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EEVA-MARIA LUOMALA

Photosynthesis, chemical composition and anatomy of
Scots pine and Norway spruce needles under
elevated atmospheric CO₂ concentration and temperature

Doctoral dissertation

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Department of Ecology and Environmental Sciences
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Series Editor: Professor Lauri Kärenlampi, Ph.D.
Department of Ecology and Environmental Science

Author's address: Finnish Forest Research Institute
Suonenjoki Research Station
Juntintie 40
FIN-77600 Suonenjoki
FINLAND
E-mail: Eeva.Luomala@utu.fi

Supervisors: Docent Elina Vapaavuori, Ph.D.
Finnish Forest Research Institute
Suonenjoki Research Station

Docent Pedro J. Aphalo, Ph.D.
University of Jyväskylä

Docent Jarmo K. Holopainen, Ph.D.
University of Kuopio

Reviewers: Gerhard Kerstiens, Ph.D.
Lancaster University
UK

Docent Kari Laine, Ph.D.
University of Oulu

Opponent: Professor Olevi Kull
University of Tartu
Estonia

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ABSTRACT

Introduction As a result of human activities, atmospheric carbon dioxide (CO₂) concentration is rising, leading to higher global surface air temperatures. The increase in atmospheric CO₂ is expected to be beneficial for photosynthesis and growth of plants, and this benefit should be greater when temperature increases also. During growth at elevated CO₂, however, reductions in photosynthetic capacity often occur. The aim of the present study was to study whether there is down-regulation of photosynthetic capacity in needles of Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.) grown at elevated CO₂ and temperature. The specific aim was to study biochemical alterations and possible mechanisms resulting in down-regulation of photosynthetic capacity, and to study whether a higher nitrogen (N) supply in the soil alleviates these responses. Finally, the aim was also to find whether elevation of CO₂ causes alterations in the anatomy and chemical composition of needles, and whether elevation of temperature counteracts some of the changes induced by elevated CO₂ concentration.

Material and Methods This study consists of three experiments conducted in different types of facilities for elevation of CO₂ and temperature, either singly or in combination. In a 50-day growth chamber experiment at the University of Kuopio with small Scots pine and Norway spruce seedlings, atmospheric CO₂ and temperature were elevated to levels that are expected to prevail in Finland at the end of this century. At the Mekrijärvi Research Station of the University of Joensuu, the responses of young Scots pine trees to elevated CO₂, elevated temperature and to N-fertilization were studied in a branch bag or in a closed-top chamber experiment over one season or 3 years, respectively. In all experiments, photosynthetic, biochemical and chemical properties of needles were studied. In the chamber experiments, anatomical and ultrastructural parameters of needles were studied and growth of the seedlings was measured.

Results and Conclusions Net photosynthesis in young Scots pine trees was, in general, not stimulated by elevated CO₂ and was reduced by elevated temperature, whereas in the combined treatment, elevated CO₂ and temperature had positive interactions, leading to an unaltered rate of net photosynthesis compared to that under ambient conditions. The lack of photosynthetic stimulation at elevated CO₂ was caused by down-regulation of photosynthetic capacity, which was mainly observed as a reduced concentration and activity of Rubisco, and also as decreases in chlorophyll concentration. Photosynthetic down-regulation was related to a reduced foliar N concentration, and could not solely be explained by an accumulation of starch and end-product inhibition or by an earlier aging of needles, although both of these mechanisms were observable at some point. Although N-fertilization alone had very little effect on the biochemical composition of needles, it counteracted the reduction in foliar N and photosynthetic down-regulation at elevated CO₂, supporting the view that low nutrient levels in the soil may restrict growth responses at elevated CO₂. The elevation of temperature alleviated this constraint by counteracting the reductions in the concentrations of biochemical components and nutrients caused by elevated CO₂, possibly because of faster nutrient mineralization in the soil. Elevated temperature tended to reduce the thickness of mesophyll, vascular cylinder and needle diameter and to decrease stomatal density, whereas elevated CO₂ had little effect on the anatomy. In small seedlings elevated CO₂ did not alter the photosynthetic properties, whereas elevated temperature enhanced reallocation of N from older needles and led to reductions in carboxylation capacity. Higher terpenoid concentrations at elevated temperature may indicate an increased production and emissions of terpenoids and an improved thermotolerance of photosynthesis. The reductions in the amount of Rubisco and in foliar N may have permitted a larger allocation of N to other plant parts to promote increased growth at elevated CO₂. Elevated temperature alone was beneficial for growth of the trees, and growth increased most when both CO₂ concentration and temperature were elevated. These results suggest that carbon sequestration of boreal forests may increase in the future climate, in spite of constraints imposed by low nutrient levels in the soils.

Universal Decimal Classification: 504.73, 581.13, 581.19, 581.4, 582.47, 632.111, 632.151

CAB Thesaurus: carbon dioxide; chlorophyll; conifer needles; plant anatomy; ultrastructure; chemical composition; temperature; growth; nutrients; nitrogen; photosynthesis; forest trees; *Picea abies*; *Pinus sylvestris*; starch; terpenoids; carboxylation; mesophyll; stomata

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Turku, April 2005

Eeva-Maria Luomala

ABBREVIATIONS

ATP	adenosine triphosphate
CBSC	carbon-based secondary compounds
CO ₂	carbon dioxide
Chl a/b	the ratio of chlorophyll a to chlorophyll b
$\Delta F/F_m$	maximal photochemical yield of PSII photochemistry in a dark-adapted state
FACE	free-air CO ₂ enrichment
NADPH	nicotinamide adenine dinucleotide phosphate
NPQ	non-photochemical fluorescence quenching
PSII	photosystem 2
Rbc/Chl	the ratio of Rubisco (Rbc) to chlorophyll (Chl)
Rubisco	ribulose-1,5-bisphosphate carboxylase-oxygenase
RuBP	ribulose-1,5-bisphosphate
SLW	specific leaf weight
TNC	total non-structural carbohydrates

LIST OF ORIGINAL PAPERS

This thesis is mainly based on the following articles, which are referred to in the text by their chapter numbers:

- Chapter 2** Sallas L., Luomala E.-M., Utriainen J., Kainulainen P. & Holopainen J.K. (2003) Contrasting effects of elevated carbon dioxide concentration and temperature on Rubisco activity, chlorophyll fluorescence, needle ultrastructure and secondary metabolites in conifer seedlings. *Tree Physiology* 23, 97-108.
- Chapter 3** Laitinen K., Luomala E.-M., Kellomäki S. & Vapaavuori E. (2000) Carbon assimilation and nitrogen in needles of fertilized and unfertilized field-grown Scots pine at natural and elevated concentrations of CO₂. *Tree Physiology* 20, 881-892.
- Chapter 4** Luomala E.-M., Laitinen K., Kellomäki S. & Vapaavuori E. (2003) Variable photosynthetic acclimation in consecutive cohorts of Scots pine needles during 3 years of growth at elevated CO₂ and elevated temperature. *Plant, Cell and Environment* 26, 645-660.
- Chapter 5** Luomala E.-M., Laitinen K., Kellomäki S. & Vapaavuori E. (2003) Acclimation in Scots pine needles during three years of growth at elevated CO₂ and temperature. *Ekológia (Bratislava)* 22, Supplement 1/2003, 197-202.
- Chapter 6** Luomala E.-M., Laitinen K., Sutinen S., Kellomäki S. & Vapaavuori E. Stomatal density, anatomy and nutrient concentrations of Scots pine needles are affected by elevated CO₂ and temperature. *Plant, Cell and Environment*, in press.

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CHAPTER 1

General introduction

General introduction

1.1 Climate is changing

Since the beginning of industrialization in the 18th century, man has progressively been causing fundamental changes in the composition of the atmosphere and, consequently, in the climate of the Earth. Mainly as a result of the burning of fossil fuels and deforestation, atmospheric CO₂ concentration has increased from the preindustrial 280 $\mu\text{mol mol}^{-1}$ to the current 370 $\mu\text{mol mol}^{-1}$ (IPCC 2001). Given the present rate of increase, 1.5 $\mu\text{mol mol}^{-1}$ per year, CO₂ concentration will reach 700 $\mu\text{mol mol}^{-1}$ by the end of this century (Schimel et al. 1996). A predicted consequence of this increase in CO₂ concentration and in the concentrations of other greenhouse gases (such as methane, nitrous oxide and chlorofluorocarbons) is higher global surface air temperatures. The global mean surface air temperature has increased by 0.6 °C during the 20th century, and recent climate change models predict a further increase of 1.4 - 5.8 °C in the present century (IPCC 2001). Because of the complex interactions between the different elements of the climate system, there will be substantial regional and seasonal variations in the warming, and climate scenarios suggest most warming at high latitudes in the northern hemisphere and during the winter. In northern Europe, this is predicted to result in an increase of 2.5 - 4.5 °C in winter temperatures. During the summer, the range of temperature alterations may be even wider, but the upper limit of the range is about 4.5 °C for southern and northern Europe (Kattenberg et al. 1996). In addition to increasing CO₂ concentration and temperature, climate change also includes alterations in e.g. precipitation, cloudiness and the duration of the snow cover, all of which are difficult to predict and will affect the growth of plants and the functioning of ecosystems.

Over the long term, even small changes in the atmospheric concentration of CO₂ and in temperature are likely to affect plant growth, since both have direct and indirect effects on carbon metabolism and plant development (see the review by Morison & Lawlor 1999). Forests cover about one third of the Earth's land area (Meyer & Turner 1992) and are estimated to account for up to 70% of the terrestrial carbon fixation (Melilo et al. 1993). Thus, even small changes in the carbon metabolism of trees are likely to have a large impact on the global carbon cycling, and will also have a bearing on whether forests will attenuate climate change by acting as a sink of atmospheric CO₂ in the near future.

1.2 Facilities for climate change studies

1.2.1 Man's greatest geochemical and ecophysiological experiment

The greenhouse effect has been and still is vital in facilitating life on the Earth by trapping outgoing infrared radiation and warming the climate, making it suitable for the present life forms to develop and exist. Warmer and colder eras have followed each other in the history of the Earth, and, as a consequence, the existence and diversity of species has changed. The current climate change caused by man differs from previous alterations in the climate in being faster and more intense in terms of warming. With reason it has been called 'man's greatest geochemical experiment' by Roger Revelle, one of the first scientists to detect the signs of climate change. Without doubt, climate change is also a large ecophysiological experiment, which is by no means to understate the economic and social effects that it will cause. Since the 1970's an increasing body of experiments have explored the effects of elevated CO₂, warming, increasing tropospheric ozone and ultraviolet-

B radiation on the physiology of individual plants, and more recent studies have been extended to include whole ecosystems. Nevertheless, our knowledge of the basic molecular and physiological mechanisms that are crucial in predicting the responses of individual plants is deficient, and we are able to give only tentative hints about how whole ecosystems may respond.

1.2.2 Climate change studies from leaves to ecosystems

Most climate change studies have been conducted in greenhouses or in growth chambers with potted plants or seedlings. The advantage of these studies is that climate factors such as temperature, CO₂ concentration and irradiation can be quite accurately controlled and also that conditions in replicate chambers are closely similar to each other. Other interactions between a plant and its biotic and abiotic environment may, however, alter the response to climate change. In addition, growth chamber studies have often been short-term, and have been conducted in small pots, which restrict the growth of the root system and may have led to an artificial acclimation response (Arp 1991, Sage 1994). The use of transparent plastic branch bags around single branches of mature trees to elevate CO₂ concentration allows the experimental set-up to be constructed with minimal disturbance to ground and surrounding vegetation. Initially, utilization of this technique for elevated CO₂ studies was based on the theory that branches are largely independent of the rest of the tree in their carbohydrate metabolism after their first year, and they do not import any carbon and satisfy their own energy and carbohydrate requirements before exporting any carbon to woody tissues and roots (Sprugel et al. 1991). Later, it has become obvious that this is generally not true, because, at the beginning of the growing season, carbohydrates stored in the stem are exported to rapidly expanding new shoots, and in general, the survival and growth of a branch are influenced not only by its own environment but also by the condition

of the whole tree and by the relative position of the branch on the tree (Sprugel 2002). Thus, experiments conducted with branch bags cannot be directly applied in the estimation of the response of entire trees to elevated CO₂. Instead, open-top or closed-top field chambers allow multiyear exposures with entire, young trees growing at their natural sites without restrictions on root growth (e.g. Norby et al. 1999) and studies with small intact ecosystems (e.g. Mooney et al. 1991). The problem with chambers is that they create artificial environmental conditions by altering e.g. radiation, humidity, wind conditions and plant-atmosphere coupling, all of which affect the physiology and growth of plants. A chamber can accommodate only one or a few, usually young, trees, and therefore the results cannot be directly extrapolated to the stand level. Free-air CO₂ enrichment (FACE) technology permits large, open-air plots to be exposed without the confounding effects of chambers, and facilitates studies with trees that may have already reached canopy closure and have a slower growth phase, and with larger communities and ecosystems (see the reviews by Long et al. 2004, Nowak et al. 2004). In the same way, CO₂ springs offer the possibility of studying acclimation in trees that have probably been exposed to elevated CO₂ throughout their lives at natural sites (see e.g. Styliniski et al. 2000 and references therein). Studies at CO₂ springs are, however, complicated by the possible nonuniformity of CO₂ concentrations and by the choice of control sites. To simulate climate warming under realistic field conditions, a technique analogue for FACE, termed free-air temperature increase, has been developed, which uses additional infrared radiation as a warming method (Nijs et al. 1996). An approach creating passive nighttime warming by covering vegetation with reflective curtains at night to reduce heat loss to the atmosphere was recently introduced (Beier et al. 2004). Such experimental studies provide large data sets at various spatial and temporal levels that can be incorporated into mathematical models to predict how individual organisms and ecosystems may respond under future conditions.

1.3 Photosynthesis

1.3.1 Present CO₂ concentration is limiting for net photosynthesis

Almost all of the carbon bound from the air by autotrophic plants is assimilated with the help of Rubisco enzyme (ribulose-1,5-bisphosphate carboxylase-oxygenase, EC 4.1.1.39). Rubisco is located in the stroma of chloroplasts and is the first enzyme to bind CO₂ in the Calvin cycle of the dark reactions of photosynthesis. In the carboxylation reaction, Rubisco catalyzes the binding of CO₂ to a five-carbon sugar, ribulose-1,5-bisphosphate (RuBP). The carboxylation of RuBP produces two molecules of 3-phosphoglycerate, which are reduced in the reactions of the Calvin cycle by the high energy (ATP) and reducing compounds (NADPH) produced in the light reactions of photosynthesis to yield triose phosphates. Triose phosphates are further used for the synthesis of the end-products of carbon fixation, mainly sucrose and starch. Starch is synthesized and stored in the stroma of chloroplasts, whereas sucrose is synthesized in the cytosol, and as a soluble sugar is also the most common transport form of the assimilates.

Oxygen is a competitive inhibitor of CO₂ at the active site of Rubisco (Bowes & Ogren 1972). The oxygenation of RuBP leads to production of 2-phosphoglycolic acid, which is metabolized in a reaction pathway termed photorespiration. Photorespiration leads to a loss of energy and a loss of carbon already bound. Because of the kinetic properties of Rubisco, the present concentration of CO₂ limits the rate of net photosynthesis of C₃ plants (Sharkey 1988, Bowes 1991, 1993, Speitzer & Salvucci 2002). Under present atmospheric conditions the ratio of carboxylation to oxygenation ranges from 2:1 to 3:1, depending on the temperature (Bowes 1991), and it has been estimated that C₃ plants lose 20-25% of the carbon already bound through photorespiration (Sharkey 1988). An increase in CO₂ concentration is thus expected to increase the rate of net

photosynthesis and the production of assimilates.

1.3.2 Photorespiration is faster at higher temperature

With increasing temperature the ratio of photorespiration to carbon fixation increases because the solubility of CO₂ in water decreases more than that of O₂ (Hall & Keys 1983), and the affinity of Rubisco to O₂ increases more than its affinity to CO₂ (Jordan & Ogren 1984, Brooks & Farquhar 1985). As a consequence, the temperature optimum for net photosynthesis increases with increasing CO₂, and the relative stimulation of assimilation by elevated CO₂ should be greater at higher temperature (Long 1991, Speitzer & Salvucci 2002). The stimulation is greater when the activity of Rubisco is limiting the rate of net photosynthesis than when the rate of RuBP regeneration is doing so (Farquhar et al. 1980, Bernacchi et al. 2001, 2003b). When photosynthesis is limited by the rate of triose-phosphate utilization, the rate is independent of CO₂ at all temperatures (Sharkey 1985, Harley & Sharkey 1991).

1.3.3 Acclimation of photosynthesis at elevated CO₂ and temperature

Short-term exposure to elevated CO₂ increases the rate of net photosynthesis in C₃ plants by stimulating the carboxylation reaction catalyzed by Rubisco and by inhibiting the competitive oxygenation reaction (Bowes 1991). Initial stimulation of photosynthesis, however, often decreases during prolonged growth at elevated CO₂, especially with low nutrient availability (e.g. Sage 1994, Thomas et al. 1994, Rogers & Ellsworth 2002). This has been termed down-regulation of photosynthesis and is often associated with reductions in photosynthetic components (mainly Rubisco), in concentrations of foliar nutrients (especially N) and with increases in concentrations of non-structural carbohydrates (mainly starch) (e.g. Sage 1994, Drake et al. 1997, Long et al.

2004). When photosynthesis is measured at ambient CO₂ concentration, down-regulation of photosynthesis leads to a lower rate of net photosynthesis in plants growing at elevated CO₂ compared to that in plants growing at ambient CO₂, which is largely attributable to a loss of active Rubisco (Rogers & Humphries 2000). In spite of a decrease in the photosynthetic capacity, net photosynthesis measured under growth conditions may still be considerably higher at elevated CO₂ than at ambient CO₂ (e.g. Medlyn et al. 1999, Norby et al. 1999, Long et al. 2004). In several experiments with field-grown trees photosynthetic rates have remained high during long-term exposure to elevated CO₂ (e.g. Gunderson et al. 1993, Tissue et al. 1996, Gunderson et al. 2002, Bernacchi et al. 2003a). A reduction in photosynthetic capacity at elevated CO₂ is not a general, nor is it a species-specific phenomenon. The long-term effect of elevated CO₂ on photosynthesis also depends on other environmental factors, e.g. temperature, water availability and nutrient supply, and on the ability of the plant to use or store carbohydrates.

Because of the kinetic properties of Rubisco, the temperature optimum for assimilation increases with increasing CO₂, and the relative stimulation of assimilation by elevated CO₂ should be greater at higher temperatures (Long 1991). This is supported by some (Kellomäki & Wang 1996, Wang & Kellomäki 1997, Tjoelker et al. 1998) but not all studies (Wang et al. 1995, Tjoelker et al. 1998, Lewis et al. 2004) conducted at elevated CO₂. Net photosynthesis at elevated temperature cannot, however, be calculated on the basis of short-term temperature responses, since photosynthesis acclimates to the growth temperature (Berry & Björkman 1980). During acclimation, the temperature optimum of photosynthesis shifts so that the highest rates of net photosynthesis are frequently measured at temperatures that are near to the growth temperature (Berry & Björkman 1980, Hikosaka et al. 1999, Teskey & Will 1999, Turnbull et al. 2002). The relative enhancement of photosynthesis by

elevated CO₂ is not necessarily greater at elevated temperature, since the temperature dependence of photosynthesis changes seasonally in many species (Bunce 2000, Lewis et al. 2001). Temperature acclimation of photosynthesis probably involves modifications in several components of the photosynthetic apparatus, such as chloroplast membrane lipids and Rubisco (see Berry & Björkman 1980). In addition to alterations in the amount of Rubisco (Hikosaka et al. 1999), the kinetic properties of Rubisco may also change during acclimation to elevated temperature (Bunce 2000), affecting the temperature dependence of RuBP carboxylation. In general, however, modest increases in temperature are considered to be beneficial for photosynthesis of temperate trees (Saxe et al. 2001).

The use of the term 'down-regulation' has sometimes been confusing, because it has been used liberally from describing a lower rate of net photosynthesis measured at any common CO₂ concentration to reductions in the amounts of photosynthetic components. The term 'acclimation' may better describe the alterations often observed at elevated CO₂ (Long et al. 2004), since concentrations of biochemical components may decrease and allocation of nutrients may change, but net photosynthesis measured under growth conditions may still be higher at elevated CO₂ than at ambient CO₂. In general, acclimation is understood as biochemical and physiological alterations that improve the performance (in this case photosynthesis and growth) of a plant under altered conditions by increasing the efficiency of the use of the resources. In this study, down-regulation is used to describe a reduction in biochemical components of photosynthesis, regardless of whether it improves N use efficiency and growth or not, while acclimation is used to describe alterations that probably are beneficial with regard to carbon fixation and growth of a plant.

1.3.4 Mechanisms and nature of photosynthetic acclimation are still unclear

In some earlier studies at elevated CO₂ it was noticed that a decline in photosynthetic capacity was often the result of a lower amount and activity of Rubisco in leaves and was associated with increased amounts of nonstructural carbohydrates (e.g. Delucia et al. 1985, Sage et al. 1989). This led to suggestions that down-regulation of photosynthesis was caused by an accumulation of carbohydrates in the source leaves, which, directly or indirectly, resulted in a feedback-inhibition of photosynthesis (Azcón-Bieto 1983, Foyer 1988). Since then, it has been shown that the expression of various photosynthetic genes, including that of the small subunit of Rubisco, are regulated by the end-products of photosynthesis (reviewed in Smeekens 2000, Rolland et al. 2002). Evidently, carbohydrates and enzymes of sugar metabolism, especially hexokinases, play a role in signal transduction pathways leading to down-regulation of photosynthetic gene expression (Smeekens 2000, Rolland et al. 2002).

The exact reasons and mechanisms leading to down-regulation of photosynthetic capacity are still under debate and active research. Reductions in photosynthetic capacity at elevated CO₂ are usually more marked when the supply of nitrogen (N) is low (e.g. Petterson et al. 1993, Petterson & McDonald 1994, Thomas et al. 1994), and down-regulation has been related to an increased demand for N and to a reduced C/N ratio of leaves at elevated CO₂ (Petterson et al. 1993, Paul and Driscoll 1997). In many studies, photosynthetic acclimation has occurred only in older foliage, or it has taken place earlier in older leaves than in younger ones (in conifers e.g. Turnbull et al. 1998, Griffin et al. 2000, Jach & Ceulemans 2000, Tissue et al. 2001, Rogers & Ellsworth 2002, Crous & Ellsworth 2004). It has been suggested that photosynthetic acclimation may in fact be an enhanced remobilization of nutrients and also an earlier aging or senescence caused by N-deficiency, or an ontogenetic drift caused by

the altered timing of growth (see the review by Stitt & Krapp 1999).

Conflicting responses and interactions with other environmental factors at elevated CO₂ have also been related to the so-called source-sink balance of plants (e.g. reviewed by Wolfe et al. 1998, Paul & Foyer 2001). A source is formed by photosynthesizing leaves that produce carbohydrates in excess of their own needs and export them to other plant parts, the sinks. The sink strength of a plant refers to the ability of a plant to use carbohydrates for respiration, growth, storage, root exudates and so on. The sink strength of a plant is regulated by e.g. temperature, water and nutrients, and by all environmental and genetic factors that affect growth and usage of assimilates (Paul & Foyer 2001). If the production of carbohydrates in the sources exceeds the rate of transport or utilization in the sinks, non-structural carbohydrates accumulate in the sources, which is thought to lead to the feed-back inhibition of photosynthesis discussed above. Carbohydrates probably do not, however, alone mediate the source-sink regulation of photosynthesis, as the source-sink balance is most probably controlled in a close interaction with the nitrogen status (Paul & Foyer 2001). The long-term response of photosynthesis to elevated CO₂ varies with the canopy position (Tissue et al. 2001, Crous & Ellsworth 2004), during the ontogenetic development of seedlings (Kellomäki & Wang 2001) and during the growing season (Hymus et al. 1999, 2001, Stylinski et al. 2000), which may be related to changes in the source-sink balance and in the demand for and allocation of nitrogen. An elevation of temperature most probably increases the rate of end-product synthesis and accelerates the transport of carbohydrates from sources to sinks, and also enhances the sink metabolism by increasing the rates of metabolic processes (Farrar & Williams 1991), and thus may prevent an accumulation of carbohydrates and down-regulation of photosynthesis at elevated CO₂.

1.3.5 Alterations in the light reactions of photosynthesis at elevated CO₂ and temperature

In the light reactions of photosynthesis, energy of solar radiation is bound to high-energy compounds (ATP) and to reducing compounds (NADPH), which are subsequently used in the dark reactions of photosynthesis for assimilation of carbon. The initial slope of the light response curve of net photosynthesis, i.e. the maximum quantum yield of CO₂ uptake, is determined by the rate of RuBP regeneration and is limited by light. An elevation in CO₂ concentration increases the quantum yield and the rate of light-limited photosynthesis because less ATP and NADPH are used for photorespiration. In accordance with these theoretical expectations, increases in quantum yield at elevated CO₂ have been observed (Long et al. 2004), but significant reductions have also occurred (Wang 1996), as well as contrasting alterations in chlorophyll concentration (Saxe et al. 1998). Reductions in the ratio of chlorophyll a to b (Chl a/b) have been related to an increased thickness and concurrently higher internal shading in leaves (Arp & Drake 1991), which leads to an increase in the proportion of the Chl b-containing light-harvesting antennas in relation to the Chl a-containing photosystem II (PSII) reaction centre complexes (Evans 1989). Soluble sugars may also regulate the expression of chlorophyll binding proteins (see Moore et al. 1999, Stitt & Krapp 1999).

When carboxylation capacity limits the rate of net photosynthesis, an elevation of CO₂ concentration should increase the utilization of the captured light energy for photochemistry and also increase the linear electron flow through PSII (Hymus et al. 1999). This would reduce the photodamage of the PSII reaction centres under high irradiance, or alternatively, decrease the employment of photoprotective mechanisms in the dissipation of excess excitation energy (Hogan et al. 1997, Hymus et al. 1999). These changes would be observable as a reduced content and activity of xanthophyll cycle

pigments and an improved photochemical efficiency of PSII, measured as chlorophyll fluorescence. Elevated CO₂ has, however, had contrasting effects on photochemical yield in tree species (e.g. Saxe et al. 1998). In some studies, alterations in electron transport and photochemical quenching reflected seasonal differences in photosynthetic acclimation at elevated CO₂ (Hymus et al. 1999, 2001, Stylinski et al. 2000). In overwintering conifers, seasonal acclimation of photosynthetic capacity also involves alterations in concentrations of chlorophyll, chlorophyll-binding protein and PSII reaction centre complexes and in the capacity of the xanthophyll cycle to dissipate excess light energy (Vogg et al. 1998, Öquist & Huner 2003), all of which are observable, for example, as a strong correlation between the daily mean temperature and the photochemical efficiency of PSII in Norway spruce during the spring recovery of photosynthesis (Lundmark et al. 1998).

1.3.6 Nitrogen and photosynthesis

Rubisco is the most common protein in the biosphere (Ellis 1979) and the major pool of N in leaves (Ellis 1979, Rintamäki et al. 1988). In general, there is a strong correlation between the concentration of N, photosynthetic capacity, and the concentration of Rubisco in leaves (Evans 1989, Evans & Seeman 1989), although in conifers this correlation may be weaker than in herbaceous plants (e.g. Vapaavuori et al. 1995, Medlyn et al. 1999).

The relative allocation of N to the different functions of photosynthesis is affected by the N concentration in the leaf, as the proportion of N bound to Rubisco decreases with a decreasing concentration of foliar N (Evans 1989, Evans & Seeman 1989). At elevated CO₂, when the carboxylation capacity may exceed the capacity of RuBP regeneration (Farquhar et al. 1980) or the capacity for end-product synthesis and triose-phosphate utilization (Sharkey 1985, Harley & Sharkey 1991), the amount of Rubisco may be reduced

even further, and the N bound in Rubisco may be reallocated to limiting components of photosynthesis, to other metabolic processes in the leaves, or to other plant parts in order to promote growth (Sage et al. 1989, Makino et al. 1997). Both elevated CO₂ (e.g. Medlyn et al. 1999, Stitt & Krapp 1999) and temperature (e.g. Tjoelker et al. 1999) may decrease foliar N concentration and lead to a reallocation of N within a plant. This has frequently been interpreted as an improved efficiency in the use of N because of higher rates of photosynthesis or greater growth per unit of N (Drake et al. 1997, Peterson et al. 1999). It is not, however, easy to distinguish whether a lower N concentration at elevated CO₂ is a direct consequence of elevated CO₂, or whether it is related to the accessibility of N (see Farage et al. 1998, Stitt & Krapp 1999). A decrease in the N concentration of a plant may simply be caused by a lower availability of N in the soil or by size-dependent dilution of N resulting from accelerated plant growth at elevated CO₂ (Stitt & Krapp 1999). Similarly, a reallocation of N within the photosynthetic apparatus may not be a specific optimization of the N use at elevated CO₂ or temperature, but rather a normal reallocation of N, reflecting a general decline in leaf N content and in the investment of N in proteins (Nakano et al. 1997, Farage et al. 1998, Theobald et al. 1998, Harmens et al. 2000). In contrast, on the basis of several FACE studies it has been proposed that the loss of Rubisco is a selective change that can be more appropriately described as an acclimatory alteration benefiting the efficiency of the N-use rather than as down-regulation of photosynthesis (Rogers & Ellsworth 2002, Long et al. 2004). Whichever is the case, flexibility in the amount of N bound to Rubisco and in the concentration of foliar N may improve the responsiveness of biomass production at elevated CO₂, but may also have serious consequences at the ecosystem level, as any changes in the quality of the leaves will have the potential to affect e.g. herbivores, decomposition and nutrient cycling.

1.4 Stomata and the use of water at elevated CO₂ and temperature

1.4.1 Stomatal density may decrease at elevated CO₂

Stomata are small pores surrounded by a pair of guard cells on the surfaces of leaves and stems that control gas exchange between plants and the atmosphere. Stomatal density sets the limit for the maximal stomatal conductance of gas exchange and thus has the potential to affect the water use efficiency of a plant (Beerling 1997) and the water economy of ecosystems. Stomatal formation and patterning differ in dicotyledous angiosperms and conifers. In dicotyledons, stomata are initiated at multiple points on the surfaces of the developing leaves, while in conifers epidermal cells and stomata are initiated at the base of the needle, developing in longitudinal files during needle growth (Croxdale 2000). Recently, factors regulating the development of the leaf epidermis and the differentiation of the stomata and guard cells have to some extent been uncovered, and it has been shown that environmental factors affecting stomatal density may be mediated by long-distance signalling from mature to newly developing leaves (Lake et al. 2001) and that the wax composition of the guard cell cuticle may be involved in the signalling (see reviews by Lake et al. 2002, Bird & Gray 2003).

Stomatal frequency of fossil plant samples has been used to estimate atmospheric CO₂ concentration in past environments (e.g. Retallack 2001, Royer 2001), since there exists an inverse correlation between stomatal frequency and growth CO₂ concentrations observed in herbarium material (e.g. Woodward 1987, Peñuelas & Matamala 1990, Woodward & Kelly 1995) and in experiments conducted in controlled environments (e.g. Woodward 1987, Woodward & Bazzaz 1988, Woodward & Kelly 1995, Beerling et al. 1998). The inverse relationship between stomatal frequency and atmospheric CO₂ concentration is more apparent at CO₂ concentrations that are lower than the ambient concentration (e.g. Woodward 1987,

Woodward & Bazzaz 1988), and in some woody shrubs the sensitivity of the stomatal response to an elevation of CO₂ concentration declines at concentrations exceeding approximately 350 μmol mol⁻¹ (Woodward & Bazzaz 1988, Woodward & Kelly 1995). In short-term experiments at approximately twice the ambient CO₂ concentration observations have been variable, ranging from no changes (e.g. Reddy et al. 1998, Vanhatalo et al. 2001, Vuorinen et al. 2004) to reductions (e.g. Ferris & Taylor 1994, Ferris et al. 1996, 2002, Lin et al. 2001, Tognetti et al. 2001) or increases (e.g. Ferris & Taylor 1994, Ferris et al. 1996, 2002, Visser et al. 1997) in stomatal density. These observations suggest that the maximum effect of rising CO₂ concentration on stomatal numbers may have already been reached. Stomatal density of Scots pine has, however, decreased in response to elevated CO₂ (Beerling 1997, Lin et al. 2001), indicating that stomatal density in Scots pine may be more sensitive to CO₂ concentrations expected to prevail in the near future than that of some other conifers (Pritchard et al. 1998, Apple et al. 2000). Studies exploring the interactive effects of elevated CO₂ and temperature on stomatal numbers are limited (Beerling & Chaloner 1993, Morgan et al. 1994, Ferris et al. 1996, Beerling 1997, Reddy et al. 1998, Apple et al. 2000). The responses of stomatal density or index to experimental or seasonal warming have been variable, displaying no alterations, increases or decreases in stomatal frequency (Beerling & Chaloner 1993, Morgan et al. 1994, Ferris et al. 1996, Beerling 1997, Reddy et al. 1998, Apple et al. 2000).

1.4.2 Stomatal opening responds to changes in CO₂ concentration

Under fluctuating environmental conditions, the opening and closure of stomata is finely attuned to maximize the CO₂ uptake and the efficiency of the light utilization for photosynthesis, and to minimize the water loss in the absence of light harvesting or during a water deficit. In the short-term, an elevation of CO₂ concentration causes

reductions in stomatal aperture and stomatal conductance, which reduces transpiration and, together with increased photosynthesis, leads to an improved water use efficiency (the amount of water transpired per the amount of carbon fixed). The mechanism by which stomata respond to changes in CO₂ concentration is still unclear, but recent studies suggest that starch degradation and carbon import from the guard cell apoplast are important in promoting and maintaining stomatal opening (reviewed in Vavasseur & Raghavendra 2005). Despite a partial closure of stomata at elevated CO₂, the ratio of intercellular CO₂ concentration to atmospheric CO₂ has been found to remain relatively constant across a wide range of conditions and plant species (Drake et al. 1997, Long et al. 2004), and thus the limitation that stomata place on photosynthesis is diminished at elevated CO₂, while transpiration is greatly reduced and the water use efficiency increased (Long et al. 2004). In field experiments stomatal conductance has exhibited large reductions during long-term growth at elevated CO₂ (Long et al. 2004). In woody plants, however, the response of stomatal conductance to elevated CO₂ has been very variable (Curtis & Wang 1998, Saxe et al. 1998, Norby et al. 1999, Medlyn et al. 2001), and stomatal conductance of conifers has decreased less than that of deciduous broadleaved species under long-term growth at elevated CO₂ (Saxe et al. 1998, Medlyn et al. 2001). It has been hypothesized that the sensitivity of stomata to close in response to increasing leaf-to-air vapour pressure difference may decrease at elevated CO₂ (Heath 1998, Maherali et al. 2003), which would lead to an increased risk of drought damage during high evaporative demand (Heath 1998). Not all studies have, however, supported this (Gunderson et al. 2002). Stomata may also acclimate to growth temperature, as plants growing in cooler conditions have shown lower stomatal conductance and intercellular CO₂ concentration, independent of the measurement temperature (Hikosaka et al. 1999).

The total water use and overall water status of a plant are affected not only by stomatal conductance but also by the total area of transpiring leaves, which has shown increases at elevated CO₂ (e.g. Riikonen et al. 2004) and reductions at elevated temperature (e.g. Olszyk et al. 2003), and also by the temperature of leaves, which may increase as a result of negative feedback from lower stomatal conductance and transpiration on evaporative cooling (see Drake et al. 1997). In general, however, stand transpiration has decreased and soil water content has increased at elevated CO₂ (Drake et al. 1997).

1.5 Anatomy and ultrastructure of leaves in a changing climate

While biochemical acclimation at elevated CO₂ has received intensive attention in recent decades, the anatomical features of leaves in a changing climate have been studied less. The anatomy of leaves is, however, highly flexible, and is modified by environmental factors such as irradiation (sun leaves/shade leaves, e.g. Lambers et al. 1998), nutrients (e.g. Jokela et al. 1998), drought (e.g. Bosabalidis & Kofidis 2002) and ozone (e.g. Oksanen et al. 2001, 2004). Anatomical changes in the mesophyll and vascular elements are likely to affect gas exchange by altering the resistance for CO₂ diffusion and to influence water transport. They are also likely to affect assimilate transport and thus the capacity to exploit extra carbon produced at elevated CO₂.

Elevated CO₂ has been found to stimulate cell division (Ferris & Taylor 1994, Kinsman et al. 1996, Masle 2000, Ferris et al. 2001) and cell expansion (Ferris & Taylor 1994, Taylor et al. 1994, Masle 2000, Ferris et al. 2001), and to result in thicker leaves (Yin 2002) with higher numbers of cells or cell layers and/or larger cells (Radoglou & Jarvis 1992, Masle 2000). Alterations in the relative volumes occupied by intercellular air spaces (Masle 2000, Oksanen et al. 2001), palisade and spongy mesophyll and vascular elements (Pritchard et al. 1997, Lin et al. 2001,

Oksanen et al. 2001, Engloner et al. 2003) have also occurred at elevated CO₂. The rate of cell division is tightly regulated by temperature, and the number of cell divisions involved in the formation of a new leaf is drastically reduced in cold climates (Körner & Larcher 1988). Plants belonging to a variety of functional types commonly have thicker leaves, thicker epidermal cell walls and higher stomatal density when growing in cool climates than in warmer climates (Körner & Larcher 1988, Loveys et al. 2002). Needle length in Scots pine is strongly dependent on the temperature of the current growing season (Junttila & Heide 1981, Junttila 1986). In Douglas fir, elevated temperature increased the elongation rate of the needles, but the net effect of temperature on needle length varied year by year (Olszyk et al. 1998, Apple et al. 2000). On the basis of these observations, an elevation of temperature could be expected to lead to formation of thinner and possibly longer needles with less stomata than at ambient temperature. The interactive effects of elevated CO₂ and temperature on the anatomy of leaves (Ferris et al. 1996) have not been widely studied.

1.6 Chemical composition of leaves is altered as well

1.6.1 Could an elevation of temperature compensate for reductions in nutrient concentrations at elevated CO₂?

Reductions in the foliar concentration of N, increases in the C/N-ratio and in concentrations of non-structural carbohydrates are evidently the most common alterations observed across different types of C₃ plants growing at elevated CO₂ (e.g. Drake et al. 1997, Poorter et al. 1997, Cotrufo et al. 1998, Curtis & Wang 1998, Medlyn et al. 1999, Yin 2002, Long et al. 2004, Nowak et al. 2004). Concentrations of other mineral nutrients, especially that of mobile nutrients have also changed, mostly by decreasing (Conroy et al. 1992, Medlyn et al. 1999, Roberntz & Linder 1999, Sigurdsson 2001). These lower concentrations of nutrients in

foliage may be related to an inadequate nutrient availability in relation to an increased growth at elevated CO₂, as has already been discussed in the case of N, or to an indirect dilution effect caused by an accumulation of non-structural carbohydrates. Mineral nutrition is indirectly coupled with alterations in stomatal frequency and in stomatal conductance, since lower transpiration stream and reduced use of water cuts down the mass flow of some nutrients (e.g. K) to the root surfaces and diminishes their uptake (Van Vuuren et al. 1997). An elevation of CO₂ and temperature may also lead to changes in the allocation of growth (Veteli et al. 2002, Olszyk et al. 2003) and in the allocation of nutrients (Makino et al. 1997, Hobbie et al. 2001) within a plant and thus alter nutrient concentrations in the leaves. At elevated temperature the nutrient supply in the soil could be expected to increase, since soil respiration and mineralisation of nutrients are strongly dependent on the temperature (Bonan & Van Cleve 1992), and experimental warming stimulates soil respiration and below-ground carbon cycling (Rustad et al. 2001, Pendall et al. 2004). Thus, elevated temperature could compensate for a decrease in nutrient concentration at elevated CO₂. Similarly, higher concentrations of foliar N have been observed in conifers growing at elevated temperature (Kellomäki & Wang 1997, Hobbie et al. 2001, Lewis et al. 2004), but reductions have also been reported (Tjoelker et al. 1999). Concentrations of nutrients are tightly linked with biochemical capacities for photosynthesis and growth, but may also regulate the anatomy of leaves (Jokela et al. 1998).

1.6.2 Production of carbon-based secondary compounds may increase in a future climate

Secondary metabolites (e.g. simple phenolics, lignin, flavonoids, tannins and terpenes) are a large, diverse array of organic compounds that function, i.a., in defence, communication and protection against extreme conditions, but many of the compounds still play unknown

roles in plant biochemistry. Several hypotheses have been put forward to predict the production of carbon-based secondary compounds (CBSC) and total non-structural carbohydrates (TNC) in relation to carbon supply, nutrient availability and growth (Loomis 1932, Bryant et al. 1983, Herms & Mattson 1992, Haukioja et al. 1998, Jones & Hartley 1998). Common to these models is that they are based on source-sink relationships, which link carbon and nitrogen metabolisms and which affect the relative carbon pool available for allocation to carbon-based secondary compounds. Frequently reported decreases in the foliar concentration of N, increases in the C/N-ratio and in concentrations of non-structural carbohydrates at elevated CO₂ (e.g. Drake et al. 1997, Poorter et al. 1997, Curtis & Wang 1998, Medlyn et al. 1999, Long et al. 2004, Nowak et al. 2004) have led to suggestions that the production of carbon-based secondary compounds would increase at elevated CO₂. Experimental evidence has, however, shown contrasting effects of elevated CO₂ on different groups of secondary compounds (reviewed by Koricheva et al. 1998, Peñuelas & Estiarte 1998, Peñuelas et al. 2002).

All plants emit a substantial fraction of their assimilated carbon into the air as phytogetic volatile organic compounds (PVOCs), which have a great effect on the chemical reactivity and composition of the atmosphere, and their functions in plants, if any, are largely unknown (Peñuelas & Llusà 2004). An elevation of temperature will most likely increase the production of PVOCs, of which isoprene is the most abundant (see Sharkey & Yeh 2001, Peñuelas & Llusà 2004). Isoprene may increase the thermotolerance of plants by stabilizing and protecting membranes (Sharkey & Singsaas 1995, Singsaas et al. 1997, Sharkey & Yeh 2001, Peñuelas et al. 2005) and may serve as an antioxidant in leaves (Loreto et al. 2001, Peñuelas et al. 2005). Emissions of monoterpene and isoprene will probably increase in Finland partly as a result of the direct effect of temperature on the emission rates and partly as a result of changes in the distribution of

Scots pine, Norway spruce and birches in the future climate (Kellomäki et al. 2001). Increased emissions may trigger further alterations in the climate and have an effect on the carbon sequestration of forests.

A few studies have been conducted on the responses of secondary metabolites in trees to a combination of CO₂ enrichment and elevated temperature (Kuokkanen et al. 2001, Veteli et al. 2002, Kuokkanen et al. 2004), in particular with conifers (Constable et al. 1999, Litvak et al. 2002, Snow et al. 2003). In these studies, elevated temperature has most commonly decreased concentrations of some phenolic compounds (Kuokkanen et al. 2001, Veteli et al. 2002, Kuokkanen et al. 2004), and generally there have been no interactions of CO₂ and temperature (Kuokkanen et al. 2001, Veteli et al. 2002, but see also Kuokkanen et al. 2004). Alterations in plant secondary metabolism at elevated CO₂ and temperature may have implications for plant-herbivore (Veteli et al. 2002, Kuokkanen et al. 2004) and plant-pathogen interactions, decomposition of litter, and carbon and nutrient cycling (Hättenschwiler & Vitousek 2000), although some studies have shown that, despite alterations in the quality of litter, the changes in decomposition rates at elevated CO₂ may be minor (Peñuelas & Estiarte 1998, Norby et al. 2001, Kainulainen et al. 2003).

1.7 Photosynthesis at canopy level and the growth of trees at elevated CO₂ and temperature

A wealth of experiments conducted at elevated CO₂ have shown substantial increases in light-saturated net photosynthesis, on average by over 50% in controlled environment and OTC studies (Curtis & Wang 1998, Medlyn et al. 1999, Norby et al. 1999), and by around 30% in FACE studies (Long et al. 2004, Nowak et al. 2004). The stimulation of net photosynthesis by elevated CO₂ has been sustained for several years, and it has occurred despite increases in starch concentration and reductions in Rubisco content and down-

regulation of photosynthetic capacity (Long et al. 2004). This poses the question of whether the stimulation of photosynthesis at leaf level extrapolates to an increased photosynthesis and production for a whole plant or at ecosystem level. Some studies with trees have suggested that the primary advantage of higher CO₂ concentration on growth is derived from an initially and temporarily increased growth rate that results in larger trees that grow at the same relative rate as trees at ambient CO₂ (e.g. Tissue et al. 1997, Centritto et al. 1999, Kellomäki & Wang 2001). In contrast, some of the FACE experiments in which trees have reached canopy closure and no longer have the additional sink provided by an exponential growth show that photosynthetic stimulation is still sustained (Gunderson et al. 2002, Crous & Ellsworth 2004). Low nutrient levels in the soil may, however, be a serious constraint limiting the carbon fixation of boreal forest ecosystems in a future climate (Oren et al. 2001). Further, as Morison and Lawlor (1999) point out, net photosynthesis per leaf area is not the most important factor in determining overall growth. The total supply of carbohydrates is a function of net photosynthesis and leaf area, and growth is ultimately regulated by the relationship of carbohydrate supply to sink demand for growth, respiration, storage and other metabolic processes. Increased photosynthetic rates at elevated CO₂ concentration have not always been associated with equivalent increases in above-ground biomass (e.g. Ceulemans & Mousseau 1994), and it has been noted that the production of PVOCs (see Peñuelas & Llusà 2004) as well as fine root turnover, mycorrhizal interaction and exudation of organic carbon into the soil (reviewed in Pendall et al. 2004) form a substantial sink of assimilated carbon. Nevertheless, greenhouse and OTC studies (Curtis & Wang 1998), as well as FACE experiments (Long et al. 2004) have shown that the overall biomass production of C₃ plants was about 31% and 20% greater, respectively, at elevated CO₂ than at ambient CO₂. On average, there were no significant increases in the leaf area index at elevated

CO₂ (Drake et al. 1997, Long et al. 2004), suggesting that an increased production results from higher photosynthesis per unit leaf area rather than from a larger assimilating leaf area.

An elevation of temperature affects carbon metabolism by increasing the rates of enzymatic reactions, such as photorespiration and dark respiration, but also by changing the timing and length of the growing season (Beuker 1994, Peltola et al. 2002) and by altering the phenology of plants (Peñuelas & Filella 2001, Badeck et al. 2004). A simulation model predicts an increase in the net primary production of coniferous stands as a result of warming in the Nordic countries, the increases being largely related to an earlier start of the growing season and a more rapid recovery of the winter-damaged photosynthetic apparatus, although temperature-driven increases in respiration would reduce the carbon gain (Bergh et al. 2003). An elevation of CO₂ further increased net primary production in this model (Bergh et al. 2003). Premature dehardening caused by warmer winter temperatures may, however, increase the risk of frost damage and cause yield losses in the boreal forests (Repo et al. 1996, Taulavuori et al. 2004).

1.7.1 Photosynthetic characteristics of Scots pine and Norway spruce

In Scots pine, 95% of the annual net photosynthesis may occur between May and October (Troeng & Linder 1982). In Scots pine and Norway spruce, variation in net photosynthesis from year to year is mainly caused by differences in regaining the full photosynthetic capacity in 1-year-old foliage and in the rate of development of current-year needles (Troeng & Linder 1982, Lundmark et al. 1998). These are closely related to the temperature (Troeng & Linder 1982, Lundmark et al. 1998), as is the photosynthetic capacity in general across the entire growing season (Öquist & Huner 2003). Temperature, along with day length, is also an important factor in regulating e.g. the

start of bud burst (Koski 1990), the rate of shoot elongation (Junttila 1986) and the length of the shoot elongation period (Oleksyn et al. 1998). Thus, even small changes in mean temperature could be of great importance for the total biomass production of Scots pine and Norway spruce, and slight differences in the responses of younger and older needles to elevated CO₂ and temperature have the potential to affect the carbon and nutrient balance of a tree and consequently, the carbon sink of boreal forests. Provenance experiments have shown that an increase in the annual mean effective temperature sum close to that expected in northern areas would increase the wood production of Scots pine (Beuker 1994, Persson & Beuker 1997). However, because of the complex interaction of CO₂ and temperature on the balance between the assimilate supply and the sink activity via changes in photosynthesis, respiration and growth of organs (see review by Morison & Lawlor 1999), the growth response in a future climate is extremely difficult to predict.

1.8 Aims and overview of the present study

The aim of the present study was to explore photosynthesis, needle composition, needle anatomy and growth in two boreal conifers, Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.), growing at elevated atmospheric CO₂ concentration and temperature. The objective was to study, whether elevated CO₂ and temperature are beneficial for photosynthesis of Scots pine and Norway spruce (Chapters 2, 3 and 4), and whether photosynthetic stimulation is sustained during prolonged growth at elevated CO₂ and temperature (Chapters 3 and 4). The specific aim was to study signs of down-regulation of photosynthetic capacity and possible mechanisms involved in down-regulation (Chapters 2, 3, 4, 5). Further, close attention was paid to whether down-regulation of photosynthetic capacity is related to reductions in foliar N concentration at elevated CO₂ (Chapters 2, 3, 4 and 5), and whether an increased supply of N in the soil

Table 1. Overview of the experiments included in this study.

Material	Exposure method	Treatments	No of replicates	Duration of exposure	Chapter
Scots pine seedlings 1-year-old	Growth chambers	Ambient CO ₂ (325±5 ppm) + ambient temperature (19/12 °C) Elevated CO ₂ (731±15 ppm) + ambient temperature Ambient CO ₂ + elevated temperature (23/16 °C) Elevated CO ₂ + elevated temperature	6 seedlings per treatment	50 days	2
Norway spruce seedlings 2-year-old	Growth chambers	Ambient CO ₂ (325±5 ppm) + ambient temperature (19/12 °C) Elevated CO ₂ (731±15 ppm) + ambient temperature Ambient CO ₂ + elevated temperature (23/16 °C) Elevated CO ₂ + elevated temperature	6 seedlings per treatment	50 days	2
Young Scots pine trees 25-30 years old Scots pine trees growing in a naturally regenerated stand	Branch bags	Ambient CO ₂ (330-420 ppm) Elevated CO ₂ (670-820 ppm) Unbagged control branch	One branch per treatment in a tree, 6 replicative trees	April 15 - Sept 15	3
		Ambient CO ₂ (330-420 ppm) Elevated CO ₂ (670-820 ppm) Unbagged control branch + N-fertilization applied in June	One branch per treatment in a tree, 6 replicative trees	April 15 - Sept 15	3
Young Scots pine trees 25-30 years old Scots pine trees growing in a naturally regenerated stand	Closed-top chambers (CTCs)	Ambient CO ₂ (362±43 ppm) + ambient temperature Elevated CO ₂ (693±30 ppm) + ambient temperature Ambient CO ₂ + elevated temperature (ambient +2.8 - +6.2°C) Elevated CO ₂ + elevated temperature	4 trees per treatment	3 years, starting in Aug 1996	4, 5, 6

(Chapter 3) or elevated temperature (Chapters 2, 4 and 5) are able to counteract these alterations. The objective was also to study whether an elevation of CO₂ causes changes in the anatomy and chemical composition of the needles that are connected with the biochemical alterations and may have implications on carbon assimilation and water use, and whether an elevation of temperature compensates for some of these changes (Chapters 2 and 6). Finally, the study concentrated on whether elevated CO₂ and temperature increase the growth of Scots pine and Norway spruce seedlings (Chapter 2).

This study consists of three experiments conducted in different types of facilities used for elevation of CO₂ concentration and temperature, either singly or in combination (Table 1). One of the experiments was conducted with small Scots pine and Norway spruce seedlings growing in growth chambers at the University of Kuopio (Chapter 2). The other two experiments were conducted at the

Mekrijärvi Research Station of the University of Joensuu with young Scots pine trees growing at a naturally regenerated forest site using either branch bags (Chapter 3) or closed- top chambers (CTC) (Chapters 4-6) as an exposure system. In the studies with seedlings growing in growth chambers (Chapter 2) and with young Scots pine trees growing in CTCs (Chapters 4-6), atmospheric CO₂ concentration and temperature were elevated to levels that are expected to prevail in Finland at the end of this century. In the study with young Scots pine trees with branch bags (Chapter 3), atmospheric CO₂ concentration was similarly doubled, and, in addition, N-fertilizer was applied to half of the trees. In all of the experiments, photosynthetic, biochemical and chemical properties of the needles were studied. In the growth chamber and CTC experiments, some anatomical and ultrastructural parameters of the needles were studied, and the growth of the seedlings was measured.

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CHAPTER 2

Contrasting effects of elevated carbon dioxide concentration and temperature on Rubisco activity, chlorophyll fluorescence, needle ultrastructure and secondary metabolites in conifer seedlings

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CHAPTER 3

Carbon assimilation and nitrogen in needles of fertilized and unfertilized field-grown Scots pine at natural and elevated concentrations of CO₂

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CHAPTER 4

Variable photosynthetic acclimation in consecutive cohorts of Scots pine needles during 3 years of growth at elevated CO₂ and elevated temperature

Luomala E.-M., Laitinen K., Kellomäki S. & Vapaavuori E. (2003)
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CHAPTER 5

Acclimation in Scots pine needles during three years of growth at elevated CO₂ and temperature

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Ekológia (Bratislava) 22, Supplement 1/2003, 197-202.

CHAPTER 6

Stomatal density, anatomy and nutrient concentrations of Scots pine needles are affected by elevated CO₂ and temperature

Luomala E.-M., Laitinen K., Sutinen S., Kellomäki S. & Vapaavuori E.
Plant, Cell and Environment, in press.

CHAPTER 7

General discussion

General discussion

7.1 Methodological considerations

7.1.1 Experimental conditions affect the interpretation of the results

A general feature of all the three experiments presented in this thesis is the low nutrient availability of the soils (see Niinistö et al. 2004 for a closer description of the soil at the Mekrijärvi site, Chapters 3-6). This offers a realistic situation for studying the responses of boreal conifers to elevation of CO₂ concentration and temperature, since it has been suggested that their growth responses at elevated CO₂ are limited by a low nutrient supply (Oren et al. 2001), and an elevation of temperature may have crucial implications for the nutrient cycling.

The presence of a chamber or a branch bag alters the microclimate around a plant. In this study, the branch bags cut about 10% of the radiation in the spectral range of 400-800 nm (Wang & Kellomäki 1997a), whereas the chambers cut the total solar radiation by 40-50% (Kellomäki et al. 2000). During the main part of the day temperature and relative humidity inside the bags were only slightly higher than ambient values, except during periods of intense sunshine, when the branch bags increased temperature by 4-6 °C and relative humidity by 10-15% (Wang & Kellomäki 1997a). The chambers increased temperature on average by 0.6 °C, relative humidity by 5-10%, and reduced vapour pressure deficit by 0-0.3 kPa compared with the conditions outside (Kellomäki & Wang 1998b, 2000, Kellomäki et al. 2000).

Even small changes in the microclimate can cause profound changes in the physiology and growth of plants and are described as chamber effects. Some of these changes may be regarded as an acclimation to lower irradiance in the chambers, such as the lower Chl a/b ratio (see Evans 1989) and higher chlorophyll concentration on a mass basis observed in all

three years, as well as reduced non-photochemical fluorescence quenching (NPQ) observed in the first year of the CTC experiment (data not shown). The chambers tended also to increase foliar concentrations of nutrients (N, K, P, S, B, Ca, Zn, Cu) and biochemical components (soluble proteins, Rubisco) on a mass basis, and to increase growth of the needles (data not shown). The chamber effect was also seen in the anatomy of the needles, as stomatal density and the proportion of mesophyll occupied by intercellular air spaces were generally reduced by the chambers (data not shown). In general, however, the crown architecture and phenology of the trees were not affected by the chambers (Kellomäki et al. 2000). The branch bags had somewhat different effects on needles from the effects of the chambers, since foliar N concentration was reduced by the bags and chlorophyll concentration remained unaltered (Chapter 3, Fig. 5, Table 2). The branch bags also tended to decrease net photosynthesis and reduced the Chl a/b ratio (Chapter 3, Fig. 2, Table 1).

In branch bag and chamber experiments it is generally assumed that the chamber (or branch bag) effects are additive with the treatment effects, since the treatment effects are explored using trees growing in chambers with ambient conditions as controls. The chamber effects may, however, be larger than the treatment effects and they are not necessarily additive with the treatment effects (Norby et al. 2001). In the CTC experiment of this study, for instance, the reductions in stomatal density and in the relative area of mesophyll occupied by intercellular air spaces may have limited the response of the trees to elevated CO₂. In addition in the branch bag experiment, the shading of a branch in an otherwise unshaded tree may have caused fewer resources (nutrients, water) to be diverted to the shaded branch (see Sprugel 2002), which is indicated here by the lower foliar N concentration of the bagged branches compared with the unbagged ones (Chapter 2, Table 2). Consequently, the effects of elevated CO₂ were studied with a branch that

probably had a poorer nutrient status than the rest of the tree, which may limit photosynthetic stimulation at elevated CO₂. Acclimation to shade on its own, in contrast, may enhance the response to elevated CO₂, since in conifers, the shaded needles in the lower parts of the canopy have exhibited greater photosynthetic stimulation at elevated CO₂ than the sunlit needles in the upper canopy (Marek et al. 2002, Crous & Ellsworth 2004), and, in general, shade-tolerant woody species have shown greater relative growth rate at elevated CO₂ than shade-intolerant ones (Kerstiens 2001).

7.1.2 Insight into the statistical analysis

In the experiment with seedlings (Chapter 2), the data was analyzed using an analysis of variance, whereas in the branch bag and CTC experiments (Chapters 3-6), the data was analysed using a less known mixed model analysis with Mln or MLwiN 1.1 program (Multilevel Models Project, Institute of Education, University of London, UK). This statistical model recognises and employs a multilevel structure in data, so that the units (observations) of data lie at different levels of a hierarchy. For example, in the branch bag experiment (Chapter 3), replicate samples (or measurements) were assigned to level 1, and the shoot which the samples were taken from to level 2, and finally, the tree to level 3. In the CTC experiment (Chapters 4-6), the replicate samples were at level 1 and the chambers (trees) at level 2. Units at one level are recognised as being grouped within units at the next higher level and to correlate with each other. For example, the shoots are grouped within trees, as the shoots within a single tree are probably more alike, on average, than shoots from another tree. The advantage of this kind of data structure is that the variances associated with the different levels of data can be estimated, and thus the model will be improved. In addition, since the formulation of the model does not assume balanced data, the observations that are occasionally missing do not interfere with the analysis as they would, for example, in

repeated measures analysis of variance.

When the data was analysed across a growing season in current-year and 1-year-old needles (Chapter 3), across the development of a specific needle cohort (Chapters 4 & 6) or across the three years of the CTC experiment (Chapter 5), the sampling date or year was included in the analysis as a fixed treatment effect. Observations made in 1996 before the start of the treatments were included in the analysis of the 1996 cohort (Chapter 4) and in the analysis of the pooled data (Chapter 5). By this means, information could be added concerning the control levels of the variables and also their variances in a specific tree.

7.2 Photosynthesis at elevated CO₂ and temperature

7.2.1 Elevated CO₂ caused reductions in the carboxylation capacity

Light-saturated net photosynthesis that was measured either at a growth CO₂ concentration of the respective treatment (Chapter 4, Fig. 1, Table 6) or at ambient CO₂ concentration (Chapter 3, Fig. 2) was not, in general, higher in young Scots pine trees growing at elevated CO₂ than in those growing at ambient CO₂. The lack of stimulation of photosynthetic rates by elevated CO₂ in these experiments can be largely related to reductions in the carboxylation capacity. A lower carboxylation capacity was caused by reductions in the amount of Rubisco (Chapter 3, Fig. 3; Chapter 4, Fig. 3), and was probably closely linked to reductions in N concentration and to increases in the C/N ratio of the needles.

In the young Scots pine trees growing in CTCs, elevated CO₂ more strongly influenced carbon fixation than light harvesting and utilization of light energy, since the amount and activity of Rubisco protein decreased during growth at elevated CO₂, most notably in the first and second year of the treatments. There were, however, only occasional reductions in chlorophyll concentration and

no consistent alterations in the photochemical yield of PSII or in non-photochemical quenching at elevated CO₂ (Chapter 4, Table 6). This seems to be a general response of trees grown at elevated CO₂, since reductions in the carboxylation capacity are common (e.g. Tissue et al. 1996, 2001, Griffin et al. 2000, Rogers & Ellsworth 2002, Crous & Ellsworth 2004, Lewis et al. 2004), but chlorophyll fluorescence is less often affected (e.g. Wang & Kellomäki 1997a, Saxe et al. 1998). Alterations in the investment of N in the components of light harvesting and electron transport resulting from elevated CO₂ are variable and difficult to interpret, probably because these processes are necessarily not rate-limiting for photosynthesis and growth at elevated CO₂ (Saxe et al. 1998).

The reductions in the carboxylation capacity at elevated CO₂ were not caused by a decline in the activation state or specific activity of Rubisco (Chapter 4, Table 6), but were associated with severe reductions in foliar N concentration and increases in the C/N ratio (Fig. 2, Tables 3, 6). A lower carboxylation capacity at elevated CO₂ was correlated with a significantly higher concentration of starch only in the first treatment year (Chapter 5, Fig. 1C), which suggests that an accumulation of non-structural carbohydrates was not the sole factor leading to reductions in the carboxylation capacity in these trees. The relative allocation of N away from Rubisco and carbon fixation in favour of thylakoids and light harvesting and other N-containing compounds of needles was significantly increased in the first year of the treatments (Chapter 4, Fig. 3, Tables 5, 6; Chapter 5, Fig. 1B), and may indicate a selective loss of Rubisco protein at elevated CO₂ (Rogers & Ellsworth 2002, Long et al. 2004). A selective loss of Rubisco protein has, indeed, occurred in several experiments with pine species at elevated CO₂, in which reductions in the carboxylation capacity have appeared without a significant decrease in foliar N concentration (Tissue et al. 1996, Turnbull et al. 1998, Griffin et al. 2000, Rogers & Ellsworth 2002, Crous & Ellsworth 2004). However, because of the severe reduction of

foliar N concentration observed during the first year at elevated CO₂ (Chapter 4, Tables 3, 6; Chapter 5, Fig. 1A), the reallocation of N within the photosynthetic apparatus in favour of light reactions may be regarded as a normal allocation of N (Nakano et al. 1997, Farage et al. 1998, Theobald et al. 1998, Harmens et al. 2000). In general, the relative allocation of N to Rubisco decreases with decreasing foliar N concentration (Evans 1989, Evans & Seeman 1989), and may do so irrespective of the growth CO₂ concentration (Nakano et al. 1997). In the second year at elevated CO₂, the reduction in Rubisco reflected a general decline in foliar N concentration with a parallel decrease in the investment of N in proteins. The evidence for this is that, although the carboxylation capacity and foliar N concentration decreased, there were no changes in the relative allocation of N to Rubisco (Chapter 4, Fig. 3, Tables 5, 6; Chapter 5, Figs. 1A, 1B).

In the seedlings of Scots pine and Norway spruce, in contrast, elevated CO₂ had little effect either on the concentration of starch, on Rubisco or on the growth of the aboveground parts (Chapter 2). Instead, elevated CO₂ reduced chlorophyll concentration in current-year needles and also increased the Chl a/b ratio (Chapter 2, Table 4). These changes may be a sign of an earlier aging of the needles, which is also supported by findings of the ultrastructural study showing an increased swelling of chloroplast thylakoids (data not shown, Chapter 2) and higher numbers of chloroplast plastoglobuli in Scots pine (Chapter 2, Table 5) at elevated CO₂. A swelling of chloroplast thylakoids and an increased size or number of plastoglobuli are considered to be non-specific stress symptoms of chloroplasts observed during the senescence of leaves (see Sillanpää 2003), and also in some studies at elevated CO₂ (Pritchard et al. 1997a). Thus, it seems that the seedlings were unable to utilize increased CO₂ concentration, although the reasons for this remain speculative in this study. A possible cause is the low nutrient availability of the soil, which in some other experiments has also led to minor growth responses in

conifers at elevated CO₂ (Murray et al. 2000, Oren et al. 2001, Olszyk et al. 2003).

7.2.2 Fertilization counteracted reductions in Rubisco caused by elevated CO₂

In young Scots pine trees grown with branch bags, there was somewhat more support for a selective loss of Rubisco protein at elevated CO₂, as foliar N concentration decreased only in 1-year-old needles, but the amount of Rubisco and the relative allocation of N to Rubisco in relation to chlorophyll, soluble proteins and other N-containing compounds decreased both in the current-year and 1-year-old needles of the unfertilized trees (Chapter 3, Figs. 6, 7, Table 2).

N-fertilization did not affect photosynthetic rates (Chapter 3, Fig. 2), which is surprising considering the importance of N in the photosynthetic machinery (Evans 1989) and the poor nutrient status of the soil at this site. Correspondingly, the foliar concentrations of N, chlorophyll, soluble proteins and Rubisco and their relations were also unaffected by fertilization (Chapter 3, Figs. 3-7, Tables 1, 2). Indirect evidence that fertilization had nevertheless increased the availability and uptake of N in the fertilized trees comes from the differential response of the fertilized and unfertilized trees to elevation of CO₂, as fertilization suppressed the reduction in foliar N and chlorophyll concentrations (Chapter 3, Fig. 5, Table 2), and partially suppressed the reduction in the carboxylation capacity induced by elevated CO₂ (Figs. 3, 4). In the fertilized trees the reductions in the amount of Rubisco (on an area basis, Chapter 3, Fig. 3) and in the Rbc/Chl ratio (Fig. 6) due to elevated CO₂ were smaller than in the unfertilized trees, and there were no changes in the other parameters related to Rubisco. Fertilization had inconsistent effects on starch concentration in needles, and reductions in photosynthetic components were not correlated with changes in the concentration of starch (Chapter 3, Fig. 8).

Smaller reductions in the carboxylation

capacity at elevated CO₂ in the fertilized trees than in the unfertilized ones support the view that photosynthetic and growth responses to elevated CO₂ may be smaller under nutrient-limited conditions. Similarly, Nowak et al. (2004) summarized in their review that the smallest enhancements of net photosynthesis under elevated CO₂ occurred in plants with the greatest reductions in N concentration. Interestingly, woody species exhibited smaller reductions in N concentration and, consequently, greater stimulations of net photosynthesis at elevated CO₂ than herbaceous species did (Nowak et al. 2004). In addition, the relative sensitivity of photosynthesis to foliar N concentration may change during growth at elevated CO₂ and temperature (Kellomäki & Wang 1997, Lewis et al. 2004), which again emphasizes the importance of considering long-term acclimation to growth conditions when predicting the responses of photosynthesis and growth to a changing climate.

7.2.3 Elevated temperature increased Rubisco in young Scots pine trees, but reduced it in seedlings

In young Scots pine trees grown at elevated temperature, the relative investment of foliar N in Rubisco and, consequently, the carboxylation capacity occasionally increased, and significantly so in the 1996 and 1999 cohorts (Chapter 4, Fig. 3, Tables 5, 6). The increased proportion of N bound to Rubisco was in some cases associated with a higher foliar N concentration and may be a normal reallocation of N that would be observed with a higher concentration of foliar N (Evans 1989, Evans & Seeman 1989), regardless of the growth temperature. Accordingly, changes in the amount of Rubisco or in the carboxylation capacity in relation to the RuBP regeneration capacity have been observed earlier during acclimation to a warmer growth temperature (Hikosaka et al. 1999, Turnbull et al. 2002) or to seasonal changes in temperature (Onoda et al. 2005). Such changes in the balance between RuBP carboxylation and RuBP regeneration affect

the extent of the CO₂ stimulation of photosynthesis and may explain part of the seasonal variation in photosynthetic enhancement at elevated CO₂ (Hikosaka et al. 1999, Onoda et al. 2005). A moderate elevation in temperature has, in general, been beneficial for the photosynthesis of conifers (e.g. Kellomäki & Wang 1997, Lewis et al. 2001, 2004, Saxe et al. 2001), and in some cases this has been associated with higher foliar N (Kellomäki & Wang 1997, Lewis et al. 2004) and chlorophyll concentration (Ormrod et al. 1999). In this study, however, despite increases in the amount of Rubisco, net photosynthesis was generally lower at elevated temperature than at ambient temperature (Chapter 4, Fig. 1, Table 6). Lower photosynthetic rates were not reflected in growth, since in the three first years of the experiment the diameter growth of trees was stimulated by elevated temperature (Peltola et al. 2002, Kilpeläinen et al. 2005).

In the seedlings of Scots pine and Norway spruce elevated temperature generally increased the maximal photochemical yield of PSII photochemistry in a dark-adapted state ($\Delta F/F_m$) (Chapter 2, Fig. 1), indicating an improvement in the functioning of PSII. An exception was found in the 1-year old needles of Scots pine at elevated temperature and in the combined treatment, where lower $\Delta F/F_m$ was associated with reductions in the concentrations of biochemical components (Rubisco, chlorophyll, soluble proteins). This probably reflects a general remobilization of N away from older needles, involving reductions also in the components of light harvesting and electron transport (Chapter 2, Tables 3, 4). Enhanced remobilization of nutrients was also observable in the 1-year-old needles of Norway spruce at elevated temperature in the form of larger reductions in the absolute and relative amounts of Rubisco than at ambient conditions. In both species these alterations were associated with a lower foliar N concentration, and in Norway spruce also with a higher concentration of starch (Chapter 2, Table 2). As in young Scots pine trees, the biomass production was increased by elevated temperature in the seedlings of

both species in spite of the reductions in the carboxylation capacity (Chapter 2, Table 1).

7.2.4 Interaction of elevated CO₂ and temperature on photosynthetic properties

In the young Scots pine trees growing in the combined treatment of elevated CO₂ and temperature, net photosynthesis was generally close to that measured in trees growing under ambient conditions (Chapter 4, Fig. 1, Table 6), which suggests that photorespiration stimulated by higher temperature was suppressed when CO₂ concentration was also elevated (Long 1991, Speitzer & Salvucci 2002). At elevated CO₂, the frequently observed unresponsiveness of photosynthesis to temperature (Wang et al. 1995, Tjoelker et al. 1998, Bunce 2000, Lewis et al. 2001, 2004, Gunderson et al. 2002) may be related to temperature acclimation and seasonal alterations in the temperature optimum of photosynthesis (Bunce 2000, Lewis et al. 2001). Seasonal variation in photosynthetic stimulation by elevated CO₂ and temperature may, alternatively, be related to temporal changes in foliar N concentration, since the largest enhancements of photosynthesis at elevated CO₂ coincided with the positive effect of elevated temperature on foliar N concentration (Lewis et al. 2004).

In young Scots pine trees the foliar concentration of starch was higher in the combined treatment of elevated CO₂ and temperature than at ambient conditions or at elevated CO₂ (Chapter 4, Table 4; Chapter 5, Fig. 1C). This suggests the existence of a source-sink imbalance. In the second and third year in the combined treatment, the accumulation of starch was associated with larger reductions in the amount and activity of Rubisco and with a greater relative allocation of N away from Rubisco than was observed at elevated CO₂ (Chapter 4, Fig. 3, Tables 5, 6; Chapter 5, Fig. 1B). Foliar N concentration in the combined treatment did not, however, decrease as much as at elevated CO₂, and significantly so only in the second year (Chapter 4, Fig. 2, Tables 3, 6; Chapter 5, Fig.

1A). Thus, the reductions in the carboxylation capacity may be regarded as a selective loss of Rubisco protein (Rogers & Ellsworth 2002, Long et al. 2004). In seedlings, in contrast, elevated CO₂ and temperature generally exhibited no interactions on Rubisco, and thus the values of these variables were close to the additive effects of the separate treatments (Chapter 2, Tables 3, 4).

Despite the reductions in the carboxylation capacity at elevated CO₂ and in the combined treatment, the total carbon gain has probably been increased by elevated CO₂ in the young Scots pine trees, since the radial growth increased at elevated CO₂ and in the combined treatment (Peltola et al. 2002, Kilpeläinen et al. 2005). Reductions in the amount of Rubisco and in foliar N concentration may have enabled larger allocation of N to other plant parts to promote increased growth at elevated CO₂ at this nutrient poor site. In this sense, the changes in N concentration and in the biochemical components of photosynthesis may be regarded as an acclimation response rather than as a detrimental down-regulation. This acclimation benefited carbon sequestration and growth, irrespective of whether the reductions in Rubisco were due to a selective loss or to a consequence of a reduction in foliar N concentration. On the basis of these results it cannot, however, be concluded whether the growth increased *in spite of* these changes or whether the increments in growth would have been even larger if these changes had been smaller or in the opposite direction.

7.2.5 Why would down-regulation be enhanced in older needles?

In several studies with tree species, photosynthetic acclimation at elevated CO₂ has been related to the age of the foliage (e.g. Turnbull et al. 1998, Griffin et al. 2000, Jach & Ceulemans 2000, Tissue et al. 2001, Rogers & Ellsworth 2002, Crous & Ellsworth 2004), and this has been suggested to be either an earlier aging caused by N-deficiency or an ontogenetic drift (see review by Stitt &

Krapp 1999). In this study, there were also some signs of enhanced remobilization of N from older needles at elevated CO₂, although these alterations were not conserved across consecutive cohorts (Chapter 4) or different experiments (Chapters 2-4). The clearest indication that needles at different ages responded differentially to elevation of CO₂ came from the branch bag experiment, in which a reduction in N concentration occurred only in the 1-year-old needles of the unfertilized trees (Chapter 3, Table 2). Other minor indications of an enhanced retranslocation of nutrients or of an ontogenetic drift at elevated CO₂ were a faster decline in chlorophyll concentration (Chapter 4, Fig. 1, Table 6) and faster declines in the concentrations of biochemical components in the 1-year-old needles of the fertilized trees late in the season at elevated CO₂ (Chapter 3, Figs. 3-5, Table 1). In the seedlings, elevated CO₂ had little effect on 1-year-old needles but, instead, induced changes in current-year needles that may be regarded as non-specific stress symptoms or alternatively, as signs of earlier aging.

Elevated temperature seemed to have a greater effect on the remobilization of N or on the aging of needles than elevated CO₂ did. In the seedlings, elevated temperature increased N reallocation from 1-year-old needles (Chapter 2, Tables 3, 4), and similarly, in young Scots pine trees, elevated temperature accelerated the reduction in the N concentration of the 1997 cohort (Chapter 4, Fig. 2, Tables 3, 6). In this same cohort at the combined treatment, N concentration was close to that observed at elevated temperature and was accompanied by a faster decline in the concentrations of biochemical components and in the relative amounts of Rubisco protein than what was observed under ambient conditions or at elevated CO₂ (Chapter 4, Figs. 1-3, Tables 3-6).

The enhancement effect of elevated temperature on the remobilization of nutrients (Chapter 4) may be related to the earlier onset of the growing season (Peltola et al. 2002), and thus could be an ontogenetic drift. It is

also likely that increased growth at elevated CO₂ and temperature (Chapter 2, Peltola et al. 2002, Kilpeläinen et al. 2005) led to an increased demand for, and enhanced remobilization of nutrients within a tree. In conifers up to 40% of the annual requirements of N, P and K for the aboveground biomass production of a tree may be supplied by retranslocation within the tree (Helmisaari 1995), and during the periods of peak growth the retranslocation of nutrients from older needles to current-year foliage may be independent of the nutrients available in the soil (Linder 1987). In other words, in conifers the internal nutrient reserves are of crucial importance for the growth of the current-year foliage, and it is not surprising that, during high demand, nutrient remobilization from older needles could be enhanced. Such alterations in the internal nutrient cycling will very probably affect the long-term response of trees to elevated CO₂ and temperature, especially when nutrients are limiting the growth. Studies of internal nutrient cycling in conifers at elevated CO₂ (Temperton et al. 2003) and at a combination of elevated CO₂ and temperature (Hobbie et al. 2001) are rare. Within Scots pine seedlings, there were no alterations in the internal N reallocation at elevated CO₂ (Temperton et al. 2003), whereas in Douglas fir, N remobilization away from the older needles increased at elevated CO₂, but influxes of N into the younger needles increased at elevated temperature (Hobbie et al. 2001). Clearly, further research in this subject is needed.

The absence of acclimation in young leaves may in part be due to the inability of the developmental programme of a young leaf, which is not fully competent as a source leaf, to allow redistribution of N away from Rubisco to a leaf that is even less developed (Moore et al. 1999). It has also been suggested that the differential responses of older and younger needles at elevated CO₂ may reflect the relationship between needle age and sink proximity, such as developing buds (Turnbull 1998, Rogers & Ellsworth 2002).

7.3 Stomatal response to elevated CO₂ and temperature

7.3.1 Stomatal density was unaffected by elevated CO₂, but reduced by elevated temperature

Stomatal density in needles of young Scots pine trees appeared to be more responsive to elevated temperature than to elevated CO₂, since at elevated CO₂, stomatal density generally was unchanged, while at elevated temperature and in the combined treatment of elevated CO₂ and temperature, stomatal density was commonly lower than in ambient conditions (Chapter 6, Table 1). In accordance, earlier studies have shown stomatal density to be lower in leaves that were formed in warmer summer temperatures than in spring (Beerling & Chaloner 1993, Ferris et al. 1996). In contrast, opposite results showing higher stomatal density at elevated temperature have been reported (Ferris et al. 1996, Reddy et al. 1998), but with varying responses in the stomatal index (the proportion of stomatal cells of all epidermal and stomatal cells) (Ferris et al. 1996, Reddy et al. 1998, Apple et al. 2000).

Higher stomatal density at elevated temperature may be related to a reduced expansion of the epidermal cells during severe water deficit (Bosabalidis & Kofidis 2002), whereas lower stomatal density may be induced by elevated temperature, when the water supply is limited but there are no abrupt periods of drought during the needle development. Slightly higher vapour pressure deficit inside the elevated temperature chambers has probably also affected stomatal density (Chapter 6). However, on the basis of these results it cannot be concluded that lower stomatal density at elevated temperature and in the combined treatment is caused by greater size of the epidermal cells (e.g. Ferris et al. 2001, 2002) or, instead, by a real reduction in the proportion of stomata of all of epidermal cells.

In some conifer species, such as Scots pine, the stomata are arranged in rows that are

grouped in bands with stomata-free spaces in between, and the extent of the stomatal bands and stomata-free regions varies. This kind of arrangement may distort the measurement of stomatal density if small counting fields are used. Hence, a measure based on the number of stomata per millimeter needle-length has been suggested as the most appropriate method for studying stomatal frequency in these species (Kouwenberg et al. 2003). In this study, stomatal density was determined on an area basis, which may have caused some inaccuracy in the results.

7.3.2 Stomatal conductance did not correlate with reduced stomatal density at elevated temperature

Lower stomatal density in needles of young Scots pine trees grown at elevated temperature (Chapter 6, Table 1) did not generally lead to lower stomatal conductance or transpiration (Chapter 4, Tables 1, 6). Stomata have apparently acclimated to elevated temperature both functionally, by an adjustment of stomatal aperture, and anatomically, by alterations in stomatal density. At elevated temperature, however, water use at the canopy level increased, as the daily total water flux from shoots (Wang et al. 2003) and the daily total sap flow (Kellomäki & Wang 1998b, 2000) were greater than in the ambient conditions. It was concluded that the greater sap flow was caused by a larger current-year needle area, by a reduced stomatal sensitivity to high levels of vapour pressure deficit, and by changes in stomatal conductance (Kellomäki & Wang 1998b, 2000). Alterations in vapour pressure deficit may explain a great proportion of variation observed in stomatal conductance, and temperature may play a minor role (Gunderson et al. 2002).

7.3.3 A trend towards lower stomatal conductance at elevated CO₂

In young Scots pine trees grown at elevated CO₂, stomatal conductance in current-year

needles was about 42% of that measured in the ambient conditions (Chapter 4, Table 1), although the reductions were not always significant (Table 6). Similarly, in several CO₂ experiments with trees, the alterations in stomatal conductance would not always have been statistically significant for individual measurement dates, but when considered over a longer period, the changes caused by environmental variables have been significant (Norby et al. 1999, Gunderson et al. 2002). The reductions in stomatal conductance in young Scots pine trees grown at elevated CO₂ (Chapter 4) probably led to decreased total water use by a tree, as in the second year of this experiment, the cumulative sap flow was smaller in the trees grown at elevated CO₂ than in those grown under ambient conditions (Kellomäki & Wang 2000). Model computations showed that alterations in the needle area or absorption of total radiation had less effect on the sap flow than reduced stomatal conductance did (Kellomäki & Wang 2000).

7.4 Chemical composition of needles at elevated CO₂ and temperature

7.4.1 Nutrient concentrations

In young Scots pine trees growing in CTCs or with branch bags, the most prominent response to the elevation of CO₂ was a reduction in N concentration and an increase in the C/N ratio of the needles (Chapters 3-5). This agrees well with several reviews and meta-analyses summarizing the effects of elevated CO₂ on woody plants and herbaceous species (Drake et al. 1997, Poorter et al. 1997, Cotrufo et al. 1998, Curtis & Wang 1998, Medlyn et al. 1999, Yin 2002, Long et al. 2004, Nowak et al. 2004). In the seedlings of Scots pine and Norway spruce, however, elevated CO₂ did not affect foliar N concentration, which may be related to the unresponsiveness of the dry mass of the seedlings to the elevation of CO₂ (Chapter 2, Table 2), and thus to a smaller demand for reallocation of N than in young trees. It is possible that the initially low N concentration

in the seedlings, especially in Norway spruce, has itself limited the responsiveness of the growth and biochemical parameters to elevation of CO₂ (Chapter 2). The deficiency of N in the seedlings is also illustrated by the lower concentrations of chlorophyll and Rubisco in current-year needles that were grown during the experimental conditions than in 1-year-old needles grown in the nursery (Chapter 2, Tables 3, 4). In general, the concentrations of biochemical constituents related to photosynthesis are greater in current-year than in 1-year-old needles (Utriainen & Holopainen 2001, Warren et al. 2003). At the Mekrijärvi site, the foliar concentrations of N and other nutrients were low (Chapters 3-6), but comparable to previous observations at this or at a similar nutrient-poor site (Helmisaari 1990, Kellomäki & Wang 1997, Kellomäki & Wang 1998a). Surprisingly, fertilization had no effect on the foliar concentrations of N or biochemical components in the branch bag experiment (Chapter 3), but the fact that elevated CO₂ reduced N concentration only in the unfertilized trees implies that fertilization had probably improved the N status of the fertilized trees.

In young Scots pine trees, growth at elevated CO₂ induced occasional reductions in the foliar concentrations of mobile nutrients P and K and of intermediately mobile nutrients S and Cu, and also occasional increases in the concentrations of less mobile nutrients B, Zn and Mn (Chapter 6, Table 5). In accordance with this, lower concentrations of mobile nutrients have been observed in trees growing at elevated CO₂ (Conroy et al. 1992, Medlyn et al. 1999, Roberntz & Linder 1999, Sigurdsson 2001), and have been related to an inadequate availability or uptake of nutrients in relation to growth, to an indirect dilution effect caused by an accumulation of non-structural carbohydrates or to changes in the allocation and translocation of nutrients within a plant. Here, changes in the concentration of N (Chapter 4, Fig. 2) and in concentrations of other nutrients on a structural dry mass basis (data not shown) were as large as the changes on a dry mass

basis (Chapter 6), and thus the reductions in nutrient concentrations are not likely to have been caused by a dilution effect resulting from an accumulation of starch. Nor were there indications of retranslocation of nutrients, since the decreases in nutrient concentrations were generally as large in current-year needles as in 1-year-old needles. The lower concentrations of foliar nutrients are more probably related to a smaller uptake of nutrients and/or to a dilution effect caused by increased growth that was observed at elevated CO₂ in young Scots pine trees (Peltola et al. 2002, Kilpeläinen et al. 2005). The higher concentrations of less mobile nutrients Zn and Mn could, in contrast, be explained by an accumulation via an increased transpiration stream. This, however, is in conflict with the reductions in the daily total water flux from shoots (Wang et al. 2003) and the daily total sap flow (Kellomäki & Wang 1998b, 2000) observed at elevated CO₂. In the seedlings, in contrast to the young trees, the concentrations of less mobile Ca and Mg decreased in current-year needles of Scots pine, and the concentration of mobile K increased in current-year needles of Norway spruce growing at elevated CO₂ (Chapter 2, Table 2). These conflicting alterations may reflect limitations on growth imposed by the low nutrient levels in the seedlings.

At elevated temperature, the higher concentrations of less mobile nutrients (Ca, Mg, B, Zn and Mn) in the needles of young Scots pine trees (Chapter 6, Table 5) could indicate an increased total transpiration. This is supported by the higher daily total water flux from shoots (Wang et al. 2003) and the daily total sap flow (Kellomäki & Wang 1998b, 2000) at elevated temperature than in ambient conditions. In young Scots pine trees, the elevation of temperature reduced the foliar concentrations of mobile nutrients N, P and K and of intermediately mobile nutrients S and Cu in the first and second year of the treatments, while increased the concentrations of N and S in the third year (Chapters 4-6). The higher foliar nutrient concentrations observed here and the increased soil CO₂ efflux at elevated temperature compared with

the ambient conditions (Niinistö et al. 2004) support the predictions that nutrient mineralization in the soil may be faster at higher temperature. The differential effect of elevated temperature from year to year may be related to weather conditions, since the third year was warmer than the previous two (the mean annual temperature in the ambient control chambers was 2.1 °C, 1.6 °C and 2.9 °C in the three years, respectively).

According to the expectations, elevated temperature compensated for reductions in foliar nutrient concentrations caused by elevated CO₂ in young Scots pine trees, since in the combined treatment of elevated CO₂ and temperature, foliar nutrient concentrations decreased less than expected on the basis of separate treatments (Chapters 4-6). Stimulation of nutrient mineralization by elevated temperature is also supported by several other experiments with conifers showing increased foliar N concentrations (Kellomäki & Wang 1997, Hobbie et al. 2001, Lewis et al. 2004) and faster rhizosphere respiration, litter decomposition and oxidation of organic matter in the soil (Lin et al. 1999, 2001b) after several years of growth at elevated temperature. There also, however, exist contrasting results showing reduced foliar nutrient concentrations in field-grown plants in response to warming (Peñuelas et al. 2004). In the seedlings, in contrast to the young trees in this study, there were no indications of the beneficial effects of elevated temperature on nutrient availability (Chapter 2, Table 2). This may be related to the increased growth (Chapter 2, Table 1) and demand for nutrients at elevated temperature and in the combined treatment, but it can also be related to limitations imposed by the experimental set-up, since it is likely that the relatively short duration of the experiment, the limited soil volume and the nature of soil media (mixture of sand and peat) do not permit natural nutrient cycling. Furthermore, in addition to temperature, other environmental factors, e.g. humidity in the soil (Emmett et al. 2004), affect the rate of mineralization and nutrient cycling in the soil. In addition, the capacity for nutrient uptake

may change at elevated CO₂ (reviewed by BassiriRad et al. 2001) and at elevated temperature (e.g. DeLucia et al. 1997).

The nutritional balance in needles of young Scots pine trees growing in CTCs was also studied in terms of the N-ratio (Linder 1995), which suggested that, at this site, P and Cu may have been even more limiting for growth than N (Chapter 6, Table 6). In northern coniferous stands, low P availability may commonly limit growth. Based on the data provided by the N-ratios, P and Cu could also be considered as limiting nutrients for growth at elevated temperature, and P at elevated CO₂ and temperature (Chapter 6). The nutritional balance in terms of N-ratio needs, however, to be considered carefully, as it is likely that changes in the growth conditions could alter the requirement of N for maximal biomass production. At elevated CO₂, the critical concentration of N required for maximal growth is likely to change because of the importance and magnitude of nitrogen in the photosynthetic machinery (Evans 1989). Requirements for other nutrients do not necessarily change in parallel with the requirements for N at elevated CO₂ (Conroy et al. 1990, Johnson et al. 1995, DeLucia et al. 1997) or at elevated temperature (DeLucia et al. 1997, Peñuelas et al. 2004).

7.4.2 Starch concentration

A reduction in the foliar N concentration of plants growing at elevated CO₂ has frequently been associated with increased amounts of non-structural carbohydrates (Poorter et al. 1997, Curtis & Wang 1998, Long et al. 2004). In this study, alterations in starch concentration in response to the elevation of CO₂ concentration and temperature and to fertilization were more variable (Chapters 2-5). In young Scots pine trees growing in CTCs, elevated CO₂ occasionally increased the concentration of starch in current-year needles, but did not affect that of 1-year-old needles (Chapter 4, Tables 4, 6), possibly because of the mobilization of carbohydrates from mature needles for the growth-needs of

current-year shoots (Ericsson 1979). When the seasonal data were pooled, the increase in starch concentration due to elevated CO₂ was significant only in the first year of the treatments (Chapter 5, Fig. 1C). In the combined treatment of elevated CO₂ and temperature, the accumulation of starch was more prominent, and the concentration of starch was 31%, 18% and 32% higher than at ambient conditions during the three years, respectively (Chapter 5, Fig. 1C). In seedlings, likewise, the concentration of starch was highest in the combined treatment of elevated CO₂ and temperature (Chapter 2, Table 2). This is surprising, since one might expect that elevated temperature would enhance the use of carbohydrates and prevent an accumulation of starch by increasing the rates of metabolic processes and transport of carbohydrates (Farrar & Williams 1991). With tree species, this assumption has been supported by lower concentrations of sugars (Hobbie et al. 2002, Turnbull et al. 2002) and starch (Turnbull et al. 1998) at elevated temperature. Additional night-time warming stimulated net photosynthesis in *Populus deltoides* more than day-time warming alone, which was assumed to be caused by a respiratory-driven reduction in leaf carbohydrate concentration (Turnbull et al. 2002). Increased net photosynthesis was associated with increased carboxylation and RuBP regeneration rates at elevated temperature (Turnbull et al. 2002).

In the young Scots pine trees with branch bags, elevated CO₂ increased the concentration of starch in 1-year-old needles of the fertilized trees (Chapter 3, Fig. 8). Fertilization alone reduced the concentration of starch in 1-year-old needles, but increased it in current-year needles. In general, any factor that limits growth more than carbon fixation, such as low nutrient availability (Ericsson 1979, Linder 1995, Paul & Driscoll 1997), low temperature or an elevation of CO₂ concentration for nutrient deficient plants (see Stitt & Krapp 1999), will very easily lead to increased amounts of non-structural carbohydrates. The accumulation of starch

observed here in fertilized trees is in discrepancy with this.

In the seedlings, the response to elevated CO₂ and temperature was, again, different from what was observed in the young trees, as elevated CO₂ had no effect on starch concentration in either species, while elevated temperature increased starch concentration in Norway spruce (Chapter 2, Table 2). In addition, there was a discrepancy between the chemical analysis of starch and the ultrastructural study, the latter showing an increased size of starch grains and an increased relative area of a chloroplast occupied by starch grains at elevated CO₂ in both species (Chapter 2, Table 5). According to the ultrastructural study, elevated temperature tended to prevent the CO₂-induced accumulation of starch. A comparable inconsistency between two methods used for the analysis of starch has also been observed elsewhere (Pritchard et al. 1997a).

7.4.3 Secondary compounds

According to the carbon-nutrient balance hypothesis (Bryant et al. 1983) and the growth-differentiation balance hypothesis (Loomis 1932, Herms & Mattson 1992), the carbon exceeding the demands for growth will be allocated to the production of non-structural carbohydrates (TNC) and carbon-based secondary compounds (CBSC). An elevation of CO₂ concentration increases the supply of carbon, whereas a lack of nutrients reduces growth and carbon demand, and thus both are expected to lead to an increased production of TNCs and CBSCs (see Peñuelas & Estiarte 1998). This hypothesis was not very strongly supported by the results of this study, as in the seedlings of Scots pine, elevated CO₂ or temperature did not affect the foliar concentration of total phenolics, while in Norway spruce, the concentration of total phenolics was lower at elevated CO₂ and higher in the combined treatment of elevated CO₂ and temperature than in the ambient conditions (Chapter 2, Fig. 2). In woody

plants growing at elevated CO₂, increases in total phenolic concentrations have frequently been observed (e.g. Pritchard et al. 1997b, Peñuelas & Estiarte 1998, Sallas et al. 2001, Coley et al. 2002, Peltonen et al. 2005), while in some studies total concentrations have remained unchanged (e.g. Kainulainen et al. 1998, Peñuelas et al. 2002, Veteli et al. 2002, Kuokkanen et al. 2004).

The concentrations of terpenes in conifer needles have either not been affected (Heyworth et al. 1998, Kainulainen et al. 1998, Peñuelas & Estiarte 1998), increased (Sallas et al. 2001) or decreased (Litvak et al. 2002) in response to elevated CO₂. In this study, the concentrations of terpenes and resin acids were generally lower at elevated CO₂ and higher at elevated temperature than in the ambient conditions in current-year needles of seedlings, while the responses of individual compounds in the stems varied more greatly (Chapter 2, Tables 6, 7). The increase in total monoterpene concentration at elevated temperature (Chapter 2, Tables 6, 7) may indicate larger monoterpene emissions and an increased protection against heat stress, as monoterpenes, in addition to isoprene (Sharkey & Singaas 1995, Singaas et al. 1997, Sharkey & Yeh 2001, Peñuelas et al. 2005), have been shown to increase the thermotolerance of photosynthesis (Delfine et al. 2000) and may also function as antioxidants in the leaves (Loreto et al. 2004). In accordance, higher monoterpene concentrations (Litvak et al. 2002) and emissions (Constable et al. 1999) have been reported in conifers growing at elevated temperature, although lower concentrations have also occurred (Snow et al. 2003). Monoterpene concentrations do not necessarily correlate with the synthesis (Litvak et al. 2002) or emission rates (Constable et al. 1999). In addition, it has been proposed that isoprene emissions may be a mechanism for maintaining metabolic homeostasis in chloroplasts by recovering phosphate from phosphorylated intermediates during periods when end-products of photosynthesis accumulate (Logan et al. 2000). Not all species emit isoprene, and

among the conifers only the *Picea* species (such as Norway spruce) emit it at moderate rates, whereas e.g. Scots pine emits only traces of isoprene (see Logan et al. 2000, Kellomäki et al. 2001). The ability to emit isoprene may be related to the strategy of phloem loading (Logan et al. 2000, Kerstiens & Possel 2001).

In young Scots pine trees, there were only minor indications that the production of resin acids would increase at elevated CO₂ or at elevated temperature, since the proportion of the needle mesophyll area occupied by resin ducts was only occasionally increased at elevated CO₂ and generally not changed at elevated temperature (data not shown). The proportion of the mesophyll area occupied by resin ducts varied between 11% and 16% in the abaxial side and between 5% and 8% in the adaxial side of the needles at ambient conditions (except in the 1999 cohort, where the relative area of resin ducts was exceptionally small).

The carbon-nutrient balance hypothesis has been criticised (Hamilton et al. 2001) because in many experiments it has failed to explain the allocation of carbon and nitrogen to secondary metabolites, and because of a large variation in the responsiveness amongst the different classes of CBSCs (e.g. Koricheva et al. 1998, Peñuelas & Estiarte 1998, Peñuelas et al. 2002). The production of CBSCs is obviously affected not only by resource availability but also to a great extent the by specific demand-side requirements that affect the production of the individual secondary metabolites differentially (Koricheva et al. 1998). Such responses are affected by the evolutionary history under different abiotic and biotic environments, leading to genotypic variation in the production and composition of secondary metabolites (Keinänen et al. 1998, Koricheva et al. 1998, Hamilton et al. 2001).

7.5 Dimensions and anatomy of needles at elevated CO₂ and temperature

7.5.1 The size of needles was generally unaffected by elevated CO₂

In young Scots pine trees growing in CTCs or with branch bags, elevated CO₂ generally had no effect on the growth of an individual needle, as the fresh weight, dry weight, structural dry weight (Chapter 3, Table 3; Chapter 6, data not shown), total projected area (Chapter 6, Fig. 2) or length of a single needle (Chapter 6, data not shown) were not affected. Contrary to general findings with plants growing at elevated CO₂ (reviewed in Yin 2002), the needle thickness studied as specific leaf area (Chapter 2, Table 1; Chapter 3, Fig. 9), specific leaf weight (SLW) (Chapter 4, Tables 3, 6) or by light microscopy (Chapter 6, Table 3) was generally not increased by the elevation of CO₂. Nor did elevated CO₂ affect the thickness of the epidermis or hypodermis, or the vascular cylinder or intercellular spaces (Chapter 6, Tables 2-4). The effects of CO₂ and temperature on the needle anatomy were in some cases interactive, and in the combined treatment the thickness of the epidermis and hypodermis decreased more than in the separate treatments (Chapter 6, Table 2).

N-fertilization tended to increase the dry weight and structural dry weight of needles (Chapter 3, Table 3), which suggests that low nutrient levels at the Mekrijärvi site restricted growth and probably affected the response to elevated CO₂. Low nutrient availability, especially that of N and P, may also limit the foliar capacity for morphological acclimation in Scots pine (Niinemets et al. 2001), and may in part explain the unresponsiveness of the needle anatomy to the elevation of CO₂ concentration.

7.5.2 Elevated temperature tended to reduce the thickness of needles

Elevated temperature reduced the growth of the 1997 cohort, since those needles were shorter (data not shown, Chapter 6) and thinner, and their total projected area (Chapter 6, Fig. 2, Table 3) was smaller at elevated temperature than at ambient temperature. Elevated temperature also tended to reduce SLW in the cohorts of 1996 - 1998 (Chapter 4, Tables 3, 6), which is in accordance with several experiments with plants grown at different temperatures (Loveys et al. 2002) and is confirmed by the anatomical analysis showing significantly reduced needle thickness in the 1997 and 1998 cohorts (Chapter 6, Table 3). Reduced needle thickness was associated with a thinner mesophyll on the abaxial side and with a thinner vascular cylinder (Chapter 6, Tables 2, 3). As the reductions in needle thickness were greater than the alterations in SLW or dry weight of the needles (Chapters 4 & 6), the density of the needles may have been greater at elevated temperature than at ambient temperature. A higher density of needles may be brought about by an accumulation of starch, by thicker cell walls, by smaller intercellular air spaces or by increased secondary lignification and secondary phloem formation (Ewers 1982). An accumulation of starch (Chapter 4, Table 4) or a reduction in intercellular air spaces (Chapter 6, Table 4) were not the mechanisms leading to higher density here. Rather, a thickening of cell walls and/or increased secondary lignification and secondary phloem formation may have occurred.

In the seedlings of Scots pine and Norway spruce, elevated temperature increased the amount of cytoplasmic lipid bodies (data not shown, Chapter 2) and tended to prevent the accumulation of starch (studied as the starch grain area of chloroplasts) induced by elevated CO₂, and the increase in the number of plastoglobuli observed in Scots pine (Chapter 2, Table 5).

7.5.3 Extension of intercellular air spaces or vascular cylinder unaltered by elevated CO₂ or temperature

Mesophyll resistance, the resistance to CO₂ transfer from the intercellular air spaces of the leaf to the site of carboxylation within the chloroplast, is a significant limitation on photosynthesis (e.g. Aalto & Juurola 2002, Bernacchi et al. 2002). It is also dependent on temperature (Aalto & Juurola 2002, Bernacchi et al. 2002) and may be altered by growth at elevated CO₂ (Singsaas et al. 2003). Mesophyll resistance is partly regulated by the extent the mesophyll cells face intercellular air spaces. This was studied here by measuring the proportion of mesophyll occupied by intercellular air spaces (Chapter 6, Table 4). In general, elevated CO₂ and temperature had little impact on intercellular air spaces, which implies that the internal resistance to gas exchange at this level was not altered. The relative area of mesophyll occupied by the vascular cylinder also remained unchanged by the treatments (data not shown, Chapter 6), which suggests that at anatomical level, the capacities for water transport and for the translocation of carbohydrates were not affected, and that the proportion of photosynthetic mesophyll cells was unaltered. In Scots pine the increased thickness of phloem has been related to the increased production and transport of photoassimilates at elevated CO₂ (Lin et al. 2001a). In older trees, the larger volume occupied by vascular cylinder has been explained by a higher demand for water transport (Apple et al. 2002).

7.6 Consecutive needle cohorts responded differentially to elevation of CO₂ and temperature

In young Scots pine trees grown for three years at elevated CO₂ and temperature, the alterations in biochemical and chemical composition and needle structure were most distinct in the needle cohort grown in the first treatment year in 1997 (Chapters 4 & 6). The more severe changes in the 1997 cohort,

compared with the other cohorts, may be related to the lower foliar concentrations of P and K in the current-year needles of this cohort (Chapter 6, Table 5). A deficiency of K reduces the expansion of cells via low cell turgor pressure, and a deficiency of P will also impair the extension of the epidermal cells and reduce the expansion of leaves (Marschner 1995). In addition, K is involved in photosynthesis at various levels, such as in the functioning of the stomata, in ATP synthesis as a counter-ion to the light-induced proton flux across the thylakoid membranes of chloroplasts, and in phloem loading and transport by creating an osmotic pressure to drive the mass flow in the sieve cells (Marschner 1995). On this basis, the more distinct reduction in photosynthetic components observed at elevated CO₂ in the 1997 cohort may be linked to the lower concentration of K in this cohort.

In contrast, in the 1999 cohort the concentrations of P, K, S and Cu were higher, and the needles were bigger than those in the other cohorts (Chapter 6). Warmer conditions during the needle expansion period in 1999 than in the two previous years (the mean temperatures in the ambient chamber in April - July were 11.8 °C, 11.2 °C and 13.2 °C in the three years, respectively) has probably stimulated the growth of the needles, since Helmisaari (1990) noted that the needles of Scots pine were shorter and lighter after a cool growing season. In the 1999 cohort, elevated temperature also increased the concentrations of foliar nutrients (Chapter 6), which was reflected in the photosynthetic capacity (Chapter 4). Thus, it seems that the differential responses of the cohorts to elevated CO₂ and temperature may be linked to the general physiological state of the tree and the cohorts, which was observable here as differences in the concentrations of nutrients and the size of the needles. According to the data of this study it seems that the down-regulative effect of elevated CO₂ was greatest in the needle cohort with initially poorest nutritional status, whereas the beneficial effect of elevated temperature was greatest in

the needle cohort with initially good nutrient levels.

7.7 Growth of trees at elevated CO₂ and temperature

Elevated temperature has enhanced the photosynthetic response to elevated CO₂ in some (Kellomäki & Wang 1996, Wang & Kellomäki 1997b, Tjoelker et al. 1998) but not all studies with conifers grown at elevated CO₂ and temperature (Wang et al. 1995, Tjoelker et al. 1998, Lewis et al. 2004). The growth of woody plants has frequently increased more in the combined treatment of elevated CO₂ and temperature than in the separate treatments (Kuokkanen et al. 2001, Veteli et al. 2002, Kilpeläinen et al. 2003, Kuokkanen et al. 2004). This was also the case with the young Scots pine trees growing in CTCs during the three first years of the treatments (Peltola et al. 2002, Kilpeläinen et al. 2005), despite the absence of photosynthetic stimulation (Chapter 4), and with the seedlings of Scots pine and Norway spruce growing in growth chambers (Chapter 2), indicating that the total growth cannot simply be predicted from the leaf-level net photosynthetic rates.

In considering the carbon balance and biomass production of plants in a changing climate, changes in the proportion of assimilated carbon lost in respiration is of vital importance. Estimates of the fraction of the net photosynthetic production consumed by respiration in trees vary from 40% to 75% (see Saxe et al. 2001). Earlier studies have suggested that the rate of dark respiration decreases in response to an elevation of CO₂ concentration, but later measurements have shown that the reductions observed were probably artefacts of the measurement systems (see Jahnke & Krewitt 2002, Davey et al. 2004, and references therein). More recently, there has been some evidence that the rate of dark respiration may increase during the long-term growth at elevated CO₂ concentration (Davey et al. 2004), while Gonzales-Meler et al. (2004) summarize in

their review that specific respiration rates were generally unchanged in field-grown plants at elevated CO₂. Whole ecosystem studies show, however, that canopy respiration does not increase proportionally with increases in biomass in response to elevated CO₂, although a larger proportion of respiration takes place in the root system (Gonzales-Meler et al. 2004). An elevation of temperature initially increases the rate of dark respiration, and thus could be expected to reduce growth, but metabolic acclimation to growth temperature leads to lower respiration rates than expected on the basis of short-term temperature elevation (Atkin & Tjoelker 2003). In the fourth year of the CTC experiment with Scots pines, respiration rates in shoots were reduced by elevated CO₂ and increased by elevated temperature (Zha et al. 2002, 2003), although acclimation of respiration to elevated temperature was evident (Zha et al. 2003). The temperature acclimation of respiration may be a possible reason why the total biomass of seedlings was not reduced at a higher growth temperature (Teskey & Will 1999). Recent studies suggest that respiration is closely linked to net photosynthesis (see Saxe et al. 2001, Atkin & Tjoelker 2003) and that the partitioning of photosynthates to respiration and growth may remain largely stable regardless of the growth temperature (see Saxe et al. 2001). Alternatively, a higher night-time temperature may increase photosynthesis and growth by stimulating dark respiration and preventing possible down-regulation of photosynthetic capacity caused by accumulating carbohydrates (Turnbull et al. 2002). The present study has shown that the elevation of temperature alone was beneficial for the growth of Scots pine and Norway spruce seedlings (Chapter 2), as well as for the growth of young Scots pine trees (Peltola et al. 2002, Kilpeläinen et al. 2005), and that the growth of the trees increased most when both CO₂ concentration and temperature were elevated. These results suggest that the carbon sequestration of boreal forests may increase in the future climate, in spite of constraints imposed by low nutrient levels in the soils.

7.8 Conclusions

Net photosynthesis in the young Scots pine trees was not, in general, stimulated by elevated CO₂ and was reduced by elevated temperature. In accordance with the theoretical expectations, elevated CO₂ and temperature had positive interactions in the combined treatment, leading to an unaltered rate of net photosynthesis compared with that under ambient conditions. The lack of photosynthetic stimulation at elevated CO₂ was caused by down-regulation of photosynthetic capacity and was related to a reduced foliar N concentration. The down-regulation of photosynthetic capacity could not be explained solely by an accumulation of starch and end-product inhibition, by the length of the exposure or by an earlier aging of the needles. Although N-fertilization alone had very little effect on the biochemical composition of needles, it counteracted the reduction in foliar N concentration and down-regulation of photosynthetic capacity at elevated CO₂, supporting the view that low nutrient levels in the soil may restrict growth responses at elevated CO₂. An elevation of temperature may alleviate this constraint, since in the young Scots pine trees elevated temperature tended to counteract the reductions in the concentrations of biochemical components and nutrients caused by elevated CO₂, possibly because of faster mineralization and increased nutrient release in the soil. In the small seedlings, in contrast to the young trees, elevated CO₂ had little effect on photosynthetic properties, while

elevated temperature enhanced reallocation of N away from older needles and led to reductions in the carboxylation capacity. In some cases in this study, the alterations in Rubisco reflected general changes in foliar N concentration and in the investment of N in proteins at elevated CO₂ or at elevated temperature, whereas in some other cases a selective reduction of Rubisco protein was evident. Reductions in the amount of Rubisco and in foliar N concentration may have permitted a larger allocation of N to other plant parts to promote increased growth at elevated CO₂. Elevation of temperature alone was beneficial for the growth of the trees, and the growth increased most when both CO₂ concentration and temperature were elevated. In the combined treatment, elevated CO₂ and temperature frequently had interactions that led to differing responses from those that might have been expected on the basis of separate treatments, a fact which points to the continuing need for multifactorial experiments in climate change research. In the young Scots pine trees, changes in the biochemical, chemical and anatomical properties of needles differed between cohorts and were most evident in the needle cohort grown in the first year of the treatments. This emphasizes that long-term experiments are vital for studying the responses of physiological processes over the course of years, so that the fate of carbon sequestration of boreal forests in the future climate may be predicted.

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