Photosynthesis, CO_2 and temperature – an approach to analyse the constraints to acclimation of trees to increasing CO_2 concentration

Eija Juurola
Department of Forest Ecology
Faculty of Agriculture and Forestry
University of Helsinki

Academic dissertation

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Author: Eija Juurola

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Thesis Supervisors:
Pertti Hari
Department of Forest Ecology, University of Helsinki, Finland
Timo Vesala
Department of Physical Sciences, University of Helsinki, Finland

Pre-examiners:
Veijo Kaitala
Department of Biological and Environmental Sciences, University of Helsinki, Finland
Elina Vapaavuori
Finnish Forest Research Institute, Suonenjoki Research Station, Finland

Opponent:
Reinhart Ceulemans
Department of Biology, University of Antwerpen, Belgium

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The aim of this thesis was to analyse the effects of temperature and increasing CO_2 concentration on the processes involved in photosynthesis and on acclimation of the photosynthetic machinery within the constraints set by three-dimensional (3D) leaf structure. These processes include both the transport of CO_2 into and within a leaf and the photosynthetic CO_2 sink in the chloroplasts.

A detailed 3D model of silver birch leaf photosynthesis was constructed to study the transport of gases into and inside a leaf as well as the light attenuation inside a leaf. To understand the role of temperature in apparent CO_2 assimilation, the temperature dependencies of essential biochemical reactions in photosynthesis were experimentally determined for silver birch and for boreal conditions utilising a conventional model of photosynthesis.

The role of temperature dependent physical phenomena in the apparent CO_2 assimilation was analysed in detail using the 3D model. Based on these results, new chloroplast related temperature dependencies describing the biochemical processes were determined that take into account the specific effects exerted by leaf structure and CO_2 diffusion. Finally, the patterns of acclimation of photosynthesis to increasing CO_2 concentration were experimentally studied in silver birch and Scots pine.

The developed model is a powerful tool for studying photosynthesis in a 3D leaf. The results showed clearly that the physical phenomena together with leaf structure play an important role in leaf CO_2 assimilation and that these have to be included in the analysis of photosynthesis in a changing environment. It was also concluded that besides other factors, leaf structure may significantly influence the acclimation patterns of different tree species when atmospheric CO_2 concentration is increasing. Due to the structural differences, in contrast to silver birch, Scots pine may be able to take full advantage of increased CO_2, at least temporarily.

Keywords: climatic change, CO_2 diffusion, leaf structure, modelling, temperature dependence

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It feels like I have spent most of my adult years at the Department of Forest Ecology, first as an undergraduate student and then many years working for the PhD. I was surprised to realise that all the work I have been carrying out was, somehow, interconnected in the end. I am most satisfied to see how the loose ends were tied, finally. However, much of scientific work is never seen in publications; I do not even want to remember those endless days of testing and checking the data over and over again.

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Tuula Aalto has been the co-author in three of the four articles included in this thesis. Our cooperation has been most fruitful and educative and I have always sensed the ease when we have worked together. I also want to thank my other co-author Tea Thum, for participating in the modelling part in Study III.

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LIST OF ORIGINAL ARTICLES

The thesis is based on the following articles which are referred to in the text by their Roman numerals:

I. Aalto, T. and **Juurola, E.** 2001. Parametrization of a biochemical CO_2exchange model for birch (*Betula pendula* Roth.). *Boreal Environment Research* 6: 53–64.

II. Aalto, T. and **Juurola, E.** 2002. A three dimensional model of CO_2 transport in airspaces and mesophyll cells of a silver birch leaf. *Plant, Cell and Environment*, 25: 1399-1409.

III. **Juurola, E.,** Aalto, T., Thum, T., Vesala T. and Hari P. 2005. Temperature dependence of leaf-level CO_2 fixation: revising biochemical coefficients through analysis of leaf three-dimensional structure. *New Phytologist* 166: 205-215.

IV. **Juurola, E.** 2003. Biochemical acclimation patterns of *Betula pendula* Roth. and *Pinus sylvestris* seedlings to elevated carbon dioxide concentrations. *Tree Physiology*, 23: 85-95.

Eija Juurola participated in planning the research, was responsible for conducting the experiments and the measurements in all studies and was the main author in Studies **III** and **IV**. In Studies **I** and **II** Eija Juurola participated in the writing and analysing the results, but mathematical modelling was done mainly by Tuula Aalto. In study **III** the mathematical modelling was done mainly by Tuula Aalto and Tea Thum.

TABLE OF CONTENTS

LIST OF SYMBOLS

Symbol	*Unit*	*Description*
A	μmol m^{-2} s^{-1}	CO_2 exchange rate
A_{350}	μmol m^{-2} s^{-1}	CO_2 exchange rate at 350 μmol mol^{-1} of CO_2
A_{growth}	μmol m^{-2} s^{-1}	CO_2 exchange rate at growth CO_2 concentration
A_c,	μmol m^{-2} s^{-1}	Rubisco-limited rate of CO_2 assimilation
A_j	μmol m^{-2} s^{-1}	RuBP regeneration-limited rate of CO_2 assimilation
B	μmol m^{-2} s^{-1}	constant
c	mol m^{-3}	CO_2 concentration in gas or in cells
c_i	μmol mol^{-1}	CO_2 concentration in leaf air spaces
D	m^2 s^{-1}	Binary diffusion coefficient in the carrier gas or in cells
D_g	cm^2 s^{-1}	Binary diffusion coefficient of CO_2 in air
D_l	cm^2 s^{-1}	Binary diffusion coefficient of CO_2 in the mesophyll
E_f	J mol^{-1}	Activation energy of the specific variable f ($V_{c(max)}$, K_o, K_c, R_d)
E_j	J mol^{-1}	Activation energy for J_{max}
g_s	mmol m^{-2} s^{-1}	Stomatal conductance
H	dimensionless	Henry's law coefficient
H*	dimensionless	Effective Henry's law coefficient
H_{298K}	dimensionless	Henry's law constant at 25 °C
H_j	J mol^{-1}	Deactivation energy for J_{max}
I_0	μmol m^{-2} s^{-1}	Incident irradiance
J	μmol m^{-2} s^{-1}	Potential electron transport rate
J_{max}	μmol m^{-2} s^{-1}	Maximum electron transport rate
K_c	μmol mol^{-1}	Michaelis-Menten constant for CO_2
K_o	μmol mol^{-1}	Michaelis-Menten constant for O_2
M_{H2O}	g mol^{-1}	Molecular weight of H_2O
o	μmol mol^{-1}	oxygen concentration in intercellular air spaces
q	mol e^- (mol quanta)$^{-1}$	Light use effectivity factor
R	J mol^{-1} K^{-1}	Gas constant
R_d	μmol m^{-2} s^{-1}	Rate of mitochondrial respiration
S	mol m^{-3} s^{-1}	Source or sink of CO_2
S_j	J mol^{-1} K^{-1}	Entropy of the denaturation equilibrium for J_{max}
T	K	Temperature
$V_{c(max)}$	μmol m^{-2} s^{-1}	Maximum rate of carboxylation
ν_{CO2}	cm^3 mol^{-1}	Molar volume of CO_2 at its normal boiling point
z	μm	Distance from the surface of the leaf
z_0	μm	Thickness of the leaf
Γ^*	μmol mol^{-1}	CO_2 compensation point in the absence of mitochondrial respiration
η_{H2O}	cP	Dynamic viscosity of water
ϕ	dimensionless	Association factor of H_2O
Θ	dimensionless	Convexity factor of the light response curve

1. BACKGROUND

1.1. Introduction

Fixing of atmospheric CO_2 through photosynthetic light and dark reactions in chloroplasts of green organisms is a fundamental process on Earth. In short, during photosynthesis light energy is converted into chemical energy by using atmospheric CO_2 as a substrate. Concurrently O_2 is released into the atmosphere. The hierarchical structure of a leaf sets the physical boundaries for photosynthetic reactions in chloroplasts as well as many other processes within a leaf. The complicated three-dimensional (3D) structure includes both physical factors such as stomata, cell walls and plasmamembranes, and a chemical medium where reactions occur, such as apoplastic fluid, cytoplasm and chloroplast stroma (Figure 1).

Leaf structure has an essential role in diffusion of CO_2 in intercellular airspaces and in mesophyll, as well as in the relative contribution of diffusion and dissolution of CO_2 in the leaf CO_2 exchange (e.g. Terashima et al. 2001). Leaf structure also plays an important role in light absorption and attenuation inside the leaf (Nishio et al. 1993, Parkhurst 1994, Ustin et al. 2001) affecting the CO_2 fixation and eventually plant production. On the other hand, the CO_2 flux to a leaf, i.e. the apparent CO_2 assimilation, which is a result of both photosynthesis and respiration, is influenced by light, temperature, ambient CO_2 concentration, photosynthetic capacity of the chloroplasts in the mesophyll cells, size of the stomatal opening, and diffusion rates in different parts of the system. Therefore, to understand the underlying temperature dependent biochemical phenomena of photosynthesis in green plant leaves, physical and biochemical factors should be distinguished.

Temperature is an essential factor affecting both the transport of gaseous substances into and inside a leaf and all biochemical processes occurring inside a leaf. The temperature dependence of photosynthesis is often studied using detailed biochemical models for which CO_2 conductance, and consequently intercellular CO_2 concentration (c_i), can be modified (originally Farquhar et al. 1980, see also von Caemmerer 2000). Recently there have been several studies addressing the effect of temperature on the variables in the model of Farquhar et al. (1980). Substantial variation in the temperature dependence of essential biochemical reactions in photosynthesis has been recorded among and within species (Wullschleger 1993, Dreyer et al. 2001, Leuning 2002, Medlyn et al. 2002). However, the temperature response of the apparent CO_2 assimilation results from all its component processes within the boundaries set by the leaf structure. This creates a contradiction as the biochemical variables are usually determined at the leaf level assuming that the CO_2 concentration in the chloroplasts equals that in intercellular air spaces (e.g. Ethier and Livingston 2004). Thus, empirically estimated biochemical parameters implicitly include CO_2 dissolution and transport in mesophyll cells. There have been attempts to experimentally verify the effect of mesophyll conductivity on biochemical parameters (Bernacchi et al. 2002, 2003, Ethier and Livingston 2004). The resulting empirical chloroplastic temperature dependencies for the biochemical processes were more temperature dependent than the original ones (Bernacchi et al. 2002).

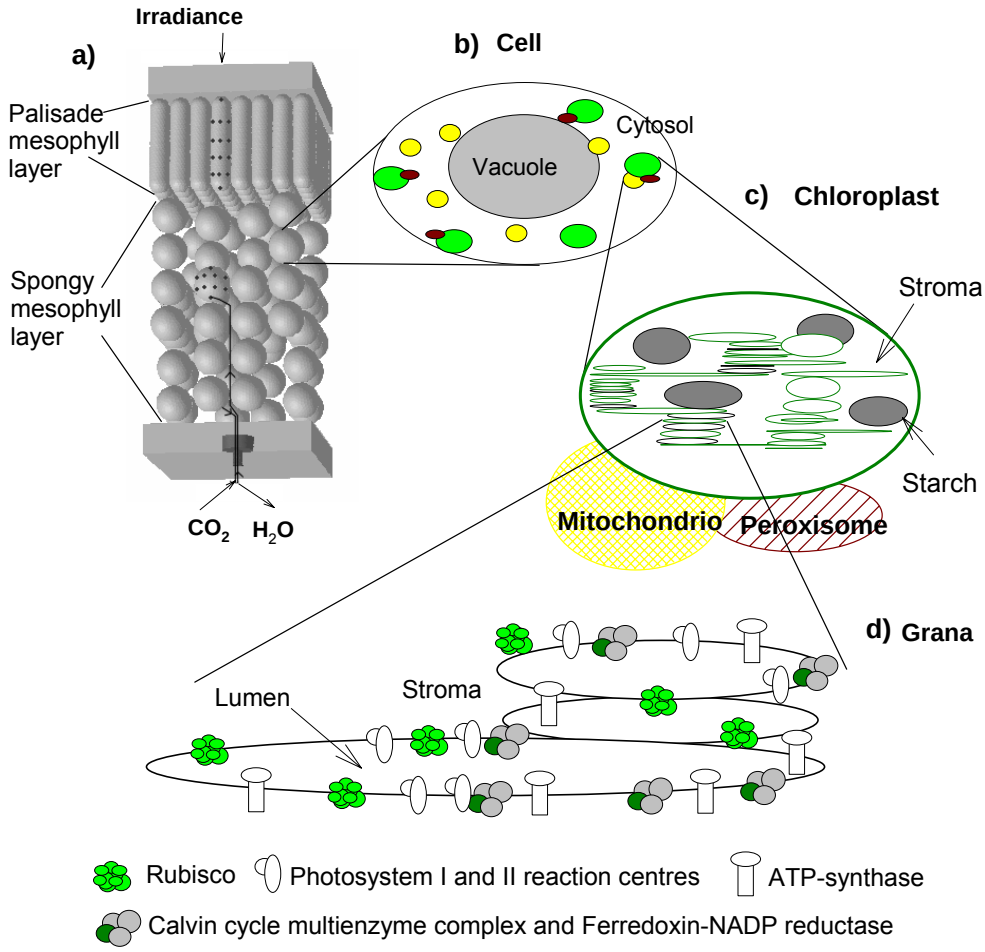

Figure 1. The structural hierarchy of a leaf. a) three-dimensional structure of a leaf and stomatal gateway to the leaf, adopted from Study **II**, b) general structure of a cell, c) schematic structure of a chloroplast and d) schematic structure of part of a thylakoid system.

Interestingly, significant differences in the temperature response of electron transport rate within the same species were established when plants were grown at different temperatures (Bernacchi et al. 2003) implying that the acclimation to growth conditions may be an important factor affecting the biochemistry of photosynthesis.

In nature plants are exposed to extremely variable environmental conditions. Light intensity fluctuates tremendously, from no light during the night to over 1700 μmol m^{-2} s^{-1} during cloudless days. In the boreal zone the temperature may vary yearly from -40 °C to +35 °C. In contrast, yearly variation in atmospheric CO_2 concentration is fairly low, at the global level about 5 to 15 μmol mol^{-1} depending on latitude (Keeling and Whorf 2004). The variation inside the canopy is somewhat higher. However, during the last 250 years the atmospheric CO_2 concentration has increased from near 280 μmol mol^{-1} in the pre-industrial era (i.e. before 1750, Houghton et al. 2001, Keeling and Whorf 2004), to 376 μmol mol^{-1} (Figure 2), and it is expected to reach 700-1000 μmol mol^{-1} by the end of the

21st century (Houghton et al. 2001). Such a rapid increase in atmospheric CO_2 concentration is unprecedented in the genetic history of tree species.

An increase in the atmospheric CO_2 concentration affects C_3 plants in a complex way (Long 1991, Long and Hutchin 1991). Initially, with increasing CO_2 concentration photosynthesis is accelerated due to the biochemical properties of CO_2 fixation and as a consequence the water use efficiency (WUE, water lost per CO_2 fixed) increases. In the long term, if the CO_2 concentration stays high, more complicated acclimation mechanisms arise leading to alterations in concentrations or activities of compounds involved in photosynthesis and finally to reallocation of resources within the photosynthetic apparatus or the plant and to changes in plant structure (e.g. Woodrow 1994, Drake et al. 1997, Luo et al. 1998).

Despite extensive research on long-term effects of elevated CO_2 concentration on plants, it has remained unclear why large differences in photosynthetic response to elevated CO_2 concentration exist among (Tjoelker et al. 1998) and within species (Pettersson et al. 1993, Gunderson and Wullschleger 1994, Rey and Jarvis 1998). In addition to direct effects of increasing CO_2 concentration, long term adjustments in photosynthetic machinery are affected by complicated feedbacks from other parts of a plant induced by e.g. resource reallocation, sink-source ratios and links to nutrient availability (e.g. Gielen and Ceulemans 2001, Bunce and Sicher 2003, Sholtis et al. 2004). This thesis does not focus on these themes, although it is recognised that they play an important role in acclimation to increasing CO_2 concentration. The importance of the availability of nitrogen as well as the role of reallocation within a tree or a plant is extensively studied elsewhere (see e.g. Norby et al. 1999, Gielen & Ceulemans 2001 and references therein).

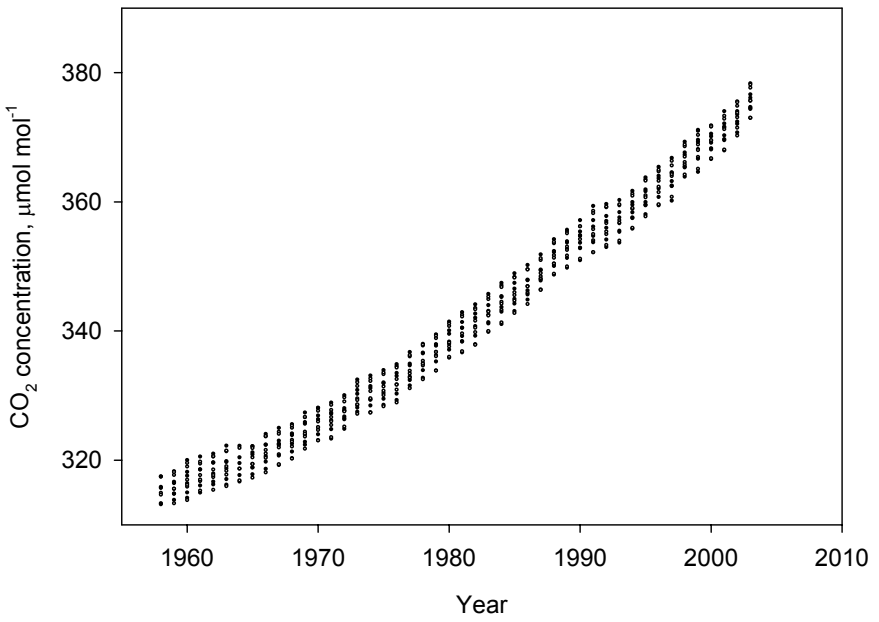

Figure 2. Yearly variation in monthly averaged CO_2 concentration at Mauna Loa observatory in Hawaii. Adopted from Keeling and Whorf (2004).

Plants are acclimated and adapted to the prevailing environmental conditions to optimize their growth and survival. In general, plants respond to a changing environment not only by altering their physiological processes, but also by adjusting their structure. Such changes are often species-specific. Initially, when atmospheric CO_2 concentration is increasing, the first effect is on the CO_2 fixing enzyme Rubisco through higher availability of substrate. All the other adjustments originate from this primary effect. For example, a species with a low diffusion rate to carboxylation sites at the current CO_2 concentration, due to long diffusion route in mesophyll or other obstructions, may have different acclimation pattern to increasing CO_2 concentration, compared to a species with a clear spongy mesophyll and fast diffusion rate in gas phase. Also, recent results show that coniferous and broadleaved tree species have different strategies in allocation of nitrogen to CO_2 fixing enzyme Rubisco (Warren and Adams 2004), which in turn may lead to different acclimation patterns. On top of all this, the Rubisco reaction is a temperature dependent process and increasing CO_2 concentration may affect the temperature dependence of apparent CO_2 assimilation that is shown to vary between species and according to growth conditions (Björkman 1981a, 1981b, Bernacchi et al. 2003).

The complexity of biochemical processes produces another problem which is rarely addressed in experimental studies on acclimation of plants to increasing CO_2 concentration. When the effects of elevated CO_2 concentration are studied in plants grown at one or two elevated CO_2 concentrations, it is implicitly assumed that the photosynthetic responses are linear, although it was suggested by Bowes (1991) and Woodrow (1994) that this is unlikely. Furthermore, structural adjustments, like stomatal density, may follow a nonlinear pattern to increasing CO_2 concentration (see Wynn 2003 and references therein). Thus, to reveal the pattern of acclimation and to establish whether there are species-specific differences, it would be essential to forget the 'double CO_2 world' predicted in one hundred years time (Houghton et al. 2001) and to study changes in plant physiology in response to a wide range of CO_2 treatments.

In conclusion, there is clearly a need for a holistic approach in predicting the effects of increasing CO_2 concentration on photosynthesis responding to prevailing light and temperature conditions, since the changing CO_2 concentration affects the plant at all hierarchical levels. This study focuses on the three dimensional (3D) structure of the leaves, where the actual processes and initial effects take place, and at the same time keeping in mind the nonlinearity of nature. In the following chapter the physical and biological aspects related to apparent CO_2 assimilation and acclimation are introduced through the hierarchical structure of a leaf.

1.2. Leaf structural boundaries for photosynthesis

The stomatal pore acts as a gateway to the leaf. It is surrounded by two guard cells, which regulate the stomatal aperture by swelling or shrinking. Stomatal closure occurs when solutes are actively transported out from the guard cells to the surrounding subsidiary cells and *vice versa* (Lambers et al. 1998). The aperture of stomata may be non-uniform in leaf epidermis, creating so called stomatal patchiness which affect leaf level stomatal conductance and further CO_2 assimilation (e.g. Pospíšilová and Šantruček 1996 and

references therein). Both the structure and organization of stomata varies between species and even within same species grown in different environments.

In silver birch the stomata are randomly distributed on the abaxial leaf surface, there is no antechamber and beneath the guard cells there is an irregularly shaped, widely spaced spongy mesophyll (Figure 1a). The cell layer closer to the upper side of the leaf, adjacent to the highest irradiance, is called the palisade parenchyma. The palisade cells are closely packed columnar cells usually rich in chloroplasts (Vogelmann et al. 1996). In Scots pine, on the other hand, the stomata are organized in rows; the stoma has a clear antechamber so that the guard cells are embedded into the cuticle (Turunen and Huttunen 1991). Beneath the guard cells and epidermis there is a substomatal cavity. Scots pine needles are amphistomatous, i.e. the stomata are located on both sides of the needle. The stomatal cavity leading into the leaf, limits strongly the CO_2 diffusion into the leaf, setting constraints for CO_2 supply to the chloroplasts. Also, for fast diffusion of CO_2 inside the leaf the essential question is the total length of the diffusion path and the length of the diffusion path in liquid phase, that depend on the percentage of total air space within a leaf.

The primary photosynthetic processes occur in chloroplasts that are surrounded by a two-layer envelope selectively allowing the passage of molecules. Chloroplasts possess an internal system of thylakoid membranes, which is surrounded by liquid stroma (e.g. Lawlor, 2001). The thylakoid system effectively occupies the chloroplast volume. The space inside the thylakoid system is called the lumen. The main reactions in photosynthesis take place in different sections of the chloroplast. The energy capture and electron transport reactions producing high-energy compounds take place in chloroplast thylakoids. The subsequent use of energy in CO_2 fixation in the Calvin cycle occurs in chloroplast stroma, where the primary step of CO_2 fixation is catalyzed by ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco) molecules, loosely attached to the thylakoid membrane (Süss et al. 1993).

The chloroplasts are usually located near the cell wall that ensures the effective transport of CO_2 molecules into the stroma. Light induced movements of chloroplasts have been observed especially in plants acclimated to shade conditions (Brugnoli and Björkman 1992, Lambers et al. 1998).

1.3. Route of CO_2 molecules into the chloroplasts

The location and the size of the chloroplasts as well as the structure of the chloroplast envelope create the boundaries for the CO_2 diffusion into the carboxylation sites and therefore a CO_2 gradient inside the leaves. When CO_2 is assimilated in light, a concentration gradient is created between the air outside a leaf and carboxylation sites in the chloroplasts that drives the flow of CO_2 into the leaf (e.g. Nobel 1999). Around the leaf surface there exists a boundary layer (typically about 1mm), where the air movement is laminar and CO_2 is transferred largely by molecular diffusion (Parkinson 1985, Nobel 1999). CO_2 molecules move down the gradient through the laminar boundary layer, stomatal pore, substomatal cavity and intercellular air spaces by diffusion in the air phase until they reach the surface of a mesophyll cell. Before entering the cell the CO_2 molecules dissolve in a thin aqueous layer at the mesophyll cell surface. From there they move across cell walls through the cytosol and chloroplast envelope, finally reaching the carboxylation

site in the chloroplasts (Nobel 1999). The transport of CO_2 molecules in the mesophyll is mainly governed by diffusion in the liquid phase.

1.3.1. Diffusion of CO_2 into the leaf

Along the gaseous part of the route, from the boundary layer to the mesophyll surface, the transport is governed by binary diffusion in a carrier gas (air) (D_g) (Lushnikov et al. 1994). The diffusivity increases with temperature due to enhanced thermal motion of molecules, and the temperature dependence of the binary diffusion coefficient of CO_2 in air is well known (Reid et al. 1987). Stomatal regulation affects the rate of the flow of CO_2 into the leaf, because decreasing stomatal aperture lowers the diffusion rate between stomatal cavity and atmosphere.

1.3.2. Dissolution of CO_2 on mesophyll cell surface

To enter the mesophyll the CO_2 molecules dissolve in the aqueous layer at the mesophyll cell surface, where a local equilibrium between CO_2 in water and air can be assumed (e.g. Nobel 1999). The dissolution of CO_2 can be described by an absorption equilibrium constant that produces a discontinuous jump in absolute concentrations (mol m^{-3}) across the interface. This constant, at the equilibrium pH of 5.6, is called Henry's law coefficient (H) (Denbigh 1971).

Dissolution of CO_2 decreases exponentially with increasing temperature (see Denbigh 1971, Seinfeld and Pandis 1998, Aalto et al. 1999). However, the effective H^*, which includes the dissociation of CO_2, is also dependent on pH of the solution (Seinfeld and Pandis 1998). For example, if the pH of the solution increases from 5.6 to 7, the Henry's law coefficient for CO_2 dissolving in liquid water increases from 0.83 to 3.88 at 25 °C (Nobel 1999). However, the pH in the cytosol is near 7, whereas on the mesophyll cell surface the pH is as low as 5 to 6 (Sze 1985). As far as the temperature dependence is concerned, dissolution into extracellular water and dissociation of CO_2 probably obey similar exponential rules.

1.3.3. Diffusion of CO_2 into the chloroplasts

The transport of CO_2 in the liquid section of the route (mesophyll) is a more complex process (Cowan 1986, Evans and von Caemmerer 1996; see also Agutter et al. 1995). In general, the diffusion in the liquid phase is slow and more temperature dependent compared to the diffusion in air. If it is assumed, that the cells are mostly water, the value as well as the temperature dependence of the diffusion coefficient (D_l) can be estimated (Aalto et al. 1999, see also Bird et al. 1960). In the cells there exist, however, macromolecules and membranes obstructing the transport of CO_2 and decreasing the diffusion coefficient even to half of the diffusion coefficient in the water (Cowan 1986). On the other hand, the aquaporins located in the plasma membrane as well as conversion of CO_2 to HCO_3^- and H_2CO_3 may accelerate transport of CO_2 through the chloroplast envelope and inside chloroplasts (Cowan 1986, Evans and von Caemmerer 1996, Nobel 1999, Terashima and

Ono 2002). In the mesophyll HCO_3^- and H_2CO_3 diffuse markedly faster than CO_2 molecules. Furthermore, dissociation of CO_2 to HCO_3^- and H_2CO_3 increases with increasing pH, and in an alkaline chloroplast stroma HCO_3^- is present at almost 100 times the concentration of CO_2 (e.g. Moroney et al. 2001).

In such environments carbonic anhydrase (CA), an enzyme that catalyses reversible hydration of CO_2, may be needed to facilitate the CO_2 supply to the sites of carboxylation (Coleman 2000, Moroney *et al*. 2001). Stromal CA and aquaporins can greatly facilitate CO_2 transport to the carboxylation sites (Moroney et al. 2001, Bernacchi et al. 2002, Terashima and Ono 2002).

1.4. Light environment inside a leaf

Absorption of PAR inside the leaf is effective, about 85 to 90% of incident light intensity is being absorbed (e.g. Lloyd et al. 1992, Nobel 1999). The absorption and penetration of light depends on leaf structure, particularly on the amount and location of the palisade and spongy mesophyll cells (Vogelmann et al. 1996). Light attenuation inside a leaf is often assumed to obey an exponential decay rule called Beer's law (Lloyd et al. 1992). The densely located palisade cells effectively shadow the spongy mesophyll layer creating less favourable light environment for CO_2 assimilation in spongy mesophyll cells. However, it is suggested that light scattering can be minimized by the organisation of the palisade mesophyll cells so that the light would be guided further into the spongy mesophyll layer where a large scattering enhances the light capture (Vogelmann and Martin 1993, Vogelmann et al. 1996, Evans 1999). Due to light scattering the photon path lengths within a leaf are commonly two to four times longer than the thickness of the leaf (Vogelmann et al. 1996). The efficient guiding of light deeper into the leaf is especially important for species acclimated to deep shade and within a canopy (Vogelmann et al. 1996).

1.5. Biochemical sink of CO_2 in chloroplasts

1.5.1. The light reactions of photosynthesis

Visible light (photosynthetically active radiation, PAR, 400-700 nm) is the driving force for photosynthesis. In a series of reactions starting from excitation of light harvesting complexes I and II (LHCI and LHCII), light energy is transformed in the chloroplasts into chemical energy, i.e. NADPH (nicotinamide adenine dinucleotide phosphate) and ATP (adenosine triphosphate), used in CO_2 fixation in stroma (Figure 3). The energy is transferred into reaction centres (photosystem I and II, PSI and PSII) (e.g. Malkin and Fork 1981, Kühlbrandt et al. 1994). The capture and transduction of energy is an extremely fast reaction (200-500 picoseconds) and almost independent of temperature (Whitmarsh and Govindjee 1995).

In electron transport reactions the electrons are transferred from PSII to PSI, where they are reenergized (Lawlor 2001, Whitmarsh and Govindjee 1995). The reenergized electrons

can then be used for the reduction of NADP+ to NADPH in an enzymatically regulated reaction. During the course of light reactions occurring in the thylakoid membrane protons are accumulating into the thylakoid lumen creating a pH difference between the chloroplast stroma and lumen (see Figure 1). Through this pH gradient ATP is synthesized by an enzyme called ATP synthase, which functions as a proton pump between lumen and stroma (Lawlor 2001, Kramer et al. 1999). Compared to light capture and the primary light reactions in PSII and PSI, the processes in the electron transport chain are clearly slower creating time dependency for the whole light reaction side (Whitmarsh and Govindjee 1995). Furthermore, although the primary light reactions are almost independent of temperature, the biochemical reactions involved in the electron transport chain create the temperature dependence for the light reaction side of photosynthesis.

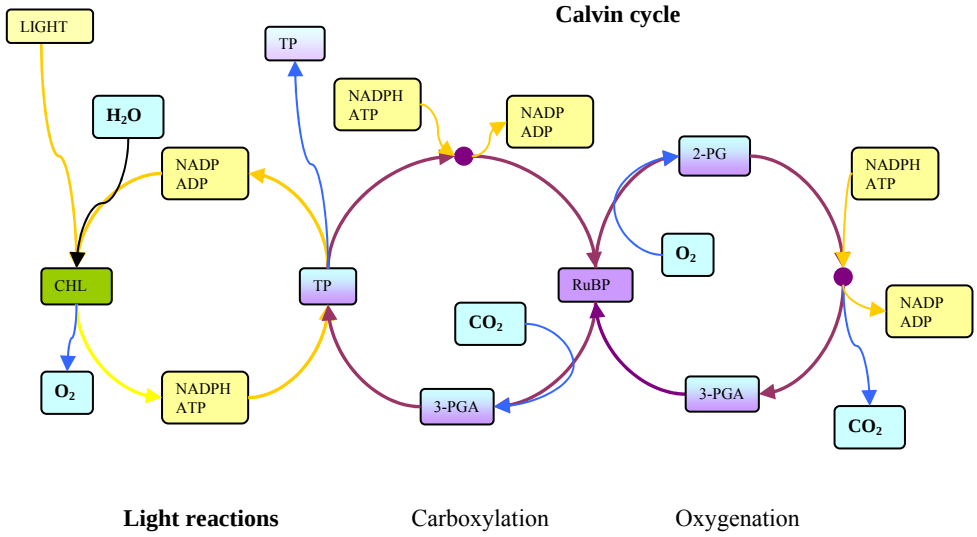

Figure 3. Schematic figure of the photosynthetic reactions showing the interconnections between light reactions, carboxylation and oxygenation processes. Light is absorbed by the reaction centres formed by chlorophylls and other pigments (CHL) and the high energy compounds like nicotinamide adenine dinucleotide phosphate, NADPH from NADP, and adenosine triphosphate, ATP, from adenosine diphosphate, ADP, are formed. These are utilized in Calvin cycle reactions for forming triose phosphates, TP, and ribulose 1,5-bisphosphate, RuBP. In carboxylation Rubisco reacts with CO_2 to form 3-phosphoglycerate, 3-PGA. When Rubisco reacts with O_2, initially 2-phosphoglycolate, 2-PG, is formed starting a series of energy demanding reactions called oxygenation or photorespiration.

1.5.2. Rubisco, the key enzyme in CO_2 fixation

Light reactions produce high energy compounds NADPH and ATP which are used in carbon-reduction in the Calvin cycle (Figure 3). These processes occur in chloroplast stroma. In the Calvin cycle Rubisco enzyme joins one molecule of CO_2 to RuBP (ribulose 1,5-bisphosphate) to yield subsequently two molecules of 3-PGA (3-phosphoglycerate) (e.g. Lorimer 1981, Stitt 1991). From 3-PGA the triose-phosphates (TP) are formed in a series of energy demanding reactions (Figure 3). Part of the formed TPs can be transported from the chloroplast for e.g. sucrose synthesis or it can be used for starch synthesis in the chloroplasts. Most of the TPs are, however, used for regenerating RuBP through a complicated series of reactions where ATP and NADPH are consumed (e.g. Raines et al. 1999, Taiz and Zeiger 2002, Poolman et al. 2000).

Rubisco is an enzyme that catalyses both the fixation of CO_2 and O_2 (oxygenation, called hereafter photorespiration) (Lorimer 1981, Woodrow and Perry 1988) (Figure 3). Photorespiration is a complicated series of energy consuming reactions occurring in three different organelles: chloroplasts, peroxisomes and mitochondria (see Figure 1). If Rubisco reacts with O_2, first one 3-PGA and one 2PG (2-phosphoglycolate) are formed. 3-PGA stays in the carboxylation cycle, but 2PG is transformed to glycolate which is transported out from the chloroplast to the peroxisome (e.g. Wingler et al. 2000). During the sequence of reactions CO_2 is eventually released in the mitochondria (Figure 3). Based on the stoichiometry of the photorespiratory process, 40 % of cyclic carbon is transported out and 75 % of that returns to the chloroplast (e.g. Taiz and Zeiger 2002). Therefore, a total of 10 % of assimilated carbon is lost in photorespiration. Eventually the photorespiration cycle closes by transporting glycerate to the chloroplast where again 3-PGA is formed in an ATP consuming reaction. Although in earlier years the photorespiration cycle was considered as a wasteful process consuming fixed carbon it is now widely agreed that photorespiration plays an important role in nitrogen assimilation in leaves as well as in stress protection (Wingler et al. 2000, Rachmilevitch et al. 2004).

The ratio of carboxylation to oxygenation primarily depends on the concentrations of CO_2 and O_2 at the carboxylation site, temperature and the Rubisco specifity factor of the species (Viil and Pärnik 1995). Like all biochemical processes, carboxylation and oxygenation of Rubisco are highly temperature dependent reactions (Jordan and Ogren 1984). In addition, as temperature increases, the solubility of CO_2 relative to O_2 decreases along with the affinity of Rubisco to CO_2 relative to O_2, thus favouring photorespiration (e.g. Jordan and Ogren 1984, Ghashghaie and Cornic 1994).

1.5. The role of mitochondrial respiration

The measurable CO_2 exchange rate of a leaf is a result of two different processes, photosynthesis which consumes CO_2 and mitochondrial activity (respiration) which produces CO_2. Respiration, occurring mainly in the cytosol and in mitochondria, is a process by which reduced organic compounds are mobilized and subsequently oxidized in a controlled manner (Taiz and Zeiger 2002). The respiration supplies much of the usable energy as ATP, NAD(P)H and the carbon skeletons that are required for growth, maintenance, transport and nutrient assimilation processes (Amthor 1994). The respiration

rate may be controlled by energy demand, or high carbohydrate concentration (Amthor 1994, Taiz and Zeiger 2002). At temperatures below ca. 40 °C, respiration depends exponentially on temperature, although the temperature coefficient (Q_{10}) changes with temperature varying between 2 to 3 at physiological temperatures (Amthor 1994).

It is often assumed that the respiration rate is reduced in light and that this reduction is almost independent of temperature (e.g. Brooks and Farquhar 1985, Lloyd et al. 1995). The decrease in respiration in light may be caused by e.g. the competition between reducing equivalents produced from photorespiration and those produced by respiration in Krebs cycle (Laisk and Loreto 1996). In light also a part of respired CO_2 is re-assimilated before it is released into the atmosphere. Respiration in light is essential to keep up with the demand for metabolites of biosynthetic processes such as nitrogen assimilation (e.g. Hoefnagel et al. 1998, Noctor and Foyer 1998). Although respiration is usually as low as 10 to 20 % of photosynthesis at current atmospheric conditions, the role of respiration in productivity increases when the diurnal or seasonal pattern of CO_2 exchange is considered. In fact, Amthor (1991) suggested that in total half of the assimilated carbon is lost via respiratory pathways.

1.6. The effect of CO_2 on photosynthetic and transport processes

Initially, an increase in atmospheric CO_2 concentration eases the diffusion into the leaf and the chloroplasts and the role of active transport diminishes. Consequently, increasing CO_2 concentration enables more effective stomatal control which decreases the transpiration rate and consequently increases water use efficiency (WUE).

With increasing CO_2 concentration the catalysing reaction of Rubisco turns in favour of carboxylation and thus the fixation of O_2 and the release of CO_2 in photorespiration diminishes (Jordan and Ogren 1984, Brooks and Farquhar 1985, Ghashghaie and Cornic 1994). As the photorespiration is relatively more enhanced with increasing temperature at current atmospheric CO_2 concentration, the temperature dependence of photosynthesis changes at elevated CO_2 concentration (e.g. Jordan and Ogren 1984, Ghashghaie and Cornic 1994) leading to a higher optimum temperature.

Direct responses of the respiration to increasing CO_2 concentration have varied from inhibition to stimulation (see Amthor 2000 and references therein). The reason for this has remained unclear, but it could be related to direct effects at any point in the respiratory enzymatic chain (Amthor 1991, Ceulemans and Mousseau 1994). However, *in situ* the direct effect of CO_2 on nocturnal respiration seems to be small and can be actually, in part, an artefact (Amthor 2000, Jahnke and Krewitt 2002).

1.7. Acclimation to increasing CO_2 concentration

1.7.1. Effects on photosynthesis and respiration

In acclimation of C_3 plants to elevated atmospheric CO_2 concentration, Rubisco plays a key role (e.g. Eamus and Jarvis 1989, Stitt 1991). In the long term, increasing CO_2 concentration can lead to reduction in Rubisco activity or concentration because the amount of Rubisco required maintaining the same assimilation rate decreases (Woodrow 1994, Drake et al. 1997). Despite this decrease, the increase in CO_2 concentration may still allow for higher CO_2 assimilation rate (e.g. Hikosaka and Hirose 1998). Furthermore, because of the general kinetics of the Rubisco-catalyzed enzymatic reactions, i.e. saturation at high substrate concentrations and two competitive substrates, the acclimation mechanism mediated through Rubisco should be nonlinear in response to increasing CO_2 concentration (Woodrow 1994, Luo et al. 1998). Finally, due to the general kinetics of Rubisco, the prolonged effect of increased CO_2 concentration on photosynthesis should be temperature dependent leading to altered optimal environmental conditions (e.g. Long 1991, Drake et al. 1997).

Due to the diminished photorespiration rate, the energy demand per fixed carbon decreases (Woodrow 1994, Wilkins et al. 1994). Consequently, the demand for compounds involved in light reactions, such as chlorophylls or carotenoids, may also decrease. The extent, to which this is actually reflected to the light reaction side of photosynthesis, depends most likely on feedbacks from the whole plant level. For example, poor nutrient status could lead to a different acclimation.

Elevated atmospheric CO_2 concentration may also lead to acclimation in respiratory processes (Ceulemans and Mousseau 1994, Luo et al. 1999). The possible changes in respiration may be due to structural changes imposed by elevated CO_2, accumulation of carbohydrates or changes in the biochemistry of respiration (Amthor 1991, Poorter et al. 1997, Amthor 1994). The acclimation of respiration can originate also from changes in both nitrogen and carbohydrate concentrations (Tjoelker et al. 1999). However, no clear trend in acclimation in respiration is evident, and diverse results on respiration rates have been found in different species on area, mass or nitrogen basis (Mitchell et al. 1995, Roberntz and Stockfors 1998, Zha et al. 2002, Tjoelker et al. 1999).

1.7.2. Structural adjustments

In the long term, changing CO_2 concentration may induce alterations in leaf structure as well as in leaf development (Pritchard et al. 1999 and references therein). The leaf dry mass to fresh mass ratio can change due to e.g. accumulation of carbohydrates into the leaf. Due to the accumulation of carbohydrates the chloroplast structure and organisation may change (Pritchard et al. 1997). Also leaf thickness might change reflecting e.g. the adjustment of altered ratio of penetrating irradiance and absorbed CO_2 (Ceulemans and Mousseau 1994). Furthermore, the increased water use efficiency can lead to decreased stomatal density of leaves (Wynn 2003). Therefore it is important to study the transport of gases in the three dimensional structure of leaves. Understanding the connections between the structural

changes in leaf and transport phenomena enables more relevant predictions on tree functioning in future climate.

1.7.3. Species specific differences

Although the basic physiological and physical processes are the same, plants differ in their structure, growth pattern and optimal growth conditions. Prevailing growth strategies of plants support optimal functioning under current environmental conditions (Björkman 1981a,b), but a change in one variable may lead to an unbalanced system (Thornley 1998). The ability to respond to such changes (e.g. the relative availability of nitrogen and carbon) by adjusting resource allocation, nutrient uptake or transport mechanisms, for example, is an important attribute for survival in a changing environment. Such an adjustment may be needed if atmospheric CO_2 concentration stays high, because in the present climate, CO_2 availability is a limiting factor for photosynthesis.

Restriction of photosynthesis through inadequate nutrient supply may lead to diverse responses of photosynthesis in elevated CO_2 concentration (Arp 1991, Stitt 1991). Because Rubisco represents the largest single nitrogen investment in a leaf, acclimation to elevated CO_2 concentration via changes in Rubisco quantity, can lead to an adjustment in nitrogen use within a plant or within the photosynthetic apparatus (Drake et al. 1997). Decreases in leaf Rubisco and nitrogen concentrations as a result of nutrient deficiency at elevated CO_2 concentration have been recorded for several species (El Kohen and Mousseau 1994, Groninger et al. 1995). However, it has become more widely accepted that the leaf nitrogen concentration decreases in response to elevated CO_2 concentration regardless of nutrient status allowing more efficient use of nitrogen within a plant (Curtis 1996, Cotrufo et al. 1998). These adjustments may be nonlinear as well.

The CO_2 concentration gradient within a leaf changes with changing CO_2 concentration. The structural differences in leaves between species can therefore also lead to different acclimation patterns in increased atmospheric CO_2 concentration.

2. AIMS OF THE STUDY

The three dimensional structure of a leaf, where the basic photosynthetic processes occur is amazingly rarely considered in studies on acclimation of trees to increasing CO_2 concentration, in contrast with studies on light acclimation of plants (see e.g. Niinemets and Tenhunen 1997). Moreover, nature is rarely linear in its behaviour in the constantly changing environment, but this also is too often omitted in studies on CO_2 acclimation of plants. These factors may affect the acclimation pattern of trees to changing environment leading to differences between species.

The aim of this thesis was to analyse the effects of temperature and increasing CO_2 concentration on the processes involved in photosynthesis and on acclimation of the photosynthetic machinery within constraints set by the leaf three-dimensional structure. These processes include the transport of CO_2 into and within the leaf as well as the photosynthetic CO_2 sink in the chloroplasts. The question of temperature was restricted to

the dependencies of physical and biochemical processes on temperature and the acclimation of plants to globally changing temperature is not considered in this thesis.

To analyse the transport of gases in the 3D structure of a leaf and to improve the understanding of optimal strategies of a plant in changing atmosphere a detailed three-dimensional model of silver birch leaf was constructed (Study **II**). To understand the role of temperature in apparent CO_2 assimilation, the temperature dependencies of essential biochemical reactions in photosynthesis were first experimentally determined for silver birch and for boreal conditions utilising widely accepted model of photosynthesis (Farquhar et al. 1980) (Study **I**). Next, the role of the temperature dependent physical phenomena in the apparent CO_2 assimilation was analysed in detail utilising the 3D model constructed in Study **II** (Study **III**). These results were, in turn, used to distinguish the effect of transport phenomena from the empirical temperature dependencies of biochemical reactions determined in Study **I**.

Finally, to understand the acclimation of photosynthesis to increasing CO_2 concentration in tree species, the relationship between leaf biochemical properties and photosynthesis during acclimation to increased atmospheric CO_2 concentration was analysed in silver birch and Scots pine (Study **IV**). Two specific hypotheses were tested: **1.** Elevated atmospheric CO_2 concentration allows reallocation of resources within the photosynthetic apparatus or within the plant (Woodrow 1994) and **2.** Due to the complexity of the acclimatory mechanisms and the dual role of CO_2 fixing enzyme Rubisco, photosynthetic responses to increasing CO_2 concentration are nonlinear (Luo et al. 1998). This leads, within the constraints created by the variable structure of leaves, to species-specific acclimation patterns to increasing CO_2 concentration.

3. MATERIALS AND METHODS

3.1. Experimental setup

3.1.1. Plant material and experimental design

One-year old nursery-grown silver birch (*Betula pendula* Roth) clones (origin Valkeakoski, Finland) were used in all experiments (Studies **I-IV**). In Study **IV** one-year old nursery grown Scots pine (*Pinus sylvestris* L.) seedlings (seeds from open pollinated trees, origin Karttula, Finland) were also used. In Study **I,** and later used also in Studies **II** and **III,** the seedlings were planted in eight-litre containers in a mixture of fertilized peat and sand (2:1 volume ratio). The seedlings were grown outdoors and exposed to ambient variation in light, temperature and air humidity.

In Study **IV** the seedlings were planted in pots, which were buried in soil to allow natural variation in root temperature. The seedlings were irrigated with nutrient solution three times per week (Ingestad 1979). Seedlings were protected from rain, and during extremely hot days they were irrigated with deionised water. The seedlings were placed outdoors on May 1994, overwintered in a cellar (0-1 °C) from September 1994 to May

1995. The aboveground plant parts were enclosed in open-top chambers. CO_2 was added to the chamber through a ventilator at the base of the chamber. A steady flow of CO_2 was maintained using a series of pressure controllers and capillaries of different lengths. Ten different CO_2 treatments were used from current ambient CO_2 concentration (350 μmol mol^{-1}) to 2000 μmol mol^{-1} of CO_2. One seedling of each species was assigned to a treatment. All data were collected in August, since acclimation most likely occurs towards the end of the growing season, and variable responses might be observed during acclimation (Jach and Ceulemans 1999, Gielen et al. 2000).

3.1.2. Gas exchange measurements

All gas exchange measurements were performed in the laboratory with a dynamic system for measuring gas exchange (described in detail in studies **I** and **IV**). To measure photosynthesis, a birch leaf or 6-8 pine needles were enclosed in a temperature-controlled cuvette with a constant airflow through it. Ambient air, from which water vapour and CO_2 were removed, was used as the base gas. Total airflow through the system and the injection of CO_2-rich gas were regulated with mass flow controllers. Water vapour was generated with a dew point generator and a mass flow controller regulated the amount of air flowing through the generator. Water vapour and CO_2 concentrations were recorded with two infrared gas analysers. The leaf CO_2 exchange rate (A) was measured separately with a differential infrared gas analyser. Light was provided by a daylight lamp and measured with a PAR-sensor. The cuvette temperature was regulated dynamically by a computer-operated temperature controller and was monitored with two constantan thermocouples. Stomatal conductance (g_s) and CO_2 concentration in the airspaces inside leaves (c_i) were calculated from the transpiration measurements.

To re-parameterise the biochemical Farquhar–model, the CO_2 response curves of CO_2 exchange were determined at full sunlight (1500 μmol m^{-2} s^{-1}) and the light response curves were determined at 1000 μmol mol^{-1} of CO_2 (Study **I**). Both responses were determined at five temperatures for three leaves. The temperature response of dark respiration was measured at 360 μmol mol^{-1} of CO_2 at ten steps each lasting ca. 30 min. Finally, the CO_2 exchange in light was measured using the same settings with light intensity of 900 μmol m^{-2} s^{-1}.

To analyse the species-specific differences in acclimation of photosynthesis to different CO_2 concentrations, the steady state CO_2 exchange rate and transpiration of silver birch and Scots pine were determined at ambient CO_2 concentration, the growth CO_2 concentration and at 2000 μmol mol^{-1} of CO_2 (Study **IV**). The measurements were done at saturating light (850-930 μmol m^{-2} s^{-1}). Mean air temperature was 19 °C and mean relative humidity was 40%. For practical reasons, attached leaves of birch and detached needles of pine were used for measurements. Immediately after cutting, needles were placed in wet cotton and wrapped in plastic. The data were analysed with linear and polynomial regression with growth CO_2 concentration as the independent variable.

To analyse the possible changes in the temperature dependence of photosynthesis during acclimation to elevated CO_2, the dynamic temperature responses of CO_2 exchange were determined at ambient CO_2 concentration and at the growth CO_2 concentration for birch seedlings. The air temperature was increased from 5-8 °C to 32 °C within an hour.

3.1.3. Biochemical determinations of photosynthesis and growth

To analyse the acclimation of photosynthetic machinery to increasing CO_2 concentration, the concentrations of Rubisco, soluble protein and chlorophyll in the leaves or needles were determined at the end of both growing season according to Rintamäki et al. (1988), Vapaavuori et al. (1992) and Ovaska et al. (1993) (Study **IV**). At the end of the experiment, specific leaf area (SLA), fresh and dry mass, nitrogen concentration and the C/N ratio of leaves, needles, stem and roots were determined.

3.2. Modelling the CO_2 assimilation within the leaf

3.2.1. Biochemical model for leaf CO_2 assimilation

As a starting point in describing the biochemical processes and their temperature dependencies, a steady state model of photosynthesis was adopted (Farquhar et al. 1980, Farquhar and von Caemmerer 1982, Harley and Baldocchi 1995, Lloyd et al. 1995) (called the Farquhar–model hereafter) that combines leaf level gas exchange with biochemical processes in chloroplasts. The Farquhar–model is based on the kinetics of Rubisco and it describes the main reactions in the biochemistry of photosynthesis. The model is widely accepted as a tool for interpreting the measured leaf level CO_2 exchange rates. Although there exist more detailed steady-state models and dynamic models that describe the biochemical reactions (Kaitala et al. 1982, Hahn 1987, Laisk and Eichelmann 1989, Pearcy et al. 1997, Lushnikov et al. 1997, Poolman et al. 2000), these models are not operational enough to be used as a functional model at different environmental conditions. At this phase, the Farquhar-model was appropriate as an easily usable, relatively simple model for studying the temperature dependencies and for application as a sub-model in further 3-dimensional modelling.

In short, Farquhar et al. (1980) formulated photosynthesis through different limitations. In ample light photosynthesis is limited by availability of CO_2 (the capacity of Rubisco to consume RuBP) and in low light it is limited by the availability of light (the capacity of RuBP regeneration). Similarly, at low CO_2 concentration the capacity of Rubisco limits photosynthesis, whereas the capacity of RuBP regeneration does so at high CO_2 concentration (Farquhar et al. 1980, Hikosaka and Hirose 1998). These two processes are considered to be co-limiting at the current atmospheric CO_2 concentration. A third limiting process, triose-phosphate utilisation (Sharkey 1985, Farquhar 1988), imposed by sink-limitation or nutrient availability (Kirschbaum and Farquhar 1984, Medlyn 1996, Hikosaka 1997) was not considered in this study.

The net rate of CO_2 exchange can be expressed as a minimum of A_j and A_c, where A_j is the RuBP regeneration-limited rate of the net CO_2 exchange and A_c is the net Rubisco-limited rate. A_j and A_c can be written as:

$$A_j = J\frac{c_i - \Gamma^*}{4(c_i + 2\Gamma^*)} - R_d \qquad (1)$$

$$A_c = V_{c(\max)} \frac{c_i - \Gamma *}{K_c\left(1 + o / K_o\right) + c_i} - R_d \qquad (2)$$

where J is the potential electron transport rate, c_i is the CO_2 concentration in leaf air spaces, Γ^* is the CO_2 compensation point in the absence of mitochondrial respiration, R_d is the rate of mitochondrial respiration, $V_{c(max)}$ is the maximum rate of carboxylation, K_c and K_o are the Michaelis-Menten constants for CO_2 and O_2, respectively, and o is the oxygen concentration in chloroplasts (assumed constant).

The temperature dependence of Γ^* was taken from Brooks and Farquhar (1985). The Arrhenius type temperature dependence was used for $V_{c(max)}$, K_o, K_c and R_d (Farquhar et al. 1980, Harley and Baldocchi 1995):

$$f_T = f_{298K} \exp\left(\frac{E_f (T - 298.15)}{298.15RT}\right) \qquad (3)$$

where f denotes the variable, E_f is the activation energy of the specific variable, T is temperature and R is gas constant.

Dark respiration was determined from the temperature dependence of CO_2 exchange in darkness and the respiration in light was determined as an intercept from linear fitting of five lowest measurement points in A/c_i curve at 25 °C.

The potential electron transport rate *(J)* is a function of incident irradiance (I_0):

$$J = \frac{qI_0 + J_{\max} - \sqrt{(qI_0 + J_{\max})^2 - 4\Theta qI_0 J_{\max}}}{2\Theta} \qquad (4)$$

where J_{max} is the maximum electron transport rate, q is the light use effectivity factor and Θ is the convexity factor.

The maximum electron transport rate, J_{max}, depends on temperature according to the following equation (Farquhar *et al*. 1980, Lloyd *et al*. 1995):

$$J_{\max} = \frac{B \exp\left(\frac{E_j (T / 298.15 - 1)}{RT}\right)}{1 + \exp\left(\frac{S_j T - H_j}{RT}\right)} \qquad (5)$$

where E_j is the activation energy, S_j is the entropy of the denaturation equilibrium, H_j is the deactivation energy for J_{max}, T is temperature, R is gas constant and B is a constant.

In Study **I** the species-specific temperature dependencies for essential Farquhar–model parameters were determined. The newly parameterised model was utilised for the description of biochemical reactions in Studies **II** and **III**.

3.2.2. 3D–model of CO_2 transport

To be able to study the role of CO_2 transport in apparent CO_2 assimilation a detailed three dimensional model (3D model) of fine structure of a birch leaf was developed, which included all the essential organelles: chloroplasts, palisade and spongy mesophyll cells, air spaces, stomatal pore and leaf boundary layer (Study **II**). The numerical 3D model described the environment around a single stoma extending from lower to upper cuticle and also including the boundary layer below the leaf. The constructed grid geometry was based on examination of silver birch leaf sections under a light microscope. The shape of a spongy mesophyll cell was approximated with a sphere and of a palisade mesophyll cell with a cylinder with spherical ends. The shape of the chloroplasts was described with a sphere and they were locating at a distance of 1 μm from the cell wall. The modelled structure of leaf section is shown in Figure 1, Chapter 1. The grid was irregular, i.e. it was dense on critical boundaries and areas facing large concentration changes and sparse in areas and volumes with small changes. The boundary conditions for cell surfaces and domain boundaries were applied according to earlier studies (Aalto et al. 1999). Above the leaf a constant CO_2 concentration was set at the top of the boundary layer.

The transfer equation for CO_2 in air and cells is governed by the following diffusion equation (Bird et al. 1960):

$$D\nabla^2 c = S \tag{6}$$

where c is the CO_2 concentration in gas or in cells, D is the binary diffusion coefficient in the carrier gas or in cells and S is the source or sink of CO_2. S is zero for transport in air and in mesophyll cells excluding chloroplasts, where the CO_2 fixation was located. The magnitude of the sink was determined by the biochemical model equations (Farquhar et al. 1980) and Beer's law for irradiation extinction (Study **I**). These equations utilized the locally defined CO_2 concentration and irradiance in each grid cell point, and a global value for temperature.

In the 3D model, the light absorption inside the leaf was assumed to obey exponential decay as described by Beer's law (Lloyd et al. 1992):

$$I = 1.1 I_0 \exp\left(-2.4\frac{z}{z_0}\right) \tag{7}$$

where I_0 is the incident irradiance, z is the distance from the surface of the leaf and z_0 is the thickness of the leaf.

3.2.3. Temperature dependent physical phenomena of CO_2 transport

The effect of temperature on the CO_2 flux through the stoma and to apparent CO_2 assimilation was studied further with the 3D model to distinguish between the physical and biochemical processes. The temperature dependencies of physical phenomena that affect the CO_2 flux were incorporated into the 3D model. The role of the diffusion of CO_2 in the mesophyll cells in the apparent CO_2 assimilation was analysed in detail. Furthermore, the

role of active transport in the chloroplasts was studied by specifically varying the chloroplastic diffusion.

The temperature dependence of the binary diffusion coefficient of CO_2 in air, D_g was approximated according to Reid et al. (1987):

$$D_g = 0.135\left(\frac{T}{273.15}\right)^{1.75} \tag{8}$$

where T is temperature.

The temperature dependence of the binary diffusion coefficient of CO_2 in the mesophyll cells, D_l, was first approximated by the diffusion coefficient of CO_2 in water (Aalto et al. 1999, after Bird et al. 1960 and Reid et al. 1987):

$$D_l = \frac{7.4\times10^{-8} T\sqrt{\phi M_{H_2O}}}{\eta_{H_2O} v_{CO_2}{}^{0.6}} \tag{9}$$

where ϕ is the association factor of H_2O, M_{H2O} is the molecular weight of H_2O, ν_{CO2} is the molar volume of CO_2 at its normal boiling point, T is temperature and η_{H2O} denotes the dynamic viscosity of water which decreases as a function of temperature.

Before entering the cell, CO_2 dissolves in the thin water layer on cell surfaces. The equilibrium between gaseous and dissolved CO_2 concentrations can be expressed by the dimensionless Henry's law coefficient H (Seinfeld and Pandis 1998):

$$H = H_{298K}\exp\left(\frac{-20256.28}{R_g}\left[\frac{1}{298.15}-\frac{1}{T}\right]\right) \tag{10}$$

where R_g is the gas constant and H_{298K} is the Henry's law constant at 25 °C = 0.83 and T is temperature. The given equation for Henry's law constant reflects only the physical solubility of CO_2 at the equilibrium pH of 5.6 ($[CO_2]_{liquid}$ /$[CO_2]_{gas}$) (Seinfeld and Pandis 1998), regardless of the subsequent fate of the dissolved gas. The value at the equilibrium pH of 5.6 was used.

The importance of each component process was studied by testing the sensitivity of the model to changes in the temperature-dependent variables. The test was done so that a constant value (at 18.5 °C, where H equals one) was applied for each variable at a time and the model was run over the whole temperature range.

First, the experimentally determined temperature responses of biochemical processes (Study **I**) were utilized in describing the sink term. Further on, based on the results of transport phenomena, new chloroplast-based values for J_{max}, $V_{c(max)}$ and q were determined taking into account the 3D structure of the leaf. The values of J_{max} and $V_{c(max)}$ were iterated so that the CO_2 sink inside the chloroplasts produced the measured CO_2 assimilation rate of the leaf when g_s was constant (adopted from Study **I**). The light use effectivity factor q was iterated using flux results at 200 μmol m^{-2} s^{-1} of incident irradiance, corresponding to an irradiance of 25 to 180 μmol m^{-2} s^{-1} in the chloroplasts after light absorption inside the leaf and subsequently, J_{max} was re-determined using newly estimated q. Finally, branches A_c and A_j were combined in order to check the resulting co-limited rate.

4. RESULTS AND DISCUSSION

4.1. The transport of CO_2 molecules into the 3D leaf

4.1.1. Structural boundaries

Flux of CO_2 into the carboxylation sites has been treated as a one-dimensional diffusion phenomenon with estimated resistance or conductance in CO_2 assimilation models (e.g. Ball, Woodrow and Berry 1987). However, the true diffusion of CO_2 in a leaf is a complex three-dimensional (3D) process that is not very well known (Parkhurst 1977, 1986, 1994). A few approaches for studying two-dimensional (2D) or 3D transport inside leaves have been reported (Parkhurst and Mott 1990, Claiborn et al. 1993, Pachepsky et al. 1995, Vesala et al. 1996, Aalto et al. 1999, Ustin et al. 2001), but these models usually include transport in the air phase only. Models that describe two phases (air and mesophyll) or porous media have assumed the sinks of the transferring gas to be continuous in mesophyll cells. None of them include chloroplasts as model objects, although the chloroplast is the site where the actual fixation of CO_2 molecules occurs. The last phases of the CO_2 transport occur in liquid phase that are regulated by other factors than those which regulate the gas-phase diffusion. The liquid phase transport is also very sensitive to actual chloroplast distribution in the liquid. Consequently, the simplifying assumptions of the actual 3D structure in 2D or resistance models can lead to misinterpretation of the behaviour of photosynthesis in variable environments (Parkhurst 1994). In Study **II**, placing the CO_2 sink realistically in the chloroplasts clearly resulted in a decrease of the actual CO_2 concentration at the sites of carboxylation. This was shown by comparing the so-called base case of the 3D model, where the CO_2 sink was placed into the chloroplasts, to the model version where the sink was continuously distributed through the cell volume. In the base case the CO_2 concentration in the chloroplast was decreased by a maximum of 28 % from the concentration in the airspaces, whereas in the model with a continuous CO_2 sink the decrease was only 18% (Study **II**).

Due to the 3D structure of leaves the CO_2 concentration within leaves is not uniform (Figure 4). During the gaseous part of the route the stomatal opening is clearly the major factor limiting the transport of CO_2 molecules. In the modelled birch leaf, the strongest gradient in air phase CO_2 concentration was found in the stomatal pore (Figure 4, Study **II**). Consequently, the stomatal regulation had a marked effect on CO_2 flux, as expected. Decreasing the stomatal opening by half led to remarkably slower gas phase diffusion compared to the liquid phase diffusion. Based on the results in Study **II**, for a leaf with a clear spongy and palisade mesophyll layer, the diffusion of CO_2 in air was efficient enough to create a quite uniform CO_2 concentration in the intercellular airspaces (Figure 4). These results agreed with Genty et al. (1999), but there exist different results in some species (Parkhurst and Mott 1990).

Importantly, for the modelled birch leaf, both the dissolution of CO_2 into water at cell surfaces (H) and the diffusion in the liquid phase (D_l) played almost as important role in CO_2 flux as the diffusion through the capillary tube, which leads through stoma to the

intercellular airspaces. The 3D structure, including the porosity of the mesophyll and the amount of absorbing surface, affects the mean length of the route for CO_2 diffusion in air and in mesophyll (Parkhurst and Mott 1990, Aalto et al. 1999). Therefore, the relative contribution of different transport phenomena varies along with the leaf structure creating species-specific differences for example in the temperature dependence of apparent CO_2 assimilation.

The constructed 3D model of a birch leaf explained the CO_2 flux to the stoma and to the chloroplast reasonably well. However, the resulting CO_2 uptake was clearly lower than that measured in birch leaves in Study **I**. In part, this was expected because the biochemical model describing the leaf CO_2 exchange was now only one component in the 3D model. The experimentally determined biochemical model parameters already included the influence of CO_2 transport that was treated separately in Study **II**. This result emphasises the problems in using an empirical biochemical model, which is parameterised at the leaf level, for detailed studies on the mechanisms affecting the apparent CO_2 assimilation within the boundaries set by leaf structure.

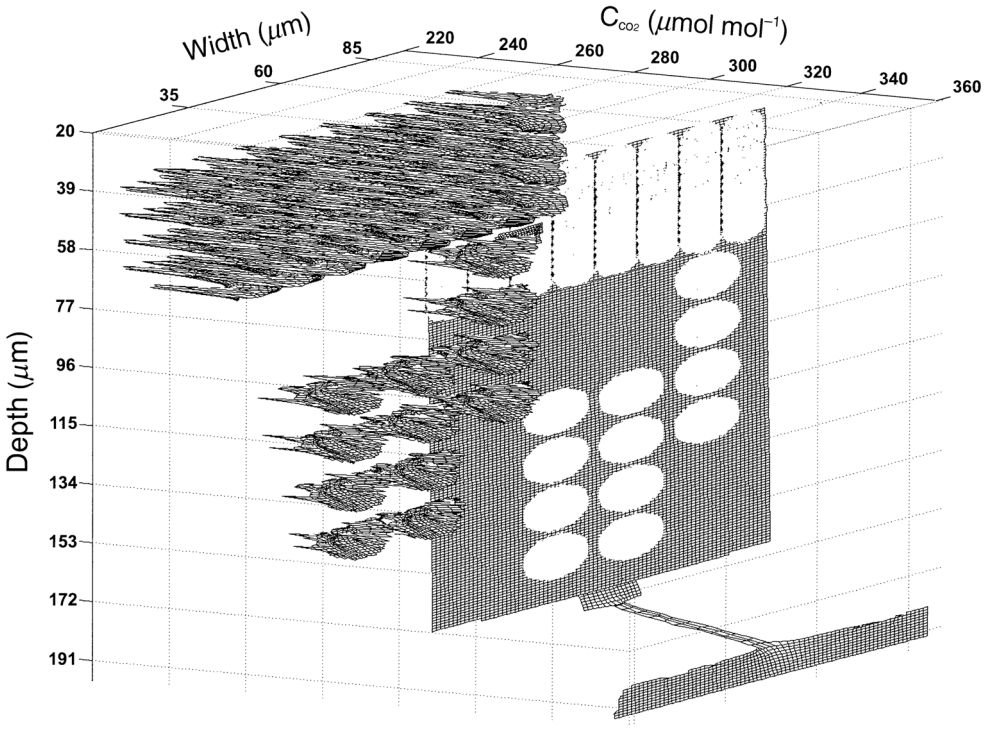

Figure 4. CO_2 concentrations in chloroplasts, cells, and air spaces and in the stomatal pore according to a 2D slice of silver birch leaf. The centres of the chloroplasts are aligned with the cutting plane in the palisade mesophyll cells, but not in the spongy mesophyll cells. Therefore the lowest concentrations vary significantly in spongy mesophyll cells. Adopted from Study **II**. Reprinted with the kind permission of Blackwell Publishing.

4.1.2. The role of temperature

Transport is a highly temperature dependent phenomenon. Leaf structure affects the relative contribution of different transport mechanisms on the overall CO_2 flux rate while temperature has very variable influence on the different mechanisms. Based on the results in Study **III**, it was clear that the most important process affecting the apparent CO_2 assimilation at different temperatures was the dissolution of CO_2 in water (H). Dissolution decreases strongly with increasing temperature so that if H equals 1 at 18.5 °C, at 8 °C it is 1.4 and at 32 °C as low as 0.7 (Seinfeld and Pandis 1998). Therefore, in light, the dissolution of CO_2 limits the CO_2 flux most strongly at high temperatures when the CO_2 sink is also strong. Consequently, omitting the dissolution of CO_2 in the model analysis, leads to an erroneous continuously increasing CO_2 assimilation rate with increasing temperature (Figure 5). Conversely, at low temperatures high H leads to a substantial increase in the CO_2 concentration in the chloroplasts. The effect on CO_2 flux remains small, however, due to low photosynthetic capacity resulting from the changes in enzymatically catalysed reactions (Figure 5). It is also noteworthy that the relative effect of H could have been even more drastic if the pH of absorbing surfaces exceeded the equilibrium pH of 5.6.

The diffusion in air (D_g) has, in general, only a minor effect on the flux of CO_2 into the mesophyll and thus its role in variation of the apparent CO_2 assimilation rate at different temperatures was small. Also the diffusion of CO_2 in mesophyll (D_l) when assumed equal to that in water, has only a minor role in the variation of the apparent CO_2 assimilation rate at different temperatures (Study **II**) in a leaf having a clear spongy mesophyll layer despite its overall importance in CO_2 transport. The combined diffusion of CO_2 in air and mesophyll had a minor effect on the temperature dependence of CO_2 exchange (Figure 5) (Study **III**). The effect was negligible at low temperatures, but ignoring both D_g and D_l from the analysis resulted in a slight decrease in the total CO_2 flux at higher temperatures. The first approximation for D_l was equal to the diffusion in pure water. However, the CO_2 flux to the chloroplasts depends heavily on the value of D_l, especially when it is small (Studies **II**, **III**). A substantially low value of D_l would have strongly limited the CO_2 transport, affecting also the temperature dependence of apparent CO_2 assimilation.

The role of carbonic anhydrase (CA) in diffusion of CO_2 in the mesophyll was analyzed with the 3D model by changing D_l only in the chloroplasts. As a result, a substantial increase in the chloroplastic diffusion increased the CO_2 flux, but only slightly increased the CO_2 concentration in the chloroplasts. Consequently, the concentration gradient was flattened inside the chloroplast. Interestingly, decreasing the D_l in the chloroplasts had a more marked effect on the CO_2 flux. Without the CA activity the diffusion in the mesophyll and chloroplast could be even less than that in water, probably due to various macromolecules obstructing diffusion in the chloroplast (Cowan 1986, Nobel 1999, Evans and Loreto 2000). In the birch leaf the role of CA appeared to be small, but it may have a more significant role in conifers such as pine, because thick leaves benefit more from high CA activity than thin leaves owing to a longer diffusion path both in gas and in liquid phase. This is indicated by the high CA hydration rates found in species with low internal conductance such as trees (as compared with herbs) (Gillon and Yakir 2000).

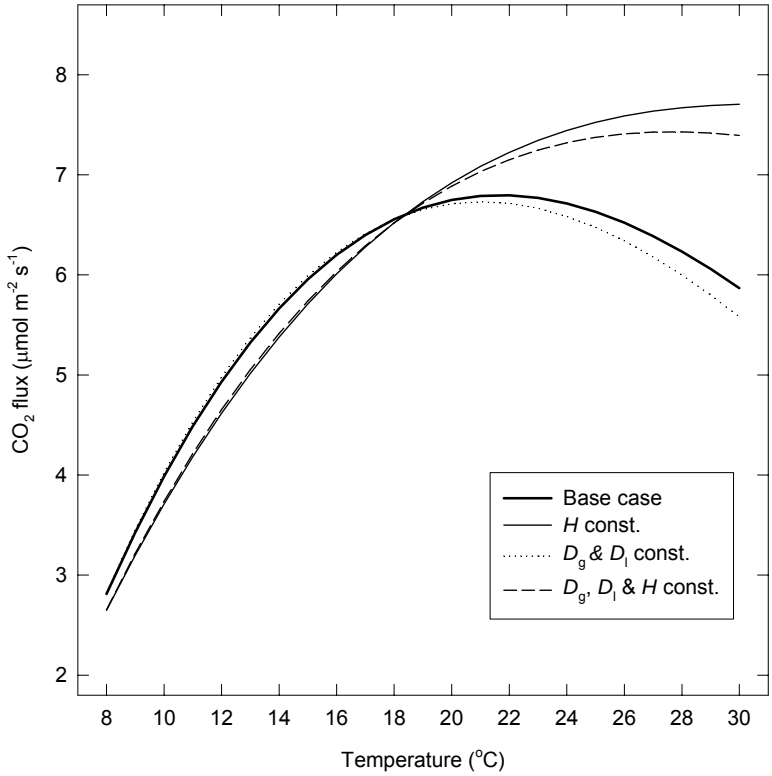

Figure 5. The sensitivity of the 3D photosynthesis model of silver birch leaf to the temperature dependent physical variables, i.e. dissolution of CO_2 (*H*), and diffusion in gas and in liquid phase (D_g and D_l, respectively), tested by applying the value of the variable(s) at 18.5 °C to the whole temperature range. The lines are defined in the legend. Lines are polynomial approximations valid from 8-30 °C. Adopted from Study **III**.

4.2. The light absorption inside a leaf

Due to the fast gas phase diffusion and equal distance from cell walls (Study **II**), chloroplasts in different parts of the leaf obtain almost equal quantities of CO_2 for photosynthesis. Instead, the 3D structure of leaves affects strongly the light environment inside leaves creating unequal conditions for photosynthesis. The effective light absorption in chloroplasts allows only 10% of the light intensity to be transmitted through the leaf where palisade mesophyll cells shadow the spongy mesophyll cells (this was experimentally verified for Study **II**). Even if the proportion of the transmitted irradiance was higher than the value used in Study **II**, the increase in light transmission from 10 to 30% results in only a marginal increase in the total CO_2 flux, at least with the current model construction. Furthermore, a limit is reached at about 50% transmission where the total CO_2 flux is saturated. The exponential decay of irradiance is a simplification of the real

situation. The palisade mesophyll cells may minimize light scattering and guide the light further into the leaf to spongy mesophyll cells where large scattering enhances light capture (e.g. Vogelmann and Martin 1993, Evans 1999). The irradiance conditions may thus be more uniform inside a leaf than Beer's law indicates.

The palisade mesophyll cells receive more irradiance and they have a larger number of chloroplasts than the spongy mesophyll layer fir effective light capture (Study **II**). This leads to higher CO_2 concentration in spongy mesophyll cells and eventually to unequal contributions to the total CO_2 flux (Figure 4), which is in agreement with the literature (e.g. Evans 1999). The effect would be amplified if the different cell types differed in their photosynthetic capacity as suggested by Nishio et al. (1993) and Nobel (1999). This was also shown in Study **II**; the effect of decreasing photosynthetic capacity was more drastic when it was decreased in palisade mesophyll layer, compared to same degree of decrease in spongy mesophyll. The light attenuation inside a leaf thus contributes significantly the apparent CO_2 assimilation at leaf level.

4.3. Photosynthesis in a three-dimensional leaf

Traditionally the apparent CO_2 assimilation is analysed by a Farquhar–type model, where the internal air space CO_2 concentration is used for biochemical parameterisation (Farquhar et al. 1980). Based on the results in Study **I**, the temperature dependence of certain parameters was specific to silver birch grown in the boreal zone. The most important differences in photosynthetic characteristics in silver birch were related to the electron transport chain. First, no optimum temperature was found in J_{max} which is in contrast to many other studies (Walcroft et al. 1997, Leuning 2002) (Figure 6a). Second, both the light-use effectivity factor q (electrons/quanta) and the convexity factor of the light response curve, Θ, showed a significant temperature dependency, which also contrasts with the literature (Terashima and Saeki 1985, Leverenz 1988, Cannel and Thornley 1998). Nevertheless, it has been shown that Θ can be lowered due to light or temperature stress (Leverenz et al. 1990, Terashima et al. 1991) and this is reflected to q as well. The results on silver birch may, in fact, originate from the same mechanism, although the temperature range was well within the natural variation. The variation in the estimates of J_{max} was considerable. The estimates were obtained from CO_2 exchange measurements. Fluorescence measurements, a more direct method for determining the properties of electron transport, probably would have given better results (June et al. 2004). In conclusion, the generalisation of the temperature dependent model parameters to different species and growth conditions is somewhat dangerous, especially if the parameters are determined on different temperature range than the plant experiences in nature.

The general problem of traditional biochemical modelling is that the biochemical parameters are determined at the leaf level, although these parameters refer to actual processes in chloroplasts. Furthermore, when the biochemical processes are characterized at the leaf level, the physical phenomena are implicitly included in the values of the parameters. Because the light attenuation is effective inside a leaf, CO_2 assimilation close to the illuminated surface is limited by CO_2 carboxylation ($V_{c(max)}$), while in the more distant parts of the leaf it becomes limited by electron transport (J_{max}) (Study **III**). Some chloroplasts function near the light compensation point, where the light effectivity factor q, plays a major role instead of J_{max}. The situation might be different for the most weakly

illuminated (spongy) cells if light scattering and channelling inside the leaf were studied in detail (Vogelmann et al. 1996, Ustin et al. 2001). The interplay between $V_{c(max)}$ and J_{max}-limited rates in different structures also changes the temperature dependence of the apparent CO_2 assimilation rate at the leaf level, even if the basic biochemical dependencies remain the same. In conclusion, when the conventional 1D model of photosynthesis is used, the 3D structure of leaves can mask the real temperature dependencies of photosynthetic CO_2 assimilation.

Excluding the transport phenomena from the variables describing the biochemical processes resulted in clearly different temperature dependencies of $V_{c(max)}$ than was expected (Figure 6a). Also, excluding both the effect of light attenuation and the transport phenomena resulted a more temperature dependent J_{max} (Figure 6b). The largest differences were found at high temperatures where the effect of CO_2 dissolution is most pronounced. The disturbing effects of structure and leaf level CO_2 exchange on J_{max} could be partly avoided by determining the properties of electron transport by fluorescence measurements. Using fluorescence techniques is problematic in its own way as the fluorescence signal comes from the uppermost layers of the palisade layer (June et al. 2004).

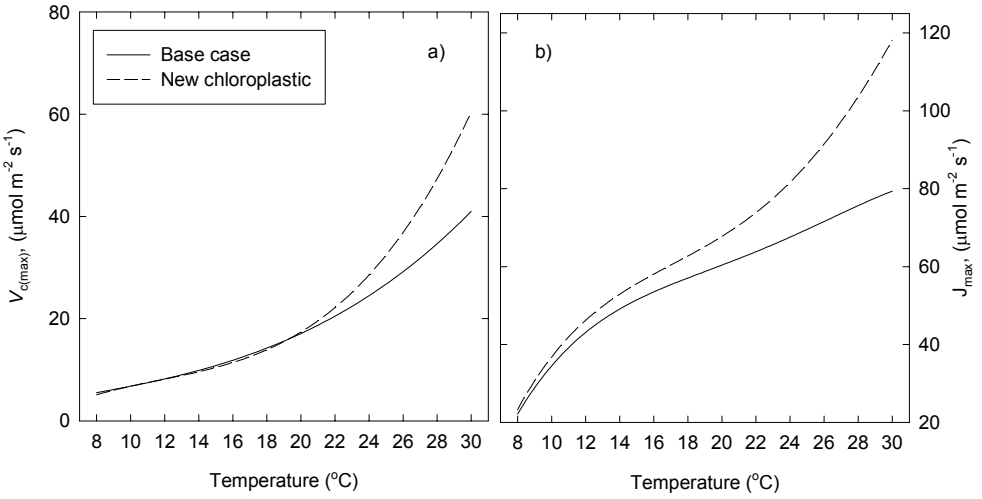

Figure 6. Temperature dependencies of a) maximum carboxylation rate, $V_{c(max)}$ and b) maximum electron transport rate, J_{max} in a silver birch leaf. The solid line represents the leaf level values determined from experiments (Base case) (Study **I**) and the dashed line the new chloroplast related values determined by utilizing the 3D model. Lines are polynomial approximations valid from 8-30 °C. Redrawn from Study **III**.

The temperature dependencies of the maximum carboxylation rate ($V_{c(max)}$) and maximum electron transport rate (J_{max}) vary along with growth conditions. The ratio between $V_{c(max)}$ and J_{max}, however, is often assumed to be preserved, because the basic photosynthetic structure is considered rather conservative and because of the strong linkage between the two processes (see e.g. von Caemmerer 2000, June et al. 2004). Recently there have been several studies where a temperature optimum has been introduced to $V_{c(max)}$ allowing for the decrease at high temperatures due to e.g. reductions in the activity of Rubisco (Dreyer et al. 2001, Leuning 2002, Medlyn et al. 2002, Haldimann and Feller 2004), similarly as with J_{max} (Farquhar et al. 1980, Lloyd et al. 1995). The new parameterisation introduced in Study **III**, was valid up to 32 °C, and no temperature optimum was found for $V_{c(max)}$ or J_{max}.

4.4. Acclimation of trees to increasing CO_2 concentration

4.4.1. Biochemical adjustments

Both acclimation to increasing CO_2 concentration, optimizing photosynthesis and growth, and down regulation, i.e. loss of enhancement during time, even to lower level than originally has been proposed. The acclimation is a complex long-term process where different mechanisms, like end-product synthesis and resource reallocation, operating at different hierarchy levels cause feedbacks to apparent CO_2 assimilation (Woodrow 1994, Luo et al. 1998). What can be considered as 'optimal behaviour' for e.g. resource reallocation may have negative influence for e.g. growth or competition between species especially if the acclimation mechanisms vary among plants (e.g. Bowes 1991, Woodrow 1994). It is logical to think that an enhancement of one key substrate, as CO_2, leads into readjustments in the photosynthetic machinery, and depending on the time scale, different feedbacks affect in turn to this readjustment. Because Rubisco represents the largest single nitrogen investment in a leaf, down regulation of Rubisco at elevated CO_2 concentration can lead to necessary and 'useful' adjustments in nitrogen use within a plant or within the photosynthetic apparatus (Drake et al. 1997).

There are a number of studies on Rubisco concentration or activity at double CO_2 concentration suggesting different acclimation patterns, from unchanged amount but decreased activity to decreased amount of Rubisco (Wilkins et al. 1994, Tissue et al. 1996, Rey and Jarvis 1998, Jach and Ceulemans 2000, Luomala et al. 2003, Riikonen et al. 2005, in tree species). In Study **IV** the non-linear decreasing trend in Rubisco concentration was clear in birch seedlings during the two growing seasons at increased CO_2 concentrations (Figure 7a). The relative change was remarkably the same in both years. The trend was similar in pine, although the variation was higher and the trend was significant only in the first year of treatment. However, the pattern of relative Rubisco concentration was similar in both years (Figure 7a).

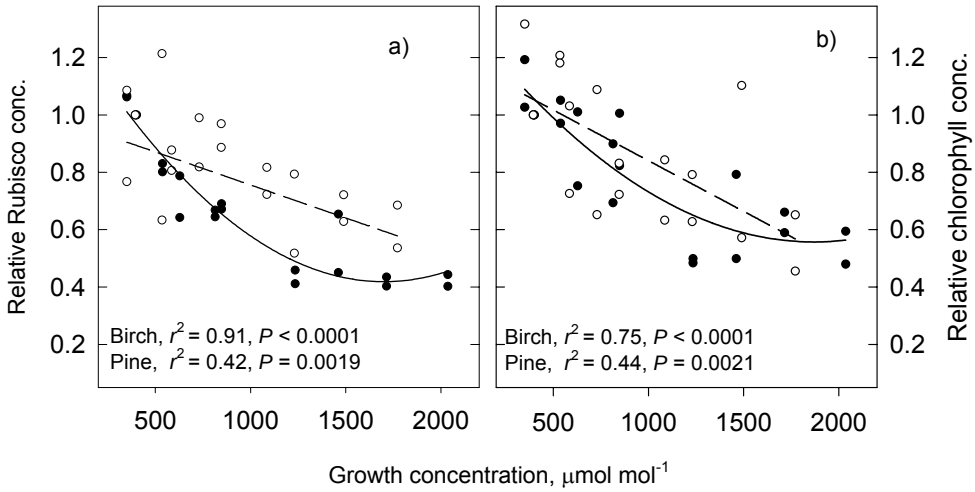

Figure 7. Patterns of a) relative Rubisco concentration and b) relative chlorophyll concentration in response to growth CO_2 concentration in silver birch (●) and Scots pine (○) when data from 2 years were pooled and expressed as relative values with respect to seedlings grown at 400 μmol mol^{-1}. Nonlinear or linear regressions were applied separately for both species. Second order regressions were applied only when the nonlinear term in the model was significant. Recalculated from Study **IV**.

For optimal growth at elevated CO_2 concentrations, greater allocation of resources to the light reaction side of photosynthesis relative to CO_2 fixation would be beneficial (Evans 1989). Acclimation to the different light environment may, however, affect the readjustments within the photosynthetic apparatus (Kubiske and Pregitzer 1996). Both an unchanged and decreased chlorophyll concentration as well as PSII photochemistry has been observed (Wilkins et al. 1994, Lawlor et al. 1995, Lewis et al. 1996, Scarascia-Mugnozza et al. 1996, Rey and Jarvis 1998, Jach and Ceulemans 2000, Gielen et al. 2000, Riikonen et al. 2005). In Study **IV**, however, a clear decreasing trend was also observed in chlorophyll concentrations in both birch and pine leading to unchanged balance between light and dark reactions in seedlings grown at elevated CO_2 concentrations (Figure 7b). This implies also that the acclimation is a complicated process not necessarily following straightforward logic.

4.4.2. Properties of apparent CO_2 assimilation

All the biochemical and structural adjustments in a leaf are manifested in the apparent CO_2 assimilation of the leaf. Accordingly, the decreased Rubisco content in response to increasing growth CO_2 concentration (Study **IV**) was reflected clearly in the steady-state CO_2 exchange as a non-linear decrease when measured at current ambient CO_2 concentration (Figure 8a,b). When measured at growth CO_2 concentration the results were

surprisingly diverse. In birch seedlings there was no clear pattern in CO_2 exchange, while in pine seedlings CO_2 exchange in growth concentration increased significantly in response to increasing CO_2 concentration (Figure 8c,d). Consequently, in contrast to birch seedlings, pine seedlings showed a clear shift from carboxylation-limited photosynthesis to electron transport limited photosynthesis, despite the substantial decrease in Rubisco concentration. The results from the 3D model analysis on the birch leaf, however, also support the theory that despite more efficient resource allocation there still may be an increase in photosynthesis (Study **II**). When the acclimation of the photosynthetic machinery to increasing CO_2 concentration was simulated by decreasing the photosynthetic capacity by 50%, the resulting total CO_2 flux was still higher than originally.

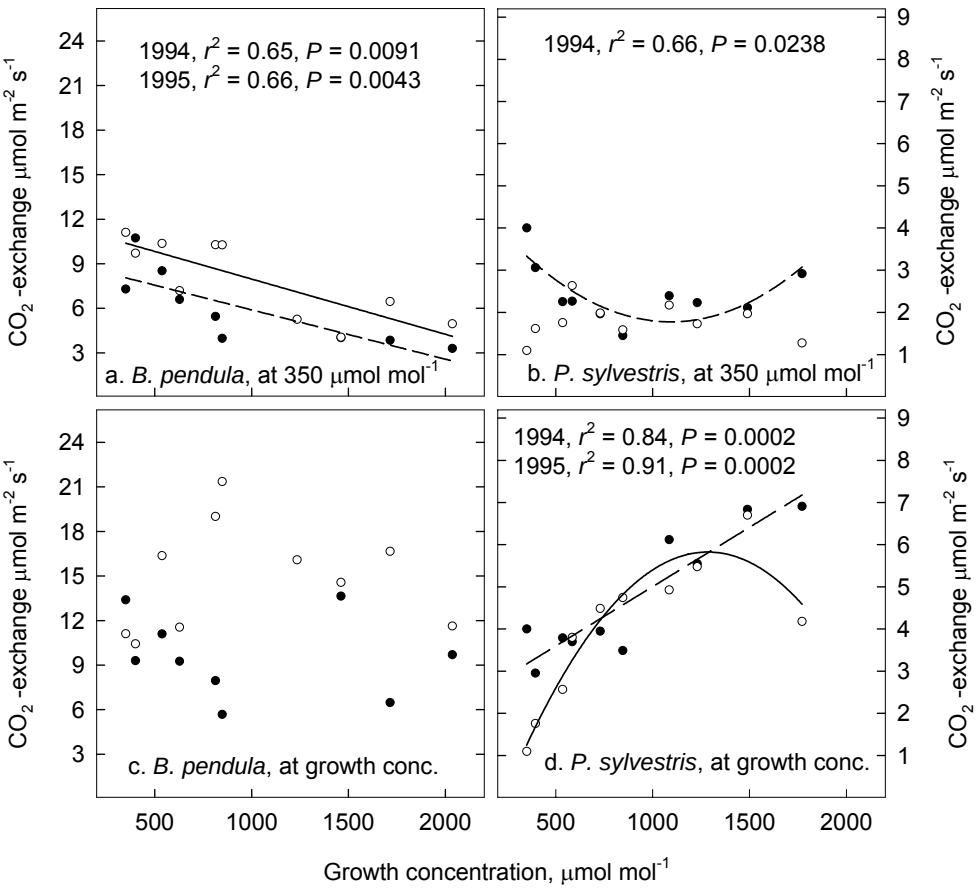

Figure 8. Response patterns of CO_2 exchange rates to growth CO_2 concentration, measured at the current atmospheric CO_2 concentration (350 μmol mol^{-1}) (a, b), and at growth CO_2 concentration (c, d), in silver birch (a, c) and Scots pine (b, d) in 1994 (●) and 1995 (○). Nonlinear or linear regressions were applied separately in 1994 (broken line) and 1995 (solid line) to illustrate the significant nonlinearity or linearity of the data ($P<0.05$). Redrawn from Study **IV**.

4.4.3. Species-specific acclimation patterns

There exist several possible reasons for the results on steady-state gas exchange in Study **IV**. First, due to the higher availability of CO_2, plants can more efficiently regulate the transpiration stream by changing stomatal aperture. The increased control of stomata is shown by the decreased stomatal conductance to water vapour (g_s) in birch seedlings in response to increasing growth CO_2 concentration; although no treatment differences were established. The response seemed to be acclimatory, because there were differences between CO_2 treatments but not between the measurement CO_2 concentrations (Morison 1998, Bunce 2001). On the other hand, in pine seedlings, no clear effect of increasing growth CO_2 concentration on g_s was observed. Instead, g_s tended to be lower when measured at increased CO_2 concentrations, implying a direct effect of CO_2 on stomatal control. In fact, a doubling of CO_2 concentration has often been reported to have no effect on g_s in conifers in contrast to deciduous species, reflecting the differences in leaf structure and in the strategy for water use (Eamus and Jarvis 1989, Roberntz and Stockfors 1998).

Second, it has now been generally accepted that the nitrogen content of leaves decreases at elevated CO_2 concentration regardless of the nutrient status of a plant, as an implication of resource reallocation, although the effects may be larger at low nutrient availability or restricted root growth (Curtis 1996, Cotrufo et al. 1998). However, in Study **IV**, the two species had a different pattern in nitrogen allocation. Scots pine showed no decline in N-content whereas in silver birch a clear non-linear decline was observed. A long-term decrease in the CO_2 exchange rate after an initial enhancement in response to elevated CO_2 concentration can also result from limitations imposed by the availability of carbon sinks (Stitt 1991). Indeed, a decreased specific leaf area (SLA) in response to increasing growth CO_2 concentration was recorded only for birch, indicating accumulation of carbohydrates in the leaf or a sink limitation. The source–sink ratio decreased in birches after the shoots were cut in spring 1995. In fact, the increase in the root/shoot ratio should have ensured sufficiently large sink strength in birch seedlings in 1995 (Rogers et al. 1988).

Third, the temperature dependence of apparent CO_2 assimilation is also an important attribute in acclimation to increasing CO_2 concentration. Therefore, the acclimation patterns in steady-state CO_2 exchange as observed in birch may also reflect the changes in the temperature response of photosynthesis originating from the combined effect of the CO_2/O_2 specificity of Rubisco (Ghashghaie and Cornic 1994, Hikosaka and Hirose 1998) and structural differences (like the role of dissolution of CO_2) between birch and pine leaves. Because silver birch seems to be more sensitive to changes in temperature than Scots pine, at least in natural conditions, the differences in steady-state CO_2 exchange may in part be due to changes in the temperature response. Indeed, the temperature response of CO_2 exchange, determined only for birch, measured at the growth CO_2 concentration, changed clearly with increasing growth CO_2 concentration leading to a significantly higher temperature optimum of CO_2 exchange in seedlings grown at elevated CO_2 concentrations (Figure 9a). Furthermore, the temperature optimum remained unchanged when measured at current ambient CO_2 concentration (Figure 9b).

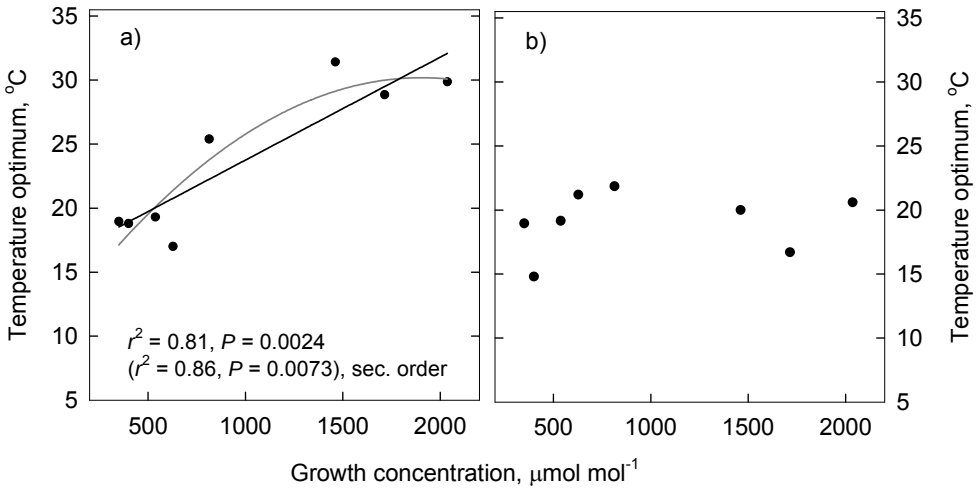

Figure 9. The temperature optimum of measured CO_2 exchange rate in silver birch seedlings exposed to different elevated atmospheric CO_2 concentrations, measured a) at growth CO_2 concentration and b) at current ambient CO_2 concentration (350 μmol mol^{-1}). Both nonlinear and linear regressions were calculated. At growth concentration the second order regression gave a better fit and is shown although the nonlinear term was not significant (grey line).

The changes in the temperature response of apparent CO_2 assimilation reflect the properties of the Rubisco-catalysed reaction, but also the acclimation of the photosynthetic machinery to increasing CO_2 concentration (e.g. Bunce 2000). This acclimatory effect is highlighted when the traditional Farquhar–model, parameterised for silver birch (Study **I**), was compared to the measured temperature responses of birch seedlings acclimated to different CO_2 concentrations. The ratio of CO_2 exchange rate at growth concentration to that at current atmospheric CO_2 concentration (A_{growth}/A_{350}) was calculated both for the birch seedlings acclimated to elevated CO_2 concentrations and by utilising the modelled temperature response in Study **I**. In the model analysis the measured respiration rates and intercellular CO_2 concentrations for each treatment in Study **IV** were taken in account to ensure that the possible differences would not originate from the differences in the respiration or stomatal conductance. The results at different temperatures show clearly that the observed changes in temperature response are not only due to the properties of Rubisco (Figure 10). The changes are also due to the acclimatory effect on the photosynthetic machinery, which is shown as a discrepancy in the calculated ratio especially at high temperatures. Thus the results imply that the Farquhar–model should be applied with caution to plants acclimated to elevated CO_2 concentration, without the careful analysis of the model parameterisation and consideration of the constraints set by leaf structure.

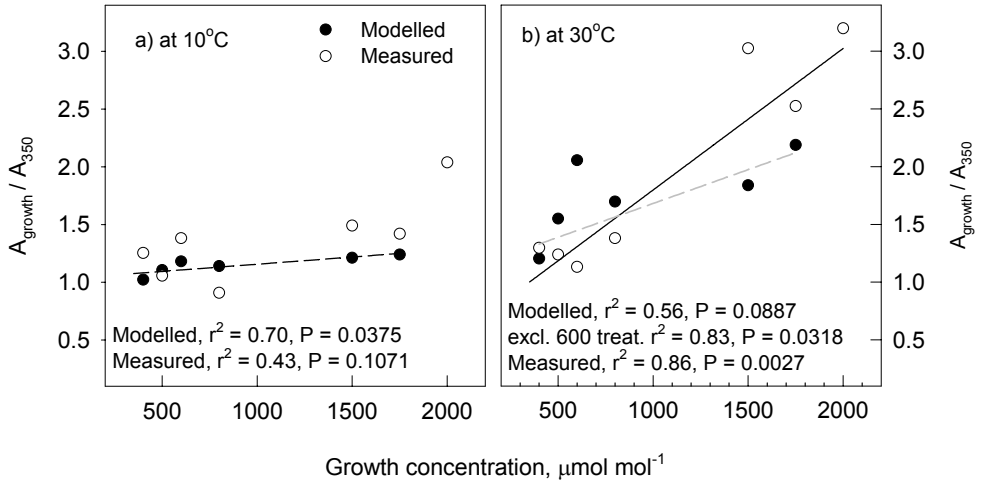

Figure 10. The modelled (●) and measured (○) ratios of CO_2 exchange at growth CO_2 concentration to that at current ambient CO_2 concentration (A_{growth}/A_{350}) in silver birch leaves at 10 °C (a) and at 30 °C (b). Linear regressions were applied separately for both modelled and measured A_{growth}/A_{350}.

Finally, the changes in steady-state CO_2 exchange rates can also reflect changes in the level of respiration or its temperature dependence, although there is no consensus on the effects of increasing CO_2 concentration on respiration (e.g. Ceulemans and Mousseau 1994, Tjoelker et al. 1999).

Practically all the observed differences in acclimation to elevated CO_2 concentration between Scots pine and silver birch can be explained by their different leaf structure, leaf longevity, and different strategy for nutrient and water conservation. The different patterns in leaf, shoot and root nitrogen concentrations in birch and pine in Study **IV** indicated different nitrogen reallocation strategies within the photosynthetic apparatus or within a plant in response to increasing CO_2 concentration. Warren and Adams (2004) suggest that evergreen trees over-invest nitrogen resources to Rubisco, using large Rubisco reserves as nitrogen storage. This creates a weaker relationship between nitrogen content and photosynthesis. For long-lived leaves that have to operate at a tremendously variable environment, this is beneficial in the long-term for optimising nutrient-use efficiency and overcoming structural limitations for CO_2 diffusion (e.g. thick leaves and cell walls, low mesophyll porosity) (Field and Mooney 1986, Warren and Adams 2004). The results from the Study **IV** support this hypothesis: although Rubisco concentration clearly decreased in response to elevated CO_2 concentration, the corresponding steady-state CO_2 exchange rate increased almost linearly in pine but not in birch. Furthermore, there was clearly a need for stomatal control in birch but not in pine, which has adaptive mechanisms for the long-term conservative use of water and nutrients, and better tolerance for nutrient and water stress (Warren and Adams 2004).

The experiment in Study **IV** was conducted with only one seedling assigned for a treatment leading to higher variability especially in gas exchange measurements. However,

because a wide range of treatments the patterns of acclimation to elevated CO_2 concentration were revealed in birch and pine. This type of approach, which recognises the nonlinearity of nature, increases the understanding of the mechanisms involved in acclimation to environmental changes and provides better tools for predicting the behaviour of the plants in future 'double CO_2' or 'more than double CO_2' world.

5. CONCLUDING REMARKS

The constraints set by leaf 3D structure determine the behaviour of apparent CO_2 assimilation at varying light, temperature and CO_2 concentration. Because plant species have adapted and acclimated to the prevailing growth environment within the range of their distribution, the temperature response of photosynthesis can vary between species and within species in different environmental conditions (e.g. Björkman 1981b, and recently Yamori et al. 2005 and references therein). It is not evident whether the differences in the apparent temperature dependencies of CO_2 assimilation would strictly result from changes in biochemical processes. These differences can also originate from different structures masking the actual biochemical dependencies. Different relative contributions of transport phenomena and biochemistry to the apparent CO_2 assimilation of a leaf may explain, for example, why silver birch is more sensitive to temperature than Scots pine.

Results of the 3D characteristics of transport phenomena (Studies **II**, **III**) showed that the physical phenomena have to be distinguished from the traditionally determined temperature dependencies of the biochemical reactions in photosynthesis (as in Study **I**). Consequently, it is important to consider the 3D structure and CO_2 transport phenomena when assessing the temperature dependence of the biochemical variables. Although an empirical bulk model gives a satisfactory result compared with measured CO_2 exchange rates, the conclusions drawn from such a model can lead to biased parameter values, and consequently a serious misunderstanding of the processes that occur inside a leaf and a chloroplast.

In general, analyses based on the 3D structure of a leaf allows the study of leaves with different structures, taking into account the light attenuation inside the leaf and the diffusion path, especially dissolution of CO_2. This approach provides a tool for thorough analysis of the variable gas exchange properties in plants acclimated to different environmental conditions.

Silver birch and Scots pine, which are adapted to growth conditions in the boreal zone, are different in many ways. Silver birch is a fast growing deciduous species with a continuous growth pattern, and may be able to respond quickly to a changing environment. In comparison, Scots pine as a genetically old, slow-growing species with a predetermined growth pattern, that retains its needles from three to seven years, may be more conservative and acclimate more slowly than silver birch. These two species differ tremendously in their leaf structure. Scots pine, in contrast to silver birch, grows on nutrient-poor, often dry sandy soils. Therefore, the needle structure of Scots pine also serves other purposes than maximising photosynthetic rate, for example water conservation and winter protection (Warren and Adams 2004). In conclusion, it is not evident that a fast growing and fast acclimating species such as silver birch would benefit more from increasing CO_2 concentration than Scots pine. In fact, due to the above-mentioned differences, Scots pine may, at least temporarily, be able to take full advantage of increased substrate availability.

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