Dissertationes Forestales 47

Wood properties of northern forest trees grown under elevated CO_2, O_3 and temperature

Katri Kostiainen

University of Oulu
Department of Biology

Academic dissertation

To be presented, with the permission of the Faculty of Science of the University of Oulu, for public criticism in Kuusamonsali (Auditorium YB210), Linnanmaa, on October 19th 2007, at 12 o'clock noon.

Title of dissertation:
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Author:
Katri Kostiainen

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Supervisors:
Doc. Elina Vapaavuori
Finnish Forest Research Institute, Suonenjoki Research Unit, Finland
Doc. Seija Kaakinen
Finnish Forest Research Institute, Suonenjoki Research Unit, Finland
Doc. Pekka Saranpää
Finnish Forest Research Institute, Vantaa Research Unit, Finland
Prof. Hely Häggman
University of Oulu, Department of Biology, Finland

Pre-examiners:
Prof. Elina Oksanen
University of Joensuu, Faculty of Biosciences, Finland
Dr. Uwe Schmitt
Federal Research Centre for Forestry and Forest Products, Institute for Wood Biology and Wood Protection, Hamburg, Germany

Opponent:
Prof. Kurt Fagerstedt
University of Helsinki, Department of Biological and Environmental Sciences, Finland

ISSN 1795-7389
ISBN 978-951-651-182-8 (PDF)

(2007)

Publishers:
Finnish Society of Forest Science
Finnish Forest Research Institute
Faculty of Agriculture and Forestry of the University of Helsinki
Faculty of Forestry of the University of Joensuu

Editorial Office:
Finnish Society of Forest Science
Unioninkatu 40A, FI-00170 Helsinki, Finland
http://www.metla.fi/dissertationes

Kostiainen, K. 2007. Wood properties of northern forest trees grown under elevated CO_2, O_3 and temperature. Dissertationes Forestales 47. 36 p. Available at http://www.metla.fi/dissertationes/df47.htm

ABSTRACT

The aim of this study was to investigate the effects of climate change on the radial growth, wood structure and chemistry of silver birch (*Betula pendula* Roth), trembling aspen (*Populus tremuloides* Michx.), paper birch (*Betula papyrifera* Marsh.), sugar maple (*Acer saccharum* Marsh.) and Norway spruce (*Picea abies* (L.) Karst.). The materials for this study were obtained from climate change studies carried out in Finland, USA and Sweden.

Elevated CO_2 concentration, O_3 concentration and temperature affected wood properties of the forest trees. In silver birch, elevated CO_2 increased ring width and the concentrations of extractives and starch, while the concentrations of cellulose and gravimetric lignin were decreased. The responses to elevated O_3 depended on the clone: vessel percentage and nitrogen concentration decreased, while cell wall percentage increased in one clone. In vessel percentage, elevated CO_2 ameliorated the O_3-induced decrease.

Responses of wood properties to elevated CO_2 and O_3 differed between 3-year-old and 5-year-old aspen and paper birch. In 3-year-old aspen clones and birch, lignin concentration increased under elevated O_3. However, elevated CO_2 ameliorated the effect, and the lignin response was no longer found with 5-year-old trees. In aspen, elevated CO_2 decreased uronic acids and elicited clone-dependent increases in concentrations of extractives and starch. Elevated O_3 reduced stem radial growth and vessel lumen diameter, while it increased cell wall thickness in aspen. In 5-year-old paper birch, elevated CO_2 increased extractives and decreased starch, while elevated O_3 increased both of them. Three-year-old maple was the least responsive of the tree species to both elevated CO_2 and O_3.

In 40-year-old Norway spruce, elevated CO_2 decreased nitrogen concentration, earlywood cell wall thickness and tracheid lumen diameter, while ring width in non-fertilised trees and latewood tracheid lumen diameter were increased. Elevated temperature decreased the concentrations of extractives and soluble sugars, and increased cell wall thickness and wood density.

The data from these exposure studies shows that wood properties may change as a result of climate change, but the responses to increasing concentrations of CO_2 and O_3 and to increasing temperature may depend on species and the age of trees. Since trees are long-living organisms, further data on long-term exposure studies are still needed before wood characteristics and material properties for different end-use purposes can be predicted for a future climate.

Key words: carbon dioxide, chemical composition of wood, climate change, ozone, ring width, wood anatomy, wood density

ACKNOWLEDGEMENTS

This work was carried out at the Suonenjoki and Vantaa Research Units of the Finnish Forest Research Institute (Metla). The Metla has provided excellent working facilities during the study; especially I wish to acknowledge the head of the Suonenjoki Unit, Doc. Heikki Smolander. The study was financed by the Foundation for Research of Natural Resources in Finland, the Wood Material Science and Engineering Research Programme promoted by the Academy of Finland, the Kone Foundation, the Finnish Society of Forest Science, the Graduate School in Forest Sciences, the University of Oulu, and the Niemi Foundation. I am thankful for the financial support.

I wish to express my deepest gratitude to my supervisors, Doc. Elina Vapaavuori, Doc. Seija Kaakinen, Doc. Pekka Saranpää and Prof. Hely Häggman, for their guidance, comments and encouragement throughout this work. Professor David F. Karnosky (the head of the Aspen FACE experiment) and Prof. Sune Linder (the head of the Flakaliden WTC experiments) are gratefully acknowledged for providing material for this study and for their helpful comments on the manuscripts. I am also grateful to all the other co-authors for their collaboration and contribution during the preparation of the manuscripts. I thank both reviewers, Prof. Elina Oksanen and Dr. Uwe Schmitt, for their constructive criticism on this thesis. Docent John A. Stotesbury is thanked for revising the English language. Special thanks belong to the technical and laboratory staff of the Suonenjoki and Vantaa Units: Mervi Ahonpää, Marja-Leena Jalkanen, Satu Järvinen, Tapio Järvinen, Irmeli Luovula, Tapio Nevalainen, Veera Pevgonen, Hanna Ruhanen, Eeva Vehviläinen and Anna-Maija Väänänen, for their skilful help in tree harvesting, sample preparation and laboratory analyses. I would also like to gratefully acknowledge the personnel of the Aspen FACE site and the Flakaliden field station for their assistance.

Finally, I want to thank my colleagues, friends and family for their support and encouragement during these years.

Suonenjoki, September 2007 Katri Kostiainen

LIST OF ORIGINAL ARTICLES

This thesis is a summary of the following papers, which are referred to in the text by their Roman numerals:

I Kostiainen, K., Jalkanen, H., Kaakinen, S., Saranpää, P. & Vapaavuori, E. 2006. Wood properties of two silver birch clones exposed to elevated CO_2 and O_3. Global Change Biology 12:1230-1240.

II Kaakinen, S., Kostiainen, K., Ek, F., Saranpää, P., Kubiske, M.E., Sober, J., Karnosky, D. F. & Vapaavuori, E. 2004. Stem wood properties of *Populus tremuloides, Betula papyrifera* and *Acer saccharum* saplings after 3 years of treatments to elevated carbon dioxide and ozone. Global Change Biology 10:1513-1525.

III Kostiainen, K., Kaakinen, S., Warsta, E., Kubiske, M.E., Nelson, N.D., Sober, J., Karnosky, D.F., Saranpää, P. & Vapaavuori, E. Wood properties of trembling aspen and paper birch after five years of exposure to elevated CO_2 and O_3. Submitted manuscript.

IV Kostiainen, K., Kaakinen, S., Saranpää, P., Sigurdsson, B.D., Linder, S. & Vapaavuori, E. 2004. Effect of elevated [CO_2] on stem wood properties of mature Norway spruce grown at different soil nutrient availability. Global Change Biology 10:1526-1538.

V Kostiainen, K., Kaakinen, S., Saranpää, P., Sigurdsson, B.D., Lundqvist, S.-O., Linder, S. & Vapaavuori, E. Stem wood properties of mature Norway spruce after three years of continuous exposure to elevated [CO_2] and temperature. Manuscript.

Katri Kostiainen participated in planning the research, was responsible for conducting the laboratory and data analyses, and was the main author of papers I, III, IV and V. Kostiainen participated in the data analysis and writing of article II.

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1 INTRODUCTION

1.1 Climate change and forest trees

Global climate change is predicted to change the growth conditions of forest trees. The global mean surface temperature has increased by 0.6–0.9°C during the past 100 years (1906-2005), and the temperature is estimated to continue rising at a rate of about 0.2°C per decade (IPCC 2007). The main reasons for the warming of the climate are the burning of fossil fuels and changes in land use. The burning of fossil fuels releases carbon dioxide (CO_2), the most abundant greenhouse gas, into the atmosphere. Greenhouse gases contribute to climate change by allowing sunlight to penetrate the atmosphere and heat the planet's surface but also by preventing some of that heat from escaping back into space. Carbon dioxide, methane (CH_4) and tropospheric ozone (O_3) are among the most important greenhouse gases. The concentration of CO_2 has increased by about 35% since the pre-industrial era, and the concentration is estimated to be increasing about 14-19 ppm (parts per million) per decade (IPCC 2007). Tropospheric O_3 is a reactive air pollutant whose concentration has risen by about 35% during the past century (IPCC 2001). Ozone is produced in the troposphere by the photochemical oxidation of air pollutants such as CO, CH_4 and non-methane volatile organic compounds VOCs (NMVOCs) in the presence of NO_x (IPCC 2007). Compared with CO_2 and most other pollutants, tropospheric O_3 concentrations vary considerably, both spatially and temporally (IPCC 2007).

Concurrently increasing concentrations of atmospheric CO_2 and tropospheric O_3, combined with increasing global mean surface temperature, are likely to have impact on forest growth and development in a wide range of biomes (e.g. Skärby et al. 1998, Medlyn et al. 1999, Morison & Lawlor 1999). Increasing CO_2 enhances the photosynthesis and growth of trees (Medlyn et al. 1999, Norby et al. 1999) and concurrently contributes to the increase in the global mean temperature. Elevated CO_2 can also affect tree phenology by causing changes in bud burst or set (Murray et al. 1994, Jach & Ceulemans 1999, Sigurdsson 2001), while in other studies no effects on phenology have been observed (Olszyk et al. 1998, Kilpeläinen et al. 2006, Slaney et al. 2007). Increasing mean temperature is also predicted to enhance the photosynthesis (Saxe et al. 2001, Hyvönen et al. 2007) and productivity of forests (Kellomäki & Kolström 1994, Talkkari 1998), and to alter tree phenology and growth period (Olszyk et al. 1998, Peltola et al. 2002, Slaney et al. 2007). In contrast, tropospheric O_3 is harmful to trees because of its oxidizing nature, and it reduces the growth and productivity of forests (Skärby et al. 1998).

The factors affecting climate change do not occur separately in natural ecosystems, and data on the responses of forest trees to multifactor studies are needed. The interaction between elevated CO_2 and temperature has been hypothesised as having a stimulating effect on plant growth, since temperature-induced photorespiration may be counteracted by an increase in the CO_2 concentration (Long 1991). Increasing CO_2 may also ameliorate the harmful effects of O_3, since elevated CO_2 can reduce stomatal conductance (Medlyn et al. 2001, Long et al. 2004) and thus smaller quantities of O_3 enter into leaves. More uncertainty about the effect of climate change on the function and growth of northern forest ecosystems in the future arise from the fact that these forests are typically nitrogen-

deficient. Soil nutrient deficiency may limit carbon sequestration under elevated CO_2 (Oren et al. 2001, Hungate 2003).

1.2 Wood properties in coniferous and deciduous trees

A thorough understanding of the climate change effects on stem wood properties in a future climate is important since tree stems play an important biological role in water, nutrient and solute transport, as storage organs and in providing physical support. Besides these physiological functions, tree stems have a substantial economic value as a raw material. The prediction of changes in wood properties is challenging because, in addition to growth conditions, the wood structure and chemical composition can vary between genotypes, within a tree, between trees and with the age of a tree.

Softwood of conifers and hardwood of deciduous trees are made up of different cell types and as a result have different structures. Softwood consists of tracheids, which are elongated cells with thick secondary walls and have no living cell contents at maturity. Tracheids provide a route for water conductance and also provide the mechanical support of the trees. Hardwoods separate the functions of conduction and support into two different cell types: vessel elements form long conduits for long-distance water transport, and elongated fibres are modified for support. Within a growth ring the vessels can be distributed throughout the ring in a diffuse-porous arrangement (e.g. *Betula*, *Populus*) or grouped in the earlywood in a ring-porous pattern (e.g. *Quercus*). Wood rays are built up of ray parenchyma cells and in some conifer species (e.g. *Picea*, *Pinus*) also contain ray tracheids (Butterfield 2003).

Northern forest trees produce growth rings that reflect the spring onset and autumn cessation of cambial division and cell differentiation. Wood produced in spring and early summer is referred to as earlywood and that produced late in the growing season is termed latewood. In softwoods, ring boundaries are usually characterised by thick-walled, small-lumened tracheids in the latewood, and thin-walled, large-lumened tracheids in the earlywood. The different properties and proportions of earlywood and latewood tracheids in softwoods have significant effects on both wood quality and tree physiology (Butterfield 2003).

Wood properties also vary within the cell wall. Middle lamella adheres the walls of adjacent cells together and is comprised mostly of lignin in differentiated tissues. The primary wall is normally very thin (0.1-0.2 μm) and remains plastic prior to lignification during the deposition of the secondary cell wall. It is capable of permanent extension during cell expansion, and as a consequence its predominant cellulose microfibril angle is random except at the cell corners. The microfibrils are bound into the matrix complex of hemicelluloses and pectinaceous material. The secondary wall is laid down after the primary wall and usually shows three distinct layers (S1, S2 and S3) with a highly ordered cellulose microfibril orientation. The S2 wall layer is by far the thickest, and its cellulose microfibril angle is a critical factor determining the mechanical properties of wood (Butterfield 2003).

The chemical composition of wood ranges broadly between 40-50% cellulose, 20-30% hemicellulose, 20-35% lignin, and 0-10% extractive components. The variation in the chemical composition is high between different species and can also vary within species as a result of environmental and genetic factors (Pereira et al. 2003). In general, softwoods

have higher lignin content (25-35%) and their hemicelluloses contain galactoglucomannan and arabinoglucuronoxylan, while hardwoods have less lignin (18-30%) and their hemicelluloses contain acetylglucuronoxylan and glucomannan. Hardwood lignin is principally made up of coniferyl and sinapyl alcohols (guaiacyl-syringyl lignin) and softwood lignin of coniferyl alcohol (guaiacyl lignin). Hardwoods usually have more extractives than softwoods, besides having different patterns of extractive composition (Pereira et al. 2003).

Wood properties vary from the pith to the bark and also with tree height. Juvenile wood is produced by young cambium. It therefore forms a continuous cylinder around the pith and occupies a larger proportion in the higher parts of the stem than in the lower part with a larger stem diameter. Juvenile wood occupies around the first 10-15 growth rings (Bonham & Barnett 2001, Butterfield 2003). In hardwoods the chemical composition shows little change from pith to bark and from base to top, while softwoods have wood cores with lower cellulose and higher lignin (Pereira et al. 2003). Compared with mature wood, juvenile wood has short tracheids with thin cell walls and a large microfibril angle (Butterfield 2003).

1.3 Responses of wood properties to elevated CO_2, O_3 and temperature

The responses of wood properties to elevated CO_2 concentration and temperature are presented in Tables 1 and 2. Most information about wood responses to elevated CO_2 and temperature has been derived from climate change studies conducted in greenhouses, in artificially illuminated controlled environment chambers or in open-top chambers in the field (Tables 1 and 2). As a result of size limitations on these systems (Long et al. 2004), most of the studies have focused on the early stages of tree growth (Tables 1 and 2). In the studies, seedlings and trees have been exposed to elevated CO_2 concentrations predicted for approximately the next 50-100 years (420-750 ppm) (Tables 1 and 2). About 20 tree species with ages ranging from 1 to 30 years have been exposed to elevated CO_2 for 1 to 6 years. Experiments with combined exposures are limited to 5 studies, in which the interactive effects of elevated CO_2 concentration and elevated temperature (ambient + 2-6°C) were studied with young beech (*Fagus sylvatica* L.), ponderosa pine (*Pinus ponderosa* L.), Scots pine (*P. sylvestris* L.) or Douglas-fir (*Pseudotsuga menziesii* Mirb.) (Tables 1 and 2).

In previous studies elevated CO_2 affected the vessel lumen diameter or area of deciduous trees, while the frequency of the vessels was unchanged (Table 1). Increased vessel lumen diameter or area observed in four tree species (Table 1) improves their capacity for water transport and can contribute to the climatic adaptation of trees (Luo et al. 2005). Wood density increased in diffuse-porous *Liquidambar* and *Populus* under elevated CO_2, while it remained unchanged, for example, in ring-porous *Quercus robur* (L.) (Table 1). In conifers, an increase in radial growth under elevated CO_2 was not accompanied by a concurrent reduction in wood density or cell wall thickness (Table 2). Exposure to elevated CO_2 either increased or did not change ring width, wood density, cell wall thickness or proportion of cell walls (Table 2). Tracheid cell dimensions (tracheid length, diameter or area) were mostly unaffected by elevated CO_2 (Table 2), indicating that the exposure in only a few cases affected the cell enlargement processes. Responses to elevated CO_2 varied in some cases (e.g. wood density in Norway spruce, tracheid diameter and cellulose concentration in Scots pine) even within the same tree species, indicating that tree

Table 1. Effects of elevated CO_2 and temperature (T, with grey background) on the wood properties of deciduous trees.

Species	Age (yrs)	Exposure method	Treatment	Ring width	Wood density	Cell wall thickness	Vessel lumen diameter/ area	Vessel length	Vessel freq.	Fibre lumen diameter	Lignin	N	Ref.
Fagus sylvatica	5	OTC	5-yr CO_2 [c]								-	-	1
	4	Semi-closed microcosm	3 -yr CO_2 [c]								-		2
	6-7	OTC	4-yr CO_2 [b]		0		-		0				3
	3	Phytotron	2.5.yr CO_2 [c]										4
			+ T (+2-4°C)				0		0				
Liquidambar styraciflua	2	OTC, in pots	>1-yr CO_2 [a-d]	+	+								5
Populus alba	4	FACE	4-yr CO_2 [b]			-	0	0	0	0			6
P. x *euramericana*						-	+	-	0	0			
P. nigra						0	+	-	0	+			
P. deltoides	3	IFM	3-yr CO_2 [d]		+					0			7
Prunus sp.	>1	Greenhouse	>1-yr CO_2 [c]				0		0				8
Quercus ilex	1.5	Greenhouse	1.5-yr CO_2 [c]		0		+		0				9
Q. robur	>1	Greenhouse	>1-yr CO_2 [c]		0		+						8

Abbreviations for Tables 1 and 2: FACE = free-air CO_2 enrichment, OTC = open-top chamber, IFM = intensive forestry mesocosm, CTC = closed-top chamber, CO_2 concentrations in the experiments: [a]420-520 ppm, [b]550-660 ppm, [c]695-750 ppm, [d]800-1200 ppm, + = increase, - = decrease, 0 = no change, lw = latewood.

References for Tables 1 and 2: [1]Cotrufo & Ineson 2000, [2]Blaschke et al. 2002, [3]Beismann et al. 2002, [4]Overdieck et al. 2007, [5]Rogers et al. 1983, [6]Luo et al. 2005, [7]Druart et al. 2006, [8]Atkinson & Taylor 1996, [9]Gartner et al. 2003, [10]Handa et al. 2006, [11]Yazaki et al. 2004, [12]Yazaki et al. 2001, [13]Hättenschwiler et al. 1996, [14]Entry et al. 1998, [15]Maherali & DeLucia 2000, [16]Atwell et al. 2003, [17]Conroy et al. 1990, [18]Ceulemans et al. 2002, [19]Kilpeläinen et al. 2003, [20]Kilpeläinen et al. 2005, [21]Ziche & Overdieck 2004, [22]Tissue et al. 1997, [23]Telewski et al. 1999, [24]Tingey et al. 2003, [25]Olszyk et al. 2005.

Table 2. Effects of elevated CO_2 and temperature (T, with grey background) on the wood properties of conifers.

Species	Age (yrs)	Exposure method	Treatment	Ring width	Wood density	Cell wall thick-ness/%	Tracheid length	Tracheid (lumen) diam./ area	Cellu-lose	Lignin	Extrac-tives	Starch	N	Ref.
Larix decidua	30	FACE	4-yr CO_2 [b]	+				0						10
L. kaempferi	2	Phytotron	>1-yr CO_2 [c]	0		0		0						11
L. sibirica	2	Phytotron	2-yr CO_2 [c]	0		0		0						12
Picea abies	7	Growth chamber	3-yr CO_2 [a,b]	0	+					0		+	-	13
	7	OTC	4-yr CO_2 [b]		0									3
Pinus palustris	2	OTC, in pots	1.5-yr CO_2 [c]						0	0	0	0	0	14
P. ponderosa	1	Growth chamber	>1-yr CO_2 [b-d]		0			0						15
			+ T (+5°C)		0			+						
P. radiata	3	OTC	3-yr CO_2 [b]	+		+		0	0	0			0	16
	2	Green-house	2-yr CO_2 [b]		+	+	0	0						17
P. sylvestris	6	OTC	3-yr CO_2 [c]	+	0			+						18
	15	OTC	3-yr CO_2 [b]	0	+[lw]		0		0	0	0			19
			+ T (amb.+2°C)	0	0		+		0	+	0			
	20	CTC	6-yr CO_2 [c]	+	0		0		-	0	0			20
			+ T (amb.+2-6°C)	0	+		+		0	0	-			
	4	Phytotron	3-yr CO_2 [c]	+	0	0		0						21
			+ T (amb.+2-4°C)	0	0	-		0						
P. taeda	4	OTC	4-yr CO_2 [b]	+	0								-	22, 23
	2	OTC, in pots	>1-yr CO_2 [a-d]	0	0									5
P. uncinata	32	FACE	4-yr CO_2 [b]	0				+[lw]						10
Pseudotsuga menziesii	6	CTC	4-yr CO_2 [b]	0	0				0	0				24, 25
			+ T (amb.+3.5°C)	0	0				0	0				

responses can also be influenced by other factors such as tree age and growth environment.

In wood chemistry the responses of deciduous trees to elevated CO_2 have been reported only for beech (Table 1), so there is obviously a need for further information. In conifers the main cell wall components (cellulose, lignin) and wood extractives have generally remained unaffected by exposure to elevated CO_2, with the exception of decreased cellulose concentration in 20-year-old Scots pine (Table 2). An accumulation of non-structural carbohydrates under elevated CO_2 is commonly associated with increased photosynthesis (Ceulemans et al. 1999, Stitt & Krapp 1999, Körner 2003); this effect was seen as increased starch concentration in 7-year-old Norway spruce (*Picea abies* (L.) Karst.) under elevated CO_2 (Table 2). Decreased nitrogen concentration resulting from elevated CO_2 is a frequent effect (Cotrufo et al. 1998, Ceulemans et al. 1999), particularly under nutrient-limiting conditions (Stitt & Krapp 1999), but responses of this kind are not consistent in wood chemistry (Table 2).

The responses of wood anatomy and density to elevated temperature have been variable. Elevated temperature reduced vessel frequency in *Eucalyptus camaldulensis* (Dehn.) (Thomas et al. 2004) and *E. grandis* (W. Hill ex Maid.) (Thomas et al. 2007), while in beech the frequency remained unchanged (Table 1). Elevated temperature had no effect on ring width (Table 2), while increased wood density was observed in *Eucalyptus* (Thomas et al. 2004, 2007) and in 20-year-old Scots pine (Kilpeläinen et al. 2005). A consistent finding in the case of Scots pine trees was that longer tracheids were produced under elevated temperature (Table 2). In the wood chemistry of Scots pine, lignin concentration either increased and extractives concentration decreased or no changes were observed (Table 2). In addition, cellulose concentration also remained unchanged (Table 2).

Reports on wood property responses to elevated O_3 are scarce and consist of 1- to 2-year exposure studies conducted with either seedlings of deciduous trees (Bertrand et al. 1999, Matyssek et al. 2002) or conifers (Andersen et al. 1997, Kurczyńska et al. 1998). Elevated O_3 reduced radial growth, which resulted in a reduction in the size of xylem cells in silver birch (*Betula pendula* Roth) (Matyssek et al. 2002). Latewood tracheid diameter decreased in non-fertilised Norway spruce (Kurczyńska et al. 1998). The concentrations of starch and soluble sugars remained unchanged under O_3 exposure in sugar maple (*Acer saccharum* Marsh.) (Bertrand et al. 1999), while starch concentration decreased in ponderosa pine (Andersen et al. 1997).

1.4 Aims of the study

The general aim of this study was to investigate the effects of climate change on the wood properties of Norway spruce, silver birch and northern American hardwoods: trembling aspen (*Populus tremuloides* Michx.), paper birch (*Betula papyrifera* Marsh.) and sugar maple. In addition, wood responses to the interactive effects of elevated CO_2 concentration with other factors occurring naturally in the field, such as an increase in the temperature and concentration of tropospheric O_3 along with nutrient limitation were determined. More specifically, the following hypotheses were posed:

(1) Elevated CO_2 (I-V), O_3 (I-III) and temperature (V) will affect radial growth, and the changes in growth may be reflected in the wood properties.

(2) Elevated CO_2 can compensate for the O_3-induced effects on the wood properties (I-III).

(3) Changes in the wood properties induced by CO_2 and O_3 can be transient, depending on the age of the tree (III).

(4) Nutrient availability will limit positive tree responses to elevated CO_2 (IV).

(5) Elevated CO_2 and temperature act synergistically, since both can have positive effects on the photosynthesis of trees (V).

2 MATERIALS AND METHODS

Attention was focused in the present study on radial growth, wood anatomy (vessel/fibre/tracheid lumen diameter and vessel percentage, cell wall thickness and percentage), wood density, fibre properties (fibre length, microfibril angle), and wood chemical composition (cellulose, lignin, uronic acids, extractives, starch, soluble sugars, C, N). In addition to their physiological function, many of these characteristics demonstrate their economic importance in the utilisation of wood as a raw material. The wood analyses have been conducted at the Suonenjoki and Vantaa Research Units of Finnish Forest Research Institute and at STFI-Packforsk (Stockholm, Sweden).

2.1 Climate change experiments

The materials (stem discs) for this study were obtained from climate change studies carried out in central Finland, northern USA and northern Sweden. All of the experiments (Table 3) were conducted with northern forest tree species during the years 1998-2004.

2.1.1 Suonenjoki OTC (I)

Two initially 7-year-old silver birch clones (*Betula pendula* Roth) were exposed to the elevated concentrations of CO_2 and O_3 alone and in combination for three growing seasons (1999-2001) in open-top chambers (OTC) at Suonenjoki (62°39´N, 27°03´E, alt. 120 m a.s.l.), Finland. Two fast-growing clones (clone 4 = V5952, clone 80 = K1659 in the Finnish forest genetic register) were selected for the experiment (Vapaavuori et al. 2002) according to their differing sensitivity to O_3 in a 2-year pot experiment with 2-year-old saplings (Pääkkönen et al. 1997), where clone 4 was classified as O_3-tolerant and clone 80 as O_3-sensitive. However, an opposing O_3 sensitivity response was reported for field-grown 10-year-old clones (Riikonen et al. 2004). Treatments were outside control, chamber control, 2*ambient CO_2, 2*ambient O_3 and 2*ambient CO_2 + 2*ambient O_3. The elevated concentration of CO_2 was 651, 720 and 729 ppm in the 1999-2001 growing seasons, respectively. AOT00 (accumulated dose over a threshold of 0 ppb) in elevated O_3 treatments was 74, 97 and 107 ppm h in the 1999-2001 growing seasons, respectively. The AOT40 (accumulated dose over a threshold of 40 ppb) in elevated O_3 treatments were 21,

Table 3. Overview of the climate change experiments included in this study. Photos, from left to right, by Erkki Oksanen, David F. Karnosky and Hanna Ruhanen, respectively.

Photo			
Location	Suonenjoki, Finland	Rhinelander, Wisconsin, USA	Flakaliden, Sweden
Studies	I	II & III	IV & V
Exposure method	OTC (open-top chamber)	FACE (free-air CO_2 enrichment)	WTC (whole-tree chamber; closed top)
Treatment	Elevated CO_2 + O_3	Elevated CO_2 + O_3	IV: Elevated CO_2 + fertilisation V: Elevated CO_2 + temperature
Duration of exposure	3 growing seasons	3 (II) or 5 (III) growing seasons	3 years
Tree species	2 silver birch (*Betula pendula*) clones	5 trembling aspen (*Populus tremuloides*; II & III) clones Paper birch (*Betula papyrifera*; II & III) Sugar maple (*Acer saccharum*; II)	Norway spruce (*Picea abies*)
No. of replicates	4	3	3
Tree age at harvest	10 years	3 (II) or 5 (III) years	41 (IV) or 45 (V) years

24 and 31 ppm h in the 1999-2001 growing seasons, respectively. The trees were exposed to CO_2 enrichment for 24 h and to O_3 enrichment for 12-14 h per day during the growing seasons of 1999-2001. The trees were exposed to treatments on 132 days in 1999 and on 148 days in 2000 and 2001.

2.1.2 Rhinelander Aspen FACE (II & III)

The material for the studies was collected from the Aspen FACE (free-air CO_2 enrichment) experiment in Rhinelander, WI, USA (45°6´N, 89°5´W). The FACE facility was constructed in 1997. It contains three FACE rings (30 m in diameter) for each of four treatments (ambient air, elevated CO_2, elevated O_3, elevated CO_2 + O_3). Exposure to the treatments was started in 1998. Trembling aspen (*Populus tremuloides* Michx.) clones and saplings of paper birch (*Betula papyrifera* Marsh.) and sugar maple (*Acer saccharum* Marsh.) were exposed during the growing season to elevated CO_2 concentration (ambient + 200 ppm) and elevated O_3 concentration (1.5*ambient) both alone and in combination. The concentration of CO_2 in ambient treatments varied between 343-361 ppm and in elevated treatments between 528-548 ppm during the 1998-2002 growing seasons (King et al. 2005). The concentration of O_3 in the ambient treatments varied between 33.1-38.8 ppb and in the elevated treatments between 49.3-54.5 ppb during the 1998-2002 growing seasons (King et al. 2005). The duration of exposure was 128-166 days in the 1998-2002 growing seasons (King et al. 2005).

Five aspen clones were included in the experiment either according to their differing sensitivity to O_3 (clones 216, 259 and 271) or according to their leaf phenology and differing response to elevated CO_2 (clones 8L and 42E). Clones 216 and 271 were classified as relatively tolerant to O_3 and clone 259 as relatively sensitive (Karnosky et al. 1996). The early leaf-fall genotype (42E) had higher photosynthesis rates under elevated CO_2 than the late leaf-fall genotype (8L) (Kubiske et al. 1998). The samples for this study were collected after three (2000, II) and five (2002, III) years of exposure.

2.1.3 Flakaliden WTC (IV & V)

The material for these studies was obtained from climate change experiments conducted at Flakaliden, northern Sweden (64°07´N; 19°17´E; alt. 310 m a.s.l.). In the first climate change study (1998-2000; IV), initially 39-year-old Norway spruce trees (*Picea abies* (L.) Karst.), grown under different nutrient availability, were exposed to elevated CO_2 concentration (2*ambient) in temperature-controlled whole-tree chambers (WTC) for three years. The treatments were: outside control, chamber control and elevated [CO_2] either in non-fertilised (control) or in irrigated-fertilised plot. Irrigated-fertilised trees were supplied with all essential macro- and micronutrients every second day during the growing season (mid-June to mid-August), and water was supplied to the plots to maintain the soil water potential above −100 kPa. The long-term nutrient optimisation experiment was established 12 years prior to the start of the chamber experiment on a stand with 27-year-old trees of local provenance (Linder 1995, Bergh et al. 1999). The CO_2 concentrations were within ± 10 ppm of the target values for more than 95% of the exposure time.

In the second climate change study (2002-2004; V), trees from non-fertilised control plots of the same experimental site were exposed to elevated CO_2 concentration (700 ppm)

and elevated temperature (ambient + 5.6 °C in winter and ambient + 2.8 °C in summer) for three years. Altogether 15 trees were assigned to five different treatments: reference (outside control without chamber), ambient temperature + CO_2 (chamber control), elevated temperature + ambient CO_2, ambient temperature + elevated CO_2, and elevated temperature + elevated CO_2. The elevated concentrations of CO_2 were within ±10 ppm of the target values for 98% and 93% of the exposure time for the winter and spring periods, respectively. Elevated temperatures were altered on a monthly time-step and were within ±0.5 °C of the target values for 99% of the exposure (Medhurst et al. 2006).

2.2 Methods

2.2.1 Harvest and preparation of samples

In order to receive representative samples from the whole tree (I, III-V) and to study the within-tree variation in the wood chemical composition in silver birch (I) and Norway spruce (IV), wood discs were sampled from three different heights along the stem. In wood structural analyses, wood discs from only one sampling height were used in analyses owing to the time-consuming preparation of samples for image analysis in studies I-IV and the high costs of the SilviScan technique used in study V. The same sampling procedure was used in studies I, IV and V. For the chemical analyses, 5 cm-thick stem discs with no knots were taken at breast height, at 40% of the total stem height, and from the base of the shoot segment, which preceded the first exposure year. In studies II and III, the wood discs for the analyses were taken from the base of stem, while for study III an additional stem disc was taken at 40% of the total stem height.

For the image analysis of the wood structure, cross sections of the annual rings formed during the exposures were prepared (I-IV). Small pieces (0.5-1 cm in thickness, 1 cm in width) of wood were boiled in water in a microwave oven. Following this softening, the wood samples were frozen at –21 °C and 16 (II-IV) or 20 (I) μm thick cross sections were cut at -14 °C using a Leitz 1516 cryomicrotome (Ernst Leitz, Ontario, Canada). The sections were stained with 1% solution of safranin (in 50% ethanol) for 2 minutes, rinsed with water, dehydrated with an ascending alcohol series, rinsed with xylene and mounted in Canada balsam.

For the SilviScan measurements (V), a wood strip (2 mm tangentially and 7 mm longitudinally) oriented from pith to bark was cut from each disc. The strip was air-dried, extracted with acetone and its upper surface showing the fibre cross sections was polished.

In the sample preparation for the chemical analyses (I-V), the bark, phloem and cambium were first removed from the wood discs. Then the rings formed during the treatment years were separated from the older wood. The sections of annual rings were further cut into picks (approximately 2-3 mm in thickness), which were freeze-dried to constant dry mass, then milled (Polymix A10, Kinematica AG, Switzerland) into powder at -25 °C and stored at -20 °C.

2.2.2 Wood anatomical and physical properties

Widths of annual rings were measured with a digital caliper on the stem discs. The microscopic measurements were performed using an Olympus BX60 (Olympus Optical, Tokyo, Japan) microscope connected to a Spot insight B/W video camera (Diagnostic Instruments Inc., Sterling Heigths, MI, USA) and an Image-Pro plus for Windows (Media Cybernetics, Silver Spring, MD, USA) program. The image area of the images captured was 1600 x 1200 pixels. In the analysis of the fibre and vessel lumen diameter, cells that were located either only partly in the image or whose cell walls were broken during the preparation of cross sections were excluded. The cell wall percentage was measured as a proportion of the cell walls in the total image area.

In the analyses of the wood structure of young deciduous tree (I-III), three images of cross sections from the last annual ring formed were captured about 0.05 mm from the border of the annual ring. The vessel percentage was analysed as an area occupied by vessel lumina of the total image area. In the analyses of the cell wall and vessel percentages and of the fibre and vessel lumen diameters the images were captured at 10x magnification (resolution 0.74 μm/pixel; I-III). A 40-fold magnification was used to analyse the fibre wall thickness; one pixel corresponded to 0.19 μm (II). Different threshold values were used depending on the species or tree sizes. The sampling procedures were different in the two aspen (II and III) studies: in the first study sample trees represented the mean size within treatments/clones, while in the second study trees were selected from three stem height classes. Cell lumina larger than 500 (selected as the threshold value for vessels; I), 625 (III) or 1000 μm^2 (II) were considered as vessels. The lumen diameter of the fibres was measured from cell lumens equal to 15-500 μm^2 (II, III).

In the analyses of the wood structure of 40-year-old Norway spruce (IV), three images of cross sections per annual ring formed during the exposure were captured at about 0.05 mm from the border of the annual ring. The images were captured at 20x magnification (resolution 0.37 μm/pixel) in the measurement of the tracheid lumen diameter and at 40x magnification (resolution 0.18 μm/pixel) in the analysis of the cell wall index. In the analysis of the tracheid cell wall thickness, six images were taken at 60x magnification and the resolution of the captured images was 0.12 μm/pixel. The latewood percentage was evaluated from cross sections at 2x magnification (resolution 3.73 μm/pixel). The differentiation between the early- and latewood cells was determined according to cell wall thickness, whereby the double cell wall thickness for latewood cells should be equal to or greater than the lumen diameter.

In the measurement of the fibre (I, IV) and vessel (I) length, small picks were chipped from the middle of the annual ring and then macerated overnight at 60°C in a solution of concentrated glacial acetic acid and 30% hydrogen peroxide (1:1, v/v) (Franklin 1945). The lengths of the fibres and vessels were measured using an image analysis system (Image-Pro Plus 4.0). The resolution of the captured images was 10.64 for tracheids (IV), 5.43 for vessels (I), and 2.70 μm/pixel for fibres (I).

The density of Norway spruce (IV) was analysed using x-ray densitometry as described in Mäkinen et al. (2002). Wood samples (5 mm wide and thick) were removed along the southern radius of each disc. The moisture content of the samples was stabilized to 12% by storing them for over three weeks at 20 °C and at a relative humidity of 65%. Thereafter, the samples were placed on a film and X-rayed for 5 min (16 kV, 20 mA, distance 2.5 m). The method has been described in detail by Saikku (1975). The films were scanned and analysed by WinDendroTM density-analysis software with standard material of known

physical and optical density. The resolution of the images captured was 0.021 mm/pixel. The boundary between the early- and latewood was defined using the equation: maximum density – 0.3*(maximum density – minimum density). The method is independent of differences in the density levels between the samples. However, it results in a different position of the boundary (depending on threshold value) compared with the traditional definition of Mork based on wood anatomy, i.e. cell wall thickness and lumen diameters (cf. Denne 1989).

In study V, wood density, the radial and tangential diameters of tracheids, cell wall thickness, tracheid coarseness, specific tracheid surface area (SSA) and microfibril angle (MFA) were determined using a SilviScan instrument (Evans 1994, 1999), and wood stiffness (modulus of elasticity, MOE) was estimated combining X-ray densitometry and diffraction data, according to Evans's (2006) description. All measurements were made in a conditioned atmosphere maintained at 42% RH and 22°C. The SilviScan data consisted of measured values for each characteristic at 50-μm intervals, except for MFA and MOE averages of 5-mm intervals were measured. A relative criterion was used to establish the distinction between the earlywood (ew) and latewood (lw). For each ring the maximum (max) and minimum density (min) was found and the span (= max – min) was calculated. When the density was lower than min + 0.2 x span, the wood was regarded as ew, and when the density was higher than min + 0.8 x span, the wood was regarded as lw.

2.2.3 Wood chemical composition

Milled stem wood (2 g) was extracted in acetone (150 ml) using Soxhlet method in accordance with the SCAN-CM (1994) standard for the measurement of extractives and to yield extractive-free samples. Alpha-cellulose, uronic acids, gravimetric lignin and acid-soluble lignin were analysed from the extractive-free samples, as described in Anttonen et al. (2002). Soluble sugars were extracted from freeze-dried and milled wood (100 mg) with a total volume of 15 ml of 80 % aqueous ethanol. Starch was extracted from the residue with 20 ml of 30 % perchloric acid. Soluble sugars and starch were measured using the anthrone method (Hansen & Møller 1975). Total carbon (C), nitrogen (N) concentration and C/N–ratio of the stem wood were measured with a CHN analyser (Leco Co., St. Joseph, MI, USA).

2.2.4 Statistical analyses

The data was analysed using the SPSS (10.1–14.0, SPSS Inc., Chicago, IL, USA) statistical package. The effects of the treatments on the wood properties were evaluated by analysis of variance (ANOVA, GLM Procedure). The repeated measures ANOVA was used in studies I and V for statistical analyses of the wood chemistry, and in study IV to analyse the within-tree or within-year variation. Analysis of covariance (ANCOVA) was used for statistical analyses of the wood structure in studies I and III, and the repeated measures ANCOVA was used in study V. Where necessary, the data was transformed to meet the assumptions of valid statistical tests. Treatment effects at $P<0.1$ were considered to be significant (I-II, IV-V) or to indicate a trend ($0.05<P<0.1$; III).

3 RESULTS

The responses of northern forest trees to elevated CO_2, O_3 and temperature (T) were dependent on the species, age and duration of the exposure (Tables 4 and 5). Under elevated CO_2 concentration, wood chemistry changed more in young deciduous trees than in 40-year-old Norway spruce (Table 5). Under elevated CO_2, the increases were found in ring width and in the concentrations of extractives and starch (Tables 4 and 5). Although radial growth was generally increased by elevated CO_2, no changes were found in the cell wall thickness of deciduous trees or wood density of Norway spruce (Table 4). Elevated O_3 concentration affected the wood anatomy of silver birch and trembling aspen by reducing either vessel lumen diameter or the proportion of vessels (Table 4). In 40-year-old Norway spruce, elevated temperature and fertilisation had more marked effects on the wood properties than elevated CO_2 (Tables 4 and 5).

The wood chemical composition varied between tree species. In some cases, also an age-dependent effect was found; for instance, gravimetric (Klason) lignin concentration was lower in 5-year-old aspen clones compared with 3-year-old clones. This is consistent with the finding that juvenile wood contains more lignin (Erickson & Arima 1974, Tyrväinen 1995). Mature Norway spruce trees had lower starch concentration than young deciduous trees. The wood of Norway spruce and aspen contained more cellulose than the wood of other tree species. Sugar maple had the highest concentration of soluble sugars, and silver birch the highest nitrogen concentration. The differences in the carbon partitioning to the wood chemical components e.g. in aspen and paper birch were also obvious when calculated on a stem mass basis (III, Figure 1).

In silver birch and Norway spruce the chemical composition varied with the sampling height. In spruce this was anticipated since the highest sampling point along the stem consisted of juvenile wood, while the two other sampling points lower in the stem were mature wood. In 10-year-old silver birch trees all of the samples were juvenile wood, since the transition point of juvenile-mature wood occurs at an age of ca. 15 years (Bonham & Barnett 2001). It seems that other factors, such as differential carbon supply from the canopy, also have an influence on the wood chemistry of forest trees.

The wood properties of the different clones of silver birch (I) and trembling aspen (II) showed large variation. Silver birch clone 4 had longer fibres, wider-lumen vessels and more extractives but also lower cell wall percentage and cellulose concentration than clone 80. Aspen clone 216 had a significantly different stem wood structure, since it possessed the smallest vessel lumen diameter and the lowest vessel proportion, and as a result, the highest cell wall proportion. The results indicate that wood quality in different end-use purposes may largely be determined by genotypic differences. The clones also responded differently to elevated CO_2 and O_3. The results suggest that variability can exist between different genotypes in their adaptation to future atmospheric conditions. Another factor causing variability in wood responses was the duration of the exposure and/or tree age. In the Aspen FACE studies (II and III) the responses to elevated CO_2 and O_3 were different in seedlings exposed for 3 years compared to older seedlings exposed for 5 years (Tables 4 and 5).

3.1 Wood structure under elevated CO_2, O_3, temperature and fertilisation

Elevated CO_2 concentration increased ring width in silver birch, in two clones of 3-year-old aspen (216, 259) and in 5-year-old aspen clones, but the radial growth increment did not translate into changes in the wood anatomy (Table 4). In 40-year-old Norway spruce, ring width was increased in the second exposure year (1999) in non-fertilised trees and in the same year tracheid lumen diameter in earlywood decreased, indicating that a larger number of tracheids were produced (IV). A different growth pattern was observed in the second climate change experiment with Norway spruce (V), where ring width and earlywood tracheid lumen diameter were unaffected, while the diameter in latewood increased and cell wall thickness in earlywood decreased (Table 4).

Under exposure to elevated O_3 concentration the vessel structure of silver birch and aspen changed. Vessel proportion (%) decreased and cell wall proportion concurrently increased in birch clone 80, while vessel lumen diameter remained unchanged (Table 4). However, elevated CO_2 ameliorated the O_3-induced reduction in vessel proportion (I). In contrast to silver birch, vessel lumen diameter was reduced in aspen, while vessel proportion was unaffected (Table 4). A reduction in the stem radial growth was also paralleled by increased cell wall proportion in aspen clones and reduced fibre lumen diameter in clone 271 after the 3-year exposure (Table 4). After the 5-year exposure, however, the fibre lumen diameter was conversely increased in aspen clone 8L.

Fertilisation increased radial growth in Norway spruce, and this was also reflected in the wood anatomy: tracheid lumen diameter increased while both cell wall thickness and wood density decreased. In contrast, the mean cell wall thickness and wood density were

Table 4. Effects of CO_2 (with grey background), O_3, temperature (T) and fertilisation (F) on the wood structure of the three forest tree species studied.

Parameter	Silver birch (I)		Aspen (II & III)		Norway Spruce (IV & V)		
	CO_2	O_3	CO_2	O_3	CO_2	F	T
Ring width/radial growth	+	0	$^{3\text{-yr}}$ +*	-	^{IV} +	^{IV} +	
			$^{5\text{-yr}}$ +	0	^{V} 0		^{V} 0
Fibre/tracheid (lumen) diameter	n.a.	n.a.	0	-*	-^{ew}	+^{ew}	
			0	+*	+^{lw}		0
Vessel lumen diameter	0	0	0	-			
			0	-			
Vessel %	0	-*	0	0			
			0	0			
Cell wall thickness/%	0	+*	0	+	0	-	
			0	0	-^{ew}		+
Wood density	n.a.	n.a.	n.a.	n.a.	0	-	
					0		+

+ = increase, - = decrease, 0 = no change, * = interaction with clone, n.a. = not analysed, ew = earlywood, lw = latewood, blank area = not measurable in conifers.

increased under elevated temperature, while radial growth was not significantly affected (Table 4). Several interactions between CO_2 and temperature were found in the anatomical and physical characteristics of spruce wood (V). Elevated temperature as a single factor increased microfibril angle, suggesting the possible occurrence of compression wood. Both elevated temperature and CO_2 alone reduced cell wall thickness and density in latewood, but not when they were combined.

3.2 Wood chemistry under elevated CO_2, O_3, temperature and fertilisation

Elevated CO_2 concentration significantly affected both the structural and non-structural compounds in silver birch: the concentrations (% of dry weight) of α-cellulose and lignin decreased, while the concentrations of acetone-soluble extractives and starch increased. In addition, in 5-year-old paper birch increased concentration of extractives was found under elevated CO_2 (Table 5). The response to elevated CO_2 in starch concentration differed for the 3-year-old and 5-year-old paper birch (Table 5). The main cell wall components (cellulose, lignin) were unaffected by elevated CO_2 either in paper birch or in aspen (Table 5). However, in 5-year-old aspen uronic acid concentration (constituents of e.g. hemicellulose) decreased (III). All of the other significant responses in the chemical composition of the wood depended on the particular aspen clone and the duration of the exposure. The concentration of starch increased in two clones (259, 271) and soluble sugars did so in one clone (8L) after the 3-year exposure. In 5-year-old aspen the opposite reaction

Table 5. Effects of CO_2 (with grey background), O_3, temperature (T) and fertilisation (F) on the wood chemistry of the five forest tree species studied.

Parameter	Silver birch (I)		Paper birch (II & III)		Aspen (II & III)		Maple (II)		Norway Spruce (IV & V)		
	CO_2	O_3	CO_2	O_3	CO_2	O_3	CO_2	O_3	CO_2	F	T
Cellulose	-	0	3-yr 0	0	3-yr 0	0	3-yr 0	0	IV 0	IV 0	
			5-yr 0	0	5-yr 0	0			V 0		V 0
Total/gravim. lignin	-	0	0	+	0	+*	0	0	0	+	
			0	0	0	0			0		0
Extractives	+	0	0	0	0	0	0	0	0	0	
			+	+	+*	-			0		-
Soluble sugars	0	0	0	0	+*	0	0	0	-	0	
			0	0	+/-*	-			0		-
Starch	+	0	0	0	+*	0	0	0	0	0	
			-	+	0	0			0		0
Nitrogen	0	-*	0	0	0	-*	0	0	-	+	
			0	0	0	0			0		0

+ = increase, - = decrease, 0 = no change, * = interaction with clone.

was found in clone 8L, where the concentration of soluble sugars decreased. The concentrations of soluble sugars and extractives increased in clone 42E after the 5-year exposure. Mature Norway spruce trees were relatively unresponsive to elevated CO_2 and only a few significant responses (decreases in soluble sugars and acid-soluble lignin in non-fertilised trees and in nitrogen concentration in 1 or 2 sampling heights out of 3) were observed. However, 3-year-old maple was the least responsive of the five tree species studied (Table 5), with only a few interactions between elevated CO_2 and O_3 found (III). Starch concentration decreased in the CO_2 and O_3 treatments, while in the combined treatment no changes were detected.

The effects of elevated O_3 concentration on the wood chemistry were studied only in young deciduous trees. Both silver birch clones were tolerant to O_3 in terms of their wood chemistry (Table 5) because the only significant effect was a decrease in nitrogen concentration in clone 4. Decreased nitrogen concentration was also found in two 3-year-old aspen clones (42E, 271).

Lignin concentration increased under elevated O_3 in both paper birch and four aspen clones after 3-year exposure (Table 5). However, elevated CO_2 ameliorated this effect (interaction CO_2 x O_3; II) and the lignin response was no longer found with 5-year-old trees (Table 5). Elevated O_3 increased the concentrations of extractives and starch in 5-year-old paper birch, while it decreased acid-soluble lignin and tended to decrease the concentrations of extractives and soluble sugars in 5-year-old aspen (Table 5).

Fertilisation increased lignin and nitrogen concentrations in Norway spruce trees. Elevated temperature decreased the concentrations of extractives and soluble sugars in spruce, while it had no effect on the main cell wall components (Table 5).

Interactions between elevated CO_2 and O_3 were found in the concentrations of cellulose and nitrogen in 3-year-old aspen (II). The CO_2 treatment decreased cellulose concentration, while it was increased in the combined CO_2 + O_3 treatment. In nitrogen concentration the CO_2 treatment alone had no influence, while the CO_2 + O_3 treatment decreased N concentration. In spruce, interaction between elevated CO_2 and T was found in the concentration of acid-soluble lignin, which decreased in the combined treatment (V).

4 DISCUSSION

4.1 General consideration of the experimental set-ups

Three different exposure methods were used in the experiments: free-air CO_2 enrichment (II and III), open-top (I), and whole-tree chambers (with closed top) (IV, V). Within the chambers, microclimatic conditions such as the air temperature, humidity, transmittance of solar irradiance, wind, rainfall and tree-atmosphere coupling can be altered (Long et al. 2004). In the chamber studies (I, IV-V) the polyethylene films used in the enclosure demonstrated a high transmittance of visible light (88-91%) at wavelengths of 400–800 nm, but the amount of light transmitted at wavelengths less than 400 nm declined sharply (Vapaavuori et al. 2002, Medhurst et al. 2006).

In the open-top experiment with silver birch, the mean temperature within the chambers was, on average, 2 °C higher and the relative humidity was lower than in the ambient air (Vapaavuori et al. 2002). Although there was no chamber effect on stem diameter or height growth (Riikonen et al. 2004), a different growth environment affected the wood anatomy. Longer fibres and a higher proportion of vessels, and, in turn, a lower proportion of cell walls were observed in the chamber control trees than in outside controls. The higher vessel proportion may be linked to higher water-use efficiency in the chamber-grown silver birch trees as a result of lower stomatal conductance (Riikonen et al. 2005).

A number of significant chamber effects were also observed in the experiments conducted with Norway spruce. Although the chambers were temperature-controlled, following the ambient air temperature throughout the year, the chamber-grown trees tended to have slightly higher earlywood and latewood densities in some of the exposure years (IV), higher latewood percentage, and lower cellulose concentration and tracheid lumen diameter (V) than the trees grown in the open air. The observed changes did not confirm the hypothesis put forward by Telewski et al. (1999) that the reduced wind effect on trees in chamber experiments might also reduce wood density. In contrast, this study suggested that growing the trees in chambers might have altered the carbon availability or allocation to Norway spruce stems, as well as growth patterns causing e.g. a differential transition to latewood.

The FACE experiment enabled examination of the effects of elevated CO_2 and O_3 concentration on the wood properties of three tree species grown in competitive communities (pure aspen, aspen-birch, aspen-maple) for either three (II) or five years (III). Rapid-growing, pioneer tree species (aspen and paper birch) were found to be more sensitive to elevated CO_2 and O_3 than the slower-growing, later-successional sugar maple. The wood responses to the treatments in the FACE experiment were different for the 3-year- and 5-year-old aspen clones and paper birch. The potential reasons for such differences may be acclimation, ontogenetic change (maturation), higher competition for resources as a result of canopy closure occurring at the site, or interannual climatic variability affecting the relative growth rates of aspen (Kubiske et al. 2006). According to Kubiske et al. (2006), the growth response to elevated CO_2 and O_3 occurred in parallel with declining July photosynthetic photon flux (PPF) and decreasing previous October temperature in 2001-2002 (III), but not in 1999-2000. Variation in responses to e.g. elevated O_3 between and within species (age-related response) was also found in an experiment related to European forest decline discussion in late 80's. The effects of the long-term exposure to low levels of O_3 on photosynthesis, growth rates and needle anatomy were different for Norway spruce and silver fir (*Abies alba* Mill.) (Arndt 1990, Schmitt & Ruetze 1990).

4.2 Effects of elevated CO_2 on the wood properties of northern forest trees (I-V)

Elevated CO_2 concentration increased stem diameter growth or annual ring width of silver birch and trembling aspen (I-III), but the changes in growth were not reflected in the wood anatomy. The changes observed in radial growth were in line with increased biomass production in silver birch (Riikonen et al. 2004) and with stem volume growth in aspen (Isebrands et al. 2001, Karnosky et al. 2003, Karnosky et al. 2005, King et al. 2005) under elevated CO_2. Enhanced growth in the experiments resulted from an increase in total leaf

area (Riikonen et al. 2004) and photosynthesis (Riikonen et al. 2005, Karnosky et al. 2005). The lack of wood anatomy responses in young deciduous trees to the exposure means that elevated CO_2 did not change the proportions or dimensions of the wood cells produced. The construction of the vascular and supporting system of the tree stem was well balanced with the increasing biomass of the trees and thus may indicate that both the growth and fibre properties of young deciduous trees are under strong genetic control (Stener & Hedenberg 2003).

In two studies with 40-year-old Norway spruce, elevated CO_2 did not affect mean ring density and had no constant increasing effect on ring width, although some within-ring alterations in wood anatomy were observed. Cell wall thickness and tracheid lumen diameter in earlywood decreased and tracheid diameter in latewood increased under elevated CO_2. These changes may have been controlled by the processes of tracheid differentiation: growth in the radial and longitudinal directions or secondary wall formation (Gindl et al. 2000). The results on wood density and ring width are well in line with other reports on conifers (Table 2). The decreased cell wall thickness in earlywood under elevated CO_2 was reflected in tracheid coarseness (tracheid mass per unit length) (V), a wood characteristic important for pulp and paper properties. The observed decrease in coarseness may improve pulp and paper properties as a result of the higher collapsibility of the tracheids (Paavilainen 1993).

The effects of elevated CO_2 on the main cell wall components were species- and age-specific. Changes were found only in silver birch and 5-year-old aspen, and were seen as decreased concentrations of cellulose and gravimetric lignin in birch, and uronic acids (components of e.g. hemicellulose) in aspen. This indicates that in these species the assimilates were more likely to be allocated to non-structural reserves that can be recycled, rather than to structural components. However, the observed decreases in the concentrations were not transferred to the overall yield of cellulose, lignin or hemicellulose per tree, since the woody biomass in these species increased under elevated CO_2 (Riikonen et al. 2004, Karnosky et al. 2005). Similar results for wood chemistry under elevated CO_2 have been reported previously, revealing decreased concentration of lignin in beech (Cotrufo & Ineson 2000, Blaschke et al. 2002) and cellulose in Scots pine (Kilpeläinen et al. 2005). Carbon partitioning may, however, be species-specific, and the results for paper birch and Norway spruce showed no changes in the balance between photosynthesis-driven substrate formation and substrate sequestration to cell wall.

In all of the tree species studied, elevated CO_2 affected either the concentrations of non-structural carbohydrates (soluble sugars, starch) or acetone-soluble extractives, or both of them. Within the xylem, the non-structural carbohydrates (Krabel 2000, Puech et al. 2000) and extractives (Sjöström & Westermark 1999) are located in the ray parenchyma cells. Non-structural carbohydrates represent an important pool for stored carbon (Hoch et al. 2003, Körner 2003), substrates for growth processes (Höll 2000) and wood formation (Oribe et al. 2003), and are involved in freezing tolerance (Bertrand et al. 1999, Piispanen & Saranpää 2001). Extractives mostly include secondary metabolites that play an important role in protection against pathogens or other biotic attacks (Pereira et al. 2003).

Increased concentration of non-structural carbohydrates is a common effect observed in trees grown under elevated CO_2 (Hättenschwiler et al. 1996, Hättenschwiler & Körner 1998, Ceulemans et al. 1999, Stitt & Krapp 1999). The same response was observed in this study, since starch accumulated in silver birch and in 3-year-old aspen clones under elevated CO_2. The accumulation was paralleled by enhanced photosynthesis in the experiments (Riikonen et al. 2004, Karnosky et al. 2005), indicating that assimilate

production exceeded the sink activity (i.e. structural growth) (Körner 2003). These mobile carbohydrate reserves can improve acclimatisation in winter and suggest a surplus for growth in the subsequent spring. The responses of soluble sugar concentration to elevated CO_2 varied according to the species, clones, exposure duration, and age of trees. This may partly be due to different harvest times; for this study the trees were harvested between August and November, when the trees had either already ceased, or were in the process of ceasing, their growth and were preparing for winter. Clones or individual trees may have been in a different physiological phase and thus displaying differences in the content or composition. Increased concentrations of extractives in both birch species and in one 5-year-old aspen clone under elevated CO_2 may be an indication of improved capacity for defence.

4.3 Effects of elevated O_3 on the wood properties of young deciduous trees (I-III)

The 3-year-old aspen clones demonstrated reduced stem diameter growth under elevated O_3 concentration, a detail that is in line with the other data obtained from the Aspen FACE site (Isebrands et al. 2001, Karnosky et al. 2005, King et al. 2005). A reduction in growth was reflected in the wood anatomy, where smaller vessel and fibre lumina and, respectively, higher cell wall proportion were observed. In silver birch or 5-year-old aspen, elevated O_3 had no effect on ring width. Even so, clone-dependent changes in wood anatomy were observed. The clone dependence in the O_3-responses of silver birch and aspen is unsurprising, since the clones were specifically included in the experiments because of their differing sensitivity to O_3. Fibre lumen diameter was increased in 5-year-old aspen clone 8L, which is the only clone with no reduction in its volume growth under elevated O_3 (Kubiske et al. 2007). In silver birch clone 80 the O_3-induced increment in cell wall proportion was connected with a parallel decline in vessel proportion.

A consistent finding for silver birch and aspen was that elevated O_3 affected vessel properties. Both reduced vessel proportion and smaller vessels may affect the capacity for water and nutrient transport. Narrow vessels are hydraulically less efficient in water transport (Tyree et al. 1994) since the conductivity of a vessel is proportional to the fourth power of its lumen radius (Hagen-Poiseuille law) (Zimmermann 1983, Tyree & Ewers 1991, Atkinson & Taylor 1996), but they also run a lower risk of embolism and cavitation (Zimmermann 1983, Schume et al. 2004). The morphogenesis of leaves and secondary vasculature in the stems of woody plants are closely coordinated (Isebrands 1972, Zimmermann 1983). Hence, the reduced vessel lumen diameter may be connected with lower transpirational leaf area and a lower demand for water transport (Joyce and Steiner 1995). In aspen elevated O_3 in fact had a negative impact on leaf area index and accelerated leaf senescence and abscission (Karnosky et al. 2005). In contrast, however, leaf area or mass ratios were unaffected in silver birch, although elevated O_3 accelerated leaf abscission (Riikonen et al. 2004). The reduction in vessel lumen diameter was not connected with the stomatal conductance of the trees, since elevated O_3 had no effect on conductance in silver birch (Riikonen et al. 2005). In aspen the stomatal conductance decreased in mature and old leaves, but the effect depended on the aspen clone (Noormets et al. 2001).

Elevated O_3 did not markedly affect the wood chemistry of sugar maple, and the responses of paper birch and aspen varied for 3-year-old and 5-year-old trees. In the 3-year-old trees, total lignin concentrations increased both in paper birch and four aspen clones,

but elevated CO_2 nullified the increase. The increase after the 3-year exposure could be linked to an observed up-regulation of the phenylpropanoid pathway in these O_3-exposed trees after the first growing season (Wustman et al. 2001). The PAL (phenylalanine ammonia-lyase) transcripts were increased by oxidative stress under elevated O_3 (Wustman et al. 2001). Whether the absence of a total lignin response in the 5-year-old trees is associated with a down-regulation of a protective metabolism against oxidative stress is not known.

The concentrations of non-structural compounds were affected under elevated O_3 in 5-year-old aspen and paper birch. Increased concentrations of acetone-soluble extractives and starch in paper birch indicate a greater allocation of carbon for storage or chemical defence. In aspen a different carbon allocation pattern was seen: the lower concentration of extractives may be a sign of decreased photosynthesis under elevated O_3 (Karnosky et al. 2003) and also of less carbon available for allocation to the chemical defense.

Nitrogen concentration decreased in one silver birch clone and two 3-year-old aspen clones. The decrease may be growth-related since significant growth reductions were observed both in silver birch clone 4 (Riikonen et al. 2004) and in aspen clones 42E and 271 under elevated O_3 (Kubiske et al. 2007). There may have been less nitrogen available for translocation from leaves to stems during leaf abscission, or the capacity for N storage within the stem may have been reduced as a result of a smaller number of living cells, where most of the xylem nitrogen is located (Hättenschwiler et al. 1996, Stockfors & Linder 1998). Elevated O_3 can also directly impair the autumnal resorption of nitrogen from leaves, causing a substantial loss of whole-tree N through litter fall (Uddling et al. 2005). The trees at either of the experiment sites did not suffer from nutrient deficiency, since at the Suonenjoki OTC experiment the trees were fertilised (Vapaavuori et al. 2002). Similarly, the Aspen FACE experiment was established at a fertile site used for agriculture, with soil nitrogen levels that were still relatively high (Dickson et al. 2000).

4.4 Effects of elevated temperature on the wood properties of Norway spruce (V)

The exposure of Norway spruce trees to elevated temperature altered their wood properties more than did the exposure of the same trees to elevated CO_2. An earlier commencement of photosynthesis (Slaney 2006) and growth (Slaney et al. 2007) under elevated temperature accords with changes in the physical properties of earlywood, including an increase in cell wall thickness and thus also in wood density. As a result, the mean ring variables were also changed, since earlywood occupies a major portion of the annual ring. Density is one of the major technical properties of wood, correlating well with many other physical properties, such as strength, stiffness and performance in use (Evans & Ilic 2001, Saranpää 2003). It is mainly determined by the size of the wood cells or their lumens, and by the thickness of the cell wall (Saranpää 2003). The wood density of *Eucalyptus camaldulensis* and *E. grandis* seedlings (Thomas et al. 2004, 2007) and 20-year-old Scots pine (Kilpeläinen et al. 2005) also increased under exposure to elevated temperature, which accords with our results. Tracheid coarseness increased under elevated temperature (V), probably as a result of increased cell wall thickness, since tracheid diameter remained unchanged. An increase in fibre coarseness impairs fibre bonding during pulping processes as a result of the lower conformability (flexibility and collapsibility) of fibres with thick walls (Paavilainen 1993).

In wood chemistry the allocation of carbon to non-structural compounds, i.e. acetone-soluble extractives and soluble sugars, decreased. Decreased concentration of soluble carbohydrates, which are involved in the process of cold hardening (Bertrand et al. 1999, Piispanen & Saranpää 2001), may indicate that the trees were not as far advanced in their acclimation process at the time of harvest (in September) as trees growing in ambient temperature.

4.5 Interactive effects of climate change factors on wood properties (I-V)

All the experiments in this study included 3- to 5-year exposure to elevated CO_2 concentration (530-730 ppm) combined with either elevated O_3 concentration (74-107 ppm h during the growing season; I-III), fertilisation (the respective amounts of N, P, K and Mg supplied to stands: 225, 30, 90 and 24 kg ha^{-1} in 1998-2000; IV) or elevated temperature (ambient + 5.6 °C in winter and ambient + 2.8 °C in summer; V). Relatively few interactions between elevated CO_2 and other factors on wood properties were observed.

In mature Norway spruce, ring width increased in one exposure year under elevated CO_2 only in non-fertilised trees (IV). The Flakaliden experiment was a nutrient-optimisation trial aimed at demonstrating the potential yield of Norway spruce by optimising the nutritional status of the stands (cf. Linder 1995; Bergh et al. 1999). Hence, the added levels of nutrients were higher than those normally used in practical forestry. In the case of the fertilised trees, elevated CO_2 did not induce any further increment in their ring width. Similarly, Hättenschwiler et al. (1996) found no positive interaction between elevated CO_2 and N fertilisation in ring widths in 7-year-old Norway spruce. It cannot, however, be concluded that soil nutrient limitation or N deposition might not have a substantial impact on tree growth under increasing atmospheric CO_2 concentrations. A limited availability of soil nutrients in boreal ecosystems can hamper positive growth responses under elevated CO_2 (Linder & Murray 1998, Morison & Lawlor 1999, Sigurdsson et al. 2001).

Based on the present studies of the interactive effects of elevated CO_2 and O_3 on young deciduous trees, these greenhouse gases may counteract each other's responses in wood properties, but the effect may be transient as a result of the duration of the exposure and tree age. Elevated CO_2 ameliorated an O_3-induced reduction in vessel proportion in silver birch (I) and an increase in the total lignin concentration of both 3-year-old aspen and paper birch (II), having a positive effect on both tree physiology and wood material properties.

In Norway spruce more carbon was sequestered in the cell wall in the combined CO_2 and T treatment than as a consequence of single exposures during late summer. This finding is in line with the fact that the combined treatment caused an additive effect with both earlier photosynthetic recovery and also the greater photosynthetic capacity of shoots, suggesting an increased potential for carbon uptake during the growing season (Slaney et al. 2006). In the case of other conifers no interactions between elevated CO_2 and temperature in wood density have been found (Kilpeläinen et al. 2003, Ziche & Overdieck 2004, Kilpeläinen et al. 2005, Olszyk et al. 2005).

5 CONCLUSIONS

The increasing atmospheric CO_2 concentration and mean temperature and also enhanced nitrogen deposition have the potential to increase carbon sequestration in the northern forests, while increasing tropospheric O_3 concentration is liable to reduce the productivity of the forests. Changes in growth may be reflected in the anatomy, chemical composition and physical properties of wood, thus altering the material properties of wood. Even small changes in the material properties may be significant for the forest industry. In the present study the wood properties of northern forest trees were altered by single or combined exposures to elevated CO_2, O_3 and temperature.

Elevated CO_2 generally increased radial growth, but affected wood anatomy only in 40-year-old Norway spruce, with intra-ring variations indicating changes in the tracheid enlargement and cell wall deposition processes during the growing season. In terms of stem wood chemistry, the allocation of photosynthates in young deciduous trees grown under elevated CO_2 was preferentially directed to mobile non-structural compounds (e.g. acetone-soluble extractives, starch) rather than structural cell wall components, but the pattern varied between tree species.

Elevated O_3 reduced vessel lumen diameter in aspen and also vessel proportion in one silver birch clone. These changes in vessel properties may indicate a lowered capacity to conduct water and nutrients under O_3 stress, and may be connected with a reduced demand for water transport in O_3-exposed trees. Elsewhere in the study, the responses of aspen and silver birch to elevated O_3 were often dependent on the clone under study, indicating variation in the adaptability of different genotypes to future conditions.

Elevated temperature allowed the cell wall to thicken over a longer period of time and thus increased cell wall thickness and wood density, but it had no effect on the radial growth of Norway spruce. Based on the results, the wood properties of mature Norway spruce are likely to be affected more by increasing temperature and by nutrient supply than by increasing CO_2 concentration.

Elevated CO_2 ameliorated only a few O_3-induced responses in wood properties, namely a reduction in vessel proportion in silver birch and an increase in lignin concentration in 3-year-old saplings of aspen and paper birch. In mature Norway spruce, high nutrient availability did not amplify the CO_2 responses. Under combined exposure to elevated CO_2 and temperature, higher latewood density was observed than in single exposures, indicating increased carbon sequestration in the cell walls during late summer.

Responses to the treatments were dependent on tree species, clones and tree ontogeny. The effects of elevated CO_2 and O_3 on wood properties differed between 3-year-old and 5-year-old aspen and paper birch, and thus the changes were transient, depending on the duration of the exposure, on climatic variability, or on the maturation of the trees. This finding emphasizes the importance of long-term studies, since an up-scaling of the findings of wood production and the properties of juvenile trees to mature forest ecosystems is problematic owing to the long life-span of trees.

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