24.78‰) and in central eastern Finland ($\delta^{13}C$ in average -23.60‰). This observation is in line with several studies that have observed a consistent decrease in $\delta^{13}C$ with increasing altitude or latitude (Körner et al. 1991). For $\delta^{13}C$, this decrease with latitude cannot be generally ascribed to a single factor but is likely a response to many (Warren et al. 2001) since various inter-related climatic (temperature, precipitation) and other (e.g. leaf morphology, nutrient availability) factors vary with this environmental gradient. The average level of $\delta^{18}O$ in the north, was observed to be 26.58‰ and in eastern Finland 27.30‰, thus showing a difference of 0.72‰. Similar difference has been found in a study that measured isotopic composition of shallow groundwaters in Finland (Kortelainen and Karhu 2004). In contrast to carbon, geospatial differences in stable water isotope ratios ($^2H/^1H$ and $^{18}O/^{16}O$) can directly be linked to variation in source water i.e. precipitation. The precipitating rainwater tends to become more depleted in $\delta^{18}O$ and δ^2H with increasing latitude due to partitioning of isotopes in vapour and precipitation. As moisture-laden air masses move over land, water condenses from them and the isotope composition of the remaining vapour within air mass becomes more depleted in ^{18}O and ^2H depending on the distance the air mass has travelled (e.g. Rozanski et al. 1981).

In northern Finland the inter-annual variation in $\delta^{13}C$ was observed to strongly respond to mean July and August temperature variations and not to respond to the amount of precipitation (Table 1). At the more southern site the response of $\delta^{13}C$ to summer temperature was slightly weaker than in northern Finland and in addition $\delta^{13}C$ responded negatively to July precipitation. In northern Finland current spring and summer temperatures were observed to have positive effect on $\delta^{18}O$, although from the individual months, only in July the response was statistically significant. Moreover $\delta^{18}O$ did not significantly respond to the amount of precipitation. On the contrary, in central eastern Finland current year July precipitation had significant

negative effect on $\delta^{18}O$. However, significant temperature response was observed only for October in previous year. On the whole, $\delta^{18}O$ measured from pine tree rings did not show as strong a response to climatic variables as carbon. Tree ring width in northern Finland was found to reflect annual variation in July temperatures. Also a weak response to variations in May precipitation was observed. Ring width in central Eastern Finland did not respond significantly to temperature or current-year precipitation.

Trees in which $\delta^{13}C$ values correlate positively with temperature are usually found in cool and moist environments typically at high altitudes or latitudes (Sidorova 2008, Tardif et al 2008). Whereas trees with $\delta^{13}C$ signal sensitive to the amount of precipitation (negative correlation between $\delta^{13}C$ and precipitation), are usually found in dry environments and on well drained soils (Gagen et al. 2004, Kagawa et al. 2003, Saurer et al. 1997, Warren et al. 2001). A strong correlation with temperatures, similar to that in our study, was also observed by McCarroll et al. (2003) and Gagen et al. (2007) who also studied the $\delta^{13}C$ signal in pine in northern Finland. They explained the observed correlation to be indirect and caused by dominating effect of photosynthetic rate over stomatal conductance on controlling carbon isotope ratios. This might seem controversial if the interpretation is based on the simple model of isotope discrimination that relates discrimination to C_i/C_a (Eq. 3), since most stomatal conductance models assume that C_i is a function of VPD and independent of the rate of photosynthesis. As mentioned earlier, rather than being dependent on C_i, carbon isotope discrimination is dependent on chloroplast CO_2 concentration (C_c). A possible explanation for why this correlation is so strong is the drawdown of CO_2 from substomatal cavity to the chloroplast caused by low mesophyll conductance. If mesophyll conductance is assumed to be nearly constant, variation in C_c does occur when assimilation rate varies for example with irradiance or photosynthetic capacity that co-varies with temperature, even with constant C_i, (von Caemmerer and Evans 1991, Hanba et al. 2003, Duursma and Marshall 2006). Compared to northern Finland a slightly different climate response pattern is seen for $\delta^{13}C$ in central eastern Finland. Significant response to precipitation suggests that at the more southern site stomata is more closed in the long run or more often limiting the CO_2 supply to the leaf than in the north. Following this the $\delta^{13}C$ signal in Eastern Finland is not strictly dominated by either stomatal control or photosynthetic rate.

A strong correlation between July temperatures and ring radial growth has been reported in many previous studies (e.g. Helama et al. 2004). Also several studies have shown that the influence of midsummer temperatures in the north gradually change into more governing impact of early summer rainfall in the south (Henttonen 1984, Lindholm et al. 2000, Helama et al. 2005). In paper III, in northern Finland ring width was observed to be strongly correlated with $\delta^{13}C$ during the 20th century (r = 0.59, p < 0.001) (Table 1.). In Eastern Finland correlation between $\delta^{13}C$ and ring width during last century was not statistically significant. Positive relationship between ring width and $\delta^{13}C$ is usually found in humid environments where $\delta^{13}C$ is controlled by photosynthetic rate (Kagawa et al. 2003, Kirdyanov et al. 2008, Tardif et al. 2008). This relationship shifts to negative when approaching to the other extreme, dry environments (Saurer et al. 1997, Leavitt and Long 1988). The

relationship can be explained if variations in radial growth are considered to be determined by the amount of photosynthetic production and compared with changes in δ^{13}C. In humid environment increasing photosynthetic rate increases photosynthetic production and δ^{13}C values. On the contrary, in dry environments, increasing stomatal control during warm and dry summers limits photosynthetic production and simultaneously increases δ^{13}C values.

Local conditions also potentially influence the climatic correlation of oxygen isotopic signal. Since stomatal conductance is affected by soil water availability and air humidity, these environmental controls will affect δ^{18}O through evaporative enrichment in leaf. Similar to carbon, the importance of precipitation signal for δ^{18}O seemed to be higher for the trees in the more southern site. This probably reflects the increased role of stomatal regulation in controlling evaporation in leaf. In addition water isotope signal may be affected by soil properties. Trees using well-mixed ground water, having a relatively long residence time, are expected to incorporate water isotopic signals representative of mean precipitation over several months or even years (Waterhouse et al. 2002). In contrast, trees on well-drained soils that are characterized by shorter soil water residence times may incorporate short term seasonal fluctuations in isotopic composition of precipitation or reflect changing seasonal proportions of rainfall amount. Due to cool and humid environment and flat topography, soil water residence time is expected to be rather long in the north, explaining the wide window of the temperature response starting earlier in spring than the ring growth starts. Additionally the soil water is possibly affected by recharge during autumn and winter, from which the October temperature signal in Eastern Finland might be an indication of.

3.3 Environmental controls on δ^{13}C, δ^{18}O and δ^2H in oak tree rings

Besides local conditions annual isotope signals and their environmental controls are also dependent on the species in question. Differences in isotope ratios and environmental signals are to be expected due to differences in e.g. water transport, hydraulic conductivity, rooting depth, position in canopy and leaf morphology (Leavitt 2002). Therefore how different species can be utilized as a climate indicator varies. Oak was selected since it is long lived and since in Finland it is growing on the border of its northernmost distribution range. Typically at the edges of the distribution range, one or two climatic factors become limiting to growth to the extent that they override other growth controlling factors (Fritts 1976). Such trees can potentially contain stronger and more direct climate signals than trees elsewhere (Schleser et al. 1999).

In paper IV three isotope ratios and early and latewood ring widths were measured for the last one hundred years from oak in Southern Finland. Correlation analysis between isotope and ring width time series and as well as principal component (PC) analysis indicated high coherence between δ^{13}C and δ^{18}O time series (r = 0.79 P < 0.001) (Table 1). δ^2H slightly differed from these series but still

correlated significantly with $\delta^{13}C$ and $\delta^{18}O$ (r = 0.43 P < 0.001, r = 0.30 P < 0.01, respectively). However, ring width series, measured from latewood or the total ring width did not show any similarity with $\delta^{13}C$ or $\delta^{18}O$ series. $\delta^{13}C$ and $\delta^{18}O$ chronologies correlated significantly with all studied climate variables, temperature, precipitation, cloud cover and relative humidity (Table 1). For summer temperature correlation values were positive and for cloud cover and precipitation negative. The strongest relationship (r = 0.70, P < 0.001) was observed between $\delta^{18}O$ and cloud cover, yet, r values for $\delta^{18}O$ and temperature, $\delta^{18}O$ and precipitation, $\delta^{13}C$ and precipitation and $\delta^{13}C$ and cloud cover all exceeded 0.5 and were statistically highly significant. However, obtained expressed population signal (EPS) values indicated that the common signal between trees forming the chronology was for $\delta^{13}C$ less than commonly accepted threshold of 0.85. Thus $\delta^{13}C$ chronology should be enhanced by adding trees to the chronology. With $\delta^{2}H$, the r values were positive for temperature and negative for precipitation and cloud cover. However, the relationship was weaker compared to $\delta^{13}C$ and $\delta^{18}O$. Additionally, the period that correlated the most strongly occurred slightly earlier for $\delta^{2}H$ than for $\delta^{13}C$ and $\delta^{18}O$. Ring width chronologies, total ring width and latewood ring width, correlated positively with the amount of precipitation and relative humidity but not with temperature.

Carbon isotope composition is determined by the rate of CO_2 diffusion into leaf and photosynthetic rate, whereas oxygen isotope ratios are less affected by the photosynthetic rate, but more by transpiration effect. Parallel variations in $\delta^{13}C$ and $\delta^{18}O$ thus indicate that the variations in $\delta^{13}C$ on annual scale are mainly related to stomatal regulation of CO_2 diffusion. This interpretation is further supported by the correlation between ring width and the amount of precipitation. This indicates that carbon assimilation is affected by moisture conditions. Positive influence of summer precipitation, as the most important climatic factor limiting the annual variation in radial growth, have been demonstrated in several studies on oaks growing close to their northern distribution limit, in Finland (Robertson et al. 1997, Helama et al. 2009) as well as in Sweden (Drobyshev et al. 2008). In fact, the climate signals in oak tree ring and isotope chronologies are not that different from those reported elsewhere in northern and central Europe (Siwecki and Ufnalski 1998, Kelly et al. 2002, Etien et al. 2008, Friedrichs et al. 2009). Our results suggest that, at current climate, moisture is important for the annual growth of oak at its northern distribution limit. Yet, the finding does not rule out that oak might be sensitive to sudden cold spells during the growing season or that it might suffer from low temperatures during cold winters leading to reductions in growth occasionally, and that these phenomena might limit the species from spreading further north. However, these phenomena are evidently not regulating the growth of oak on annual basis.

Although carbon and oxygen fractionating processes are fairly well known, understanding on hydrogen isotope fractionation in trees needs to be increased, before using $\delta^{2}H$ in climate reconstructions. $\delta^{2}H$, although known to have common mechanisms with $\delta^{18}O$ affecting the isotope signal, did not correlate strongly with $\delta^{18}O$. Still, we know that inter-tree variability of $\delta^{2}H$ is small (paper IV), and so it looks promising that there could be a common control for the variations in $\delta^{2}H$. This control, however, may very well vary between environments and species. A possible

next step could be to separate climatically controlled source water signal from evidently complex tree physiological signals on δ^2H. This could be achieved by measuring position specific variation in deuterium in carbohydrate molecules (Augusti et al. 2008).

Table 1 Observed relationships between proxies and current summer climate variables, temperature (T) and precipitation (P), during the period of instrumental weather observations. Strong correlation is defined as r > 0.5 and p < 0.001.

	Scots pine		Pedunculate oak
	Northern Finland	**Central-eastern Finland**	**Southern Finland**
Ring width	Strongly correlated with T	Not correlated with T or P	Correlated with P
$\delta^{13}C$	Strongly correlated with T	Strongly correlated with T and correlated with P	Strongly correlated with P and correlated with T
$\delta^{18}O$	Correlated with T	Correlated with P	Strongly correlated with P and T
δ^2H	-	-	Correlated with P and T
$\delta^{13}C$ and $\delta^{18}O$	Correlated	Correlated	Strongly correlated
$\delta^{13}C$ and Ring width	Strongly correlated	Not correlated	Not correlated

3.4 Responses of $\delta^{13}C$ to resource availability

On annual to decadal time scales tree growth and isotopic composition of the produced biomass can potentially be affected by variety of external factors that induce physiological changes in trees and affect their water relations. In paper II tree line trees on the top of Värriö I fell were studied for their responses to resource manipulation. We hypothesized that if the trees are treated by removing a needle cohort, $\delta^{13}C$ would decrease, because the trees then can afford to transpire more per unit leaf area and keep their stomata more open thus enabling more efficient CO_2 diffusion into the leaf. In addition $\delta^{13}C$ was hypothesized to increase in response to nitrogen fertilization. That is because nitrogen can enhance photosynthetic rate by increasing the Rubisco regeneration rate (Ripullone et al. 2004). The results, however, revealed no statistically significant differences in $\delta^{13}C$ between the control group and any of the treated groups. There were no differences in the case of nitrogen fertilization either, regardless of the fact, that the fertilized trees increased their leaf nitrogen concentration suggesting that the trees had increased photosynthetic efficiency. A statistically significant change would have been c. 0.81‰ and the observed differences between the treatments and the control were less than 0.45‰, though the direction of the change was as hypothesized. However, changes in growth were observed as a result of both treatments. Fertilized trees

increased height and branch extension, but not radial growth. On the other hand, defoliated trees decreased their radial growth.

Based on this and other studies it seems that trees are able to keep their leaf internal CO_2 concentrations rather constant over different conditions and despite of changes in resources: water, carbon or nitrogen availability. Several studies on defoliation caused by outbreaks of phytophagous insects, have reported unchanged $\delta^{13}C$ values despite of significantly reduced radial growth on current and often on several subsequent years (Kress et al. 2009, Ellsworth et al. 1994, Haavik et al. 2008). Some studies, on the contrary to our hypothesis, have observed increased $\delta^{13}C$ values in the early stages of defoliation (Simard et al. 2008). This has been assumed to be caused by increased photosynthetic rate, as a compensatory mechanism for defoliation. Lavigne et al. (2001) suggested that this is a result from an increased allocation of nutrients to the foliage and a result from increased chlorophyll concentrations. It has also been hypothesized that during less severe defoliation defoliated canopy could increase light availability and thus increase photosynthetic rate and $\delta^{13}C$ (Simard et al. 2008).

Also nitrogen fertilization studies have mainly observed minor to modest increases in $\delta^{13}C$ due to fertilization (Clearwater and Meinzer 2001, Högberg et al. 1993, Betson et al. 2007), moreover, the increase has not always been interpreted to be caused by increased photosynthetic rate. Brooks and Coulombe (2009) studied tree ring isotopes and ring growth in a fertilization experiment and observed a short lived increase in $\delta^{13}C$ and slightly longer increase in $\delta^{18}O$. Considering the evidence from both of these isotopes, they concluded that the changes in water use and increase in growth were probably more related to increases in leaf area following the fertilization, and less to changes in photosynthetic rate. Also the responses of $\delta^{13}C$ and $\delta^{18}O$ were significantly shorter in duration than increases in radial growth observed as a response to fertilization.

In our experiment, as well, the hypothesized change in internal CO_2 concentrations might have been compensated or modified by other processes. Differences in growth responses due to different resource manipulation treatments indicate changes in allocation. The changes in allocation suggest that the trees may actively change their architecture to maintain or restore their functional balance and thus keep their leaf internal CO_2 concentration and consequently $\delta^{13}C$ relatively stable over long run.

3.5   Decadal scale and century scale fluctuations

It is generally assumed that isotopic time series from tree rings contain climatic information in all temporal frequencies so that long-term variability in time series faithfully records low frequency variations in climate (Gagen et al. 2007). However, due to short temporal coverage of instrumental climate observations long term stability of the climate signal in isotope records is difficult to test. Long 400 year chronologies from which two isotopes and ring width were analyzed from exactly the same ring provides an opportunity to investigate the temporal stability of isotope-

isotope and isotope-ring width relationships that may give an insight into the problem (paper III). The correlation and RMSE analyses revealed time dependent shifts in correlation strength. In Northern Finland despite of the similar response to climatic variables and strong correlation between ring width and isotope time series during the 20th century the relationship between time series periodically diverged on annual and decadal frequency during the earlier centuries. Particularly low correlations between proxies were seen in late 18th and early 19th centuries. In Eastern Finland similar fluctuation were observed between time series, though this was less unexpected because the correlation between the records were lower in the instrumental period, to begin with. Also in Eastern Finland trees are expected to contain more non-climatic disturbance in the radial growth than trees close to their distributional limits. Similar changes between isotopic time series have been observed in other studies comparing long chronologies (Reynolds-Henne et al. 2007, Haupt et al. 2010, Gagen et al. 2007, Rinne et al. 2010), and several explanations have been proposed.

Fluctuations in the relationship between two proxy indicators occur if they are controlled by different set of climatic variables and the relationship between these climatic variables changes. In this case the observed change would be directly related to changes in climate or climate regimes. Young et al. (2010) studied pines at the Norwegian coast and explained the divergence in $\delta^{13}C$ and tree ring maximum density to result from decoupling of temperature and radiation signals. Tree ring maximum density was interpreted as temperature and $\delta^{13}C$ was interpreted as near surface radiation. Decoupling of temperatures in early and late summer may also cause divergence between e.g. ring growth and isotopic composition, if they respond to temperatures in slightly different part of summer. Also the length and the timing of the part of the summer that is important to ring growth or isotopic composition may vary, for example, due to increasing spring temperatures (Helama et al. 2004, Aykroyd et al. 2001, paper III). Oxygen isotope signal in tree rings is to a large extent dependent on the isotopic composition of source water. The isotopic composition of precipitation, although locally affected by condensation temperatures of rainfall, may change as a result of large-scale synoptic circulation patterns (Reynolds-Henne et al. 2007, Edwards et al. 2008). Also as rainfall amounts or monthly distribution of rainfall amounts change, it may change the soil water isotopic composition and thus the source water incorporated in oxygen isotope signal in tree rings.

Temporal change in the relationship between time series can also be observed due to differences in characteristics between time series. Tree ring indices have been observed to be substantially more autocorrelated than isotope time series (papers III, IV). In all of the three sites where long chronologies were analyzed tree ring width showed higher autocorrelation compared to isotopes, meaning that the previous year's growth has stronger effect on ring with than on isotopic composition. Although autocorrelation is usually removed by mathematical modeling, its effect cannot be entirely excluded. As was mentioned earlier, reductions (e.g. needle damage) or increases (e.g. increased light) in tree resources might induce long

lasting changes in tree radial growth, whereas the isotope signal is usually rapidly stabilized (e.g. Saurer et al. 1997, Brooks and Coulombe 2009).

Large human induced changes in the environment such as, increased atmospheric CO_2 concentrations, and increased concentrations of several pollutants have been shown to cause trends in isotopic or tree ring width time series that cannot be explained by a trend in any climate factor. Tree ring growth has been shown to increase due to fertilizing effect of human induced nitrogen deposition (Magnani et al. 2007, Savva and Berninger 2010). Also growth has potentially increased due to fertilizing effect of CO_2, especially if it has occurred with increasing nitrogen availability (Hättenschwiler et al. 1996, Medlyn et al. 1999, Knapp and Soule 2001). On the other hand, decreases in growth have been related to sulphur depositions (Savva and Berninger 2010) and also suggested to be caused by falling ozone concentrations in the stratosphere (Briffa et al. 2004). Similarly combined physiological responses (stomatal conductance, carboxylation, respiration, water uptake) to pollution have been observed to create trends especially in $\delta^{13}C$ but also to some extent in $\delta^{18}O$ in tree rings (Wagner and Wagner 2006, Savard 2010, Rinne et al. 2010). Recent studies have also demonstrated that in addition to possible long term trends, short-term responses of tree growth or isotope ratios to climate factors may be modified by the pollutants (Berninger et al. 2004, Savva and Berninger 2010, Wyckoff and Bowers 2010). This may have resulted in increased or decreased correlation with climate variables during last century compared to previous centuries.

3.6 Potential of tree ring isotopes as climate proxy

The ultimate motivation for measuring long tree ring chronologies for their isotopic composition is to use them as indirect sources of climatic information. In paper III, three climatic reconstructions were derived. In Northern Finland both $\delta^{13}C$ and tree ring width were found to be highly sensitive proxies of summer temperature variability and thus both could be used to create climate reconstruction independently. In such cases where the primary environmental controls of two proxies are similar, they might be combined to enhance the signal of the common control. In paper III, combining spline-detrended $\delta^{13}C$ and tree ring width indices from residual chronology in a multiple linear regression to reconstruct July–August temperatures, improved the estimates of summer temperature in the calibration period. Also $\delta^{18}O$ was tested in the model but was omitted, since it did not increase the strength of the correlation between proxy series and instrumental measurements or the fit of the reconstruction. The final reconstruction accounted for 59% of the observed variance in temperature in the instrumental period from 1907 to 2002.

In Eastern Finland, from the studied potential proxy indicators only $\delta^{13}C$ was sensitive to current summer temperature variability (paper III). Simple linear regression was then used to create July-August temperature reconstruction independently from spline-detrended $\delta^{13}C$. The reconstruction described 33% of the variance in temperature, and the verification statistics suggested that the model is

applicable. Since $\delta^{18}O$ responded significantly to summer precipitation in Eastern Finland it was used to derive July-August precipitation reconstruction. The final reconstruction explained only 21% of the variance and the calibration and verification indicated that the created model has some reconstruction skill.

In paper IV, oaks were studied in order to verify if they could be used as a source of information on past climate. The results from correlation analysis showed that, $\delta^{18}O$ is the most promising climate proxy, and that it reflects variations in cloud cover and precipitation. It exhibits slightly higher correlation coefficients than $\delta^{13}C$, although both correlated with the same climatic variables. Also, as a support for $\delta^{18}O$, fewer trees were needed to fulfil the EPS criteria. The climate responses of both carbon and oxygen isotope chronologies were stronger than the climatic response of ring width indices or δ^2H.

Studies presented in this thesis suggest that measuring isotopic composition from tree ring cellulose can provide previously unattained information on past climate. Although the two climate reconstructions created for eastern Finland did not perform as well as the reconstruction in northern Finland, these proxies provide significant improvement to the current literature, since in this region climatic correlations of tree ring width indices are often observed to be poor (Helama et al. 2005). Also it seems possible that the climate signals in tree ring isotope records could be further improved by selecting study sites according to the research question; for example sampling trees from wet soils to obtain temperature signal and from well drained soils to obtain precipitation signal. In northern Finland, where strong temperature proxies are available, $\delta^{13}C$ can be used as a complementary record with ring width or possibly with other tree growth derived proxies (McCarroll et al. 2011) to increase reconstructive power and to result in more reliable climate reconstructions. $\delta^{18}O$ record from northern Finland, that did not show response to year-to-year variations in temperature, were observed to record parallel variation with ring width and $\delta^{13}C$ on decadal scale (paper III, Figure 5A). This suggests that $\delta^{18}O$, although not recording temperature on annual scale contains temperature signal on lower frequencies. It is thus hypothesized that in long chronologies, $\delta^{18}O$ might give insight into long term climatic fluctuations and changes in climatic circulation patterns. In southern Finland, isotopic signal of oak tree ring cellulose seems to bear greater potential for reconstructing climatic variability than tree ring width from the same trees. Thus if long oak chronologies become available, they would make an invaluable source of climatic information.

Isotope ratios in tree ring cellulose showed many advantages as a palaeoclimate proxy. Isotope signals studied were coherent between trees, in papers III and IV only four trees were used to construct chronologies yielding significant climatic signals. In contrast, ring width chronologies that usually contain non-climatic disturbances, need to be collected in larger amounts and from large areas to capture the common climate signal (Gagen et al. 2004). Isotopes could thus be used to deduce local climate reconstructions, to be able to compare regional variations in climate, or to be used for example as background information in an archaeological context, where the amount of wood is restricted. In paper III the detrending technique that was used prohibited the analysis of century scale climatic fluctuations. However, outside

juvenile and anthropogenic periods detrending might not be necessary (Gagen et al. 2008, Esper et al. 2010), and isotopes could possibly be used to reconstruct climatic fluctuations on all frequencies. In the future, more effort needs to be taken to compare and combine tree ring proxies to other proxy types such as peat and lake sediments etc. (e.g. Helama et al. 2010). Perhaps most importantly, constantly improving understanding of the underlying causes of the isotopic variation, and the possibility to analyse multiple isotopes together with physical proxies from the same tree ring, will make more exact interpretations of the tree ring data possible.

4 Conclusions

Stable isotope ratios in trees were studied on different temporal and spatial scales. Carbon isotopes were studied in a gas exchange chamber placed around a Scots pine shoot to define the main mechanisms contributing to formation of the carbon isotope signal in newly produced photosynthates. Carbon isotopic composition of tree ring cellulose was measured from trees that were exposed to manipulation treatments in order to examine the effect of disturbances in resource availability. Annually resolved chronologies of Scots pine tree ring cellulose carbon and oxygen isotope composition at two sites were studied for the strength of climatic relations during last century. Further, four century long climate reconstructions were created utilizing these relationships. Also proxy relationships were studied for temporal stability of the signals. Chronologies of carbon and oxygen isotope ratios in tree ring cellulose and hydrogen isotopes in nitro-cellulose of oak in Southern Finland were studied to investigate their environmental correlations. The main findings and conclusion of this study are summarized as follows:

- Instantaneous carbon isotope discrimination in Scots pine CO_2 exchange was observed to portray diurnal variation which was predicted with a model. Modelling of drawdown of CO_2 from internal airspaces to the chloroplast was required to obtain full range of variability in isotopic discrimination, indicating that mesophyll conductance plays an important role in determining the carbon isotope composition in Scots pine. The value of mesophyll conductance was observed to be of similar magnitude to stomatal conductance.

- Fertilization that was expected to increase photosynthetic rate, or defoliation that was expected to cause decrease of water demand, did not significantly affect carbon isotope composition in tree rings.

- Inter-annual variation in carbon isotope composition in Scots pine tree ring cellulose was strongly related to temperature variations in Northern Finland. In Eastern Finland precipitation had more significant influence on carbon isotopic composition. This was interpreted to indicate that towards the south the role of stomatal conductance increases in limiting CO_2 diffusion into the leaf.

- Oxygen isotope composition in Scots pine tree ring cellulose showed weaker response to climate variations than carbon isotope composition. In northern Finland, the correlation with annual temperature variation was weak, although oxygen isotope ratio was found to follow fluctuations in temperature sensitive tree-ring width and carbon isotope composition on decadal scale. In eastern Finland oxygen isotope ratio responded significantly to summer rainfall amount.

- Carbon and oxygen isotopic compositions in pedunculate oak tree ring cellulose were strongly correlated with climate. Hydrogen isotopic composition in nitrated cellulose was only moderately correlated with climate. Carbon isotope fractionation was interpreted to be mainly controlled by varying stomatal conductance.

- 400 year long temperature reconstruction was derived from carbon isotopes and ring width in northern Finland. Temperature reconstruction was derived from carbon isotopes and precipitation reconstruction was derived from oxygen isotopes in Eastern Finland. Oxygen isotope composition in oak tree rings showed high potential for climate reconstruction in southern Finland.

- Isotopes exhibited higher sensitivity to climate than ring width, and fewer trees were needed to produce a reliable chronology. Isotopes also provide a possibility to apply tree rings as a source of climatic information in wider geographic areas than ring width. In areas where also tree growth based climate proxies are available, isotopic data may be used to strengthen climatic reconstructions.

- Further studies are needed to explore the reasons for temporally changing correlations observed between isotope and ring with proxies.

Acknowledgements

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