

# **Impact of Elevated Temperature and [CO<sub>2</sub>] on Spring Phenology and Photosynthetic Recovery of Boreal Norway Spruce**

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## Abstract

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In recent decades, several methods have been devised to study the effects of elevated atmospheric CO<sub>2</sub> concentration, [CO<sub>2</sub>], and temperature on tree and plant physiological responses. This has resulted in the development of a range of systems to expose tree species to elevated [CO<sub>2</sub>] and/or temperature and it has become obvious that there is a strong need for multifactor experiments, which at the tree and stand level can become both a technical and financial challenge. Most available information on the effects of elevated [CO<sub>2</sub>] and increased temperature on trees comes from single factor experiments on seedlings or saplings and the results from these studies may not be directly applicable to mature trees or forest stands.

This thesis is based on data gathered from mature Norway spruce trees growing in whole-tree chambers in northern Sweden, where both temperature and [CO<sub>2</sub>] were controlled according to climate conditions predicted to prevail in the year 2100. As spring is such an important season for boreal tree species, it was this period in which I was most interested. Spring phenology (bud burst and shoot growth) and the timing and extent of photosynthetic recovery was studied in relation to elevated temperature and [CO<sub>2</sub>] over a three-year period.

From the analysis presented herein, phenological development of buds was enhanced with elevated temperature and new shoots emerged 10 to 20 days earlier than in ambient temperature. The duration of the shoot growth period was not affected by [CO<sub>2</sub>] or temperature and while shoot length varied between treatments, neither temperature nor [CO<sub>2</sub>] had any significant effect. These differences could not be explained by an accumulation of temperature sum (T<sub>sum</sub>) nor could ecophysiological simulation models predict these events. Although the models were not able to predict the regulation of the timing of bud burst, they did provide a clear indication that rest completion in Norway spruce trees growing in northern Sweden takes place near the spring equinox, meaning that high air temperatures are not physiologically effective until late in the spring.

Elevated temperature also enhanced the recovery of the photosynthetic apparatus, as seen in variable chlorophyll fluorescence, apparent quantum yield, and light saturated photosynthesis, but elevated [CO<sub>2</sub>] had no effect during the spring period. While measurements of chlorophyll fluorescence were a reasonable indicator of photosynthetic capacity, they cannot be used as a proxy for photosynthesis measurements since net photosynthesis is so variable and responsive to temperature and [CO<sub>2</sub>]. Rising air temperatures will lead to earlier spring photosynthetic recovery of boreal Norway spruce forests and this will ultimately affect the length of the growing period. The commencement and rate of spring photosynthetic recovery are two of the most important factors governing the potential annual carbon sequestration by boreal forests.

*Keywords:* bud break, chlorophyll fluorescence, climate change, photosynthesis, *Picea abies*, temperature sum, whole-tree chambers

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We ourselves feel that what we are doing  
is just a drop in the ocean.

But the ocean would be less  
because of that missing drop.

-Mother Theresa

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# Appendix

## Papers I-V

This thesis is based on the following papers, which are referred to in the text by the corresponding Roman numerals, I-V.

**I.** Medhurst, J., Parsby, J., Linder, S., Wallin, G., Ceschia, E. & Slaney, M. A whole-tree chamber system for examining tree-level physiological responses of field-grown trees to environmental variation and climate change. *Plant, Cell and Environment* (In review)

**II.** Slaney, M., Wallin, G., Medhurst, J. & Linder, S. Impact of elevated [CO<sub>2</sub>] and temperature on bud burst and shoot growth of boreal Norway spruce. *Tree Physiology* (In review)

**III.** Hänninen, H., Slaney, M. & Linder, S. Dormancy release of Norway spruce under climatic warming: Testing ecophysiological models of bud burst with a whole tree chamber experiment. *Tree Physiology* (In review)

**IV.** Slaney, M., Medhurst, J., Rantfors, M., Hall, M. & Wallin, G. Effects of elevated [CO<sub>2</sub>] and temperature on chlorophyll fluorescence and photosynthesis in Norway spruce during spring.

**V.** Medhurst, J., Wallin, G., Slaney, M. & Linder, S. Spring photosynthetic recovery of boreal Norway spruce at the shoot- and tree-level under conditions of elevated [CO<sub>2</sub>] and air temperature.

# Introduction

The word *boreal* is derived from *Boreas*, the Greek God of the north wind. Boreal forests occur in the uppermost northern hemisphere in regions immediately south of the Arctic Circle (Fig.1). In North America the northern boreal eco-region extends from Alaska through Canada to Newfoundland, and in Europe from Scandinavia across northern Russia and Siberia to the Bering Strait, covering approximately 33% of the world's land surface and encompassing nearly one third of the planets forest area. Global boreal forests are larger than the Amazon rainforest and its forests and peatlands are said to be one of the world's largest 'carbon reservoirs' (Dixon *et al.*, 1994).

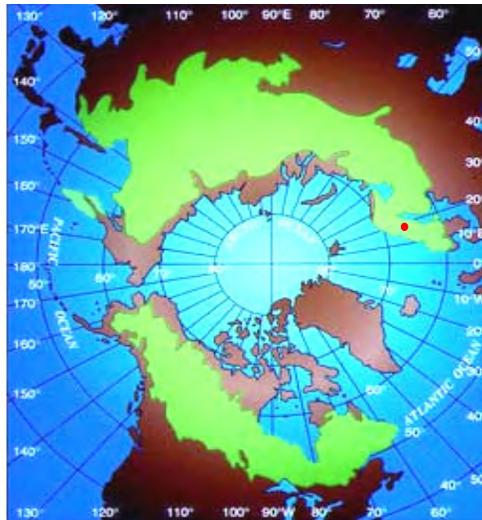


Figure 1. The circumpolar range of the Boreal forests (*i.e.* the area in green). The red dot indicates the area in which the Flakaliden study site is located. (after Hare & Ritchie, 1972, as taken from <http://www.borealforest.org>).

The boreal region typically has podzolic soils and the dominant species include spruce, pine, fir, and larch with a few broadleaved species such as birch, aspen, and alder occurring mostly along waterways and during stand initiation after disturbances such as forest fires, wind-throw or harvesting. Since the latitudinal range defining the boreal region in North America and Europe is not the same, it is more appropriately defined by its climate. The climate in the boreal region has more than one month, but less than four months with a mean temperature above 10 °C with a wide annual temperature variation (*cf.* Walter, 1973). Precipitation is primarily in the form of snow and can be within the range of 40 - 100 cm annually. Since the rate of evapotranspiration generally does not exceed precipitation, the climate in the boreal zone is humid (*e.g.* Havranek & Tranquillini, 1995).

## The global carbon cycle

Carbon dioxide (CO<sub>2</sub>) in the atmosphere is a 'greenhouse gas', which traps some of the long-wave radiation that would otherwise be lost to space, thus causing the Earth's atmosphere to be warmer than it would otherwise be. Human activities, such as the burning of fossil fuels, and land-use change affect the natural exchange of carbon between terrestrial systems and the atmosphere. Since pre-industrial times the burning of fossil fuels and other human activities have released CO<sub>2</sub> into the atmosphere, which has increased the concentration of atmospheric carbon dioxide [CO<sub>2</sub>] by 30% over the last 150 years (IPCC, 2001). This human-induced change in [CO<sub>2</sub>] is unarguably a significant contributor to global warming and changes in climate have a significant effect on the carbon cycle.

An increased assimilation of carbon by plants (*i.e.* CO<sub>2</sub> + H<sub>2</sub>O + light → CH<sub>4</sub>O + O<sub>2</sub>), can be expected with an elevation of [CO<sub>2</sub>] and this could increase the amount of carbon stored in vegetation and below ground (*i.e.* roots and soil organic matter). While photosynthesis (and ocean biota) takes up much of the anthropogenically released CO<sub>2</sub>, it is not able to do so at the same rate that CO<sub>2</sub> is released (IPCC, 2001).

Carbon is retained in terrestrial ecosystems in the living biomass and in soil organic matter and these pools play an important role in the global carbon cycle. The magnitude of additional carbon that can be stored in the terrestrial ecosystems is, however, still quite uncertain. Ecosystem models indicate that terrestrial ecosystems will continue to take up some of the carbon dioxide emissions arising from human activities for a number of decades, but this trend may gradually diminish and forest ecosystems could even become a source of CO<sub>2</sub> (Watson *et al.*, 2000). There are several possible reasons for this. The capacity of ecosystems to sequester carbon may be limited by nutrients or biophysical factors. The rate of photosynthesis for all types of plants will not necessarily increase as [CO<sub>2</sub>] continues to rise, whereas heterotrophic respiration is expected to increase with increasing temperature (IPCC, 2001). It must be said that projections beyond a few decades are highly uncertain due to our current limited understanding of climatic constraints as well as acclimation of the physiological processes and feedbacks among these processes (Watson *et al.*, 2000).

Henceforth, the rapidly rising [CO<sub>2</sub>] has a direct impact on the future climate and this has become an issue of global political and economic importance. Owing to this concern, there has been much research done on the carbon cycle and the global carbon budget as a whole, during the past few decades (*c.f.* Saxe, Ellsworth & Heath, 1998 and references therein). This requires specific knowledge on ecosystem carbon budgets (*i.e.* the amount of carbon that can be stored in a particular ecosystem over a certain time period) and flows and fluxes of carbon in ecosystems. To determine carbon sinks and sources, human impacts such as afforestation and deforestation, fossil fuel combustion, and land-use change have to be studied as well as understanding the complexities in the pathways of carbon in terrestrial biomass.

## Climate Change

Since global warming is now firmly recognised as a serious threat to natural systems (e.g. Watson *et al.*, 2000), the reaction of the global community is to minimise the impact on nature and society by finding ways of lowering greenhouse gas emissions and sequestering some of the atmospheric CO<sub>2</sub> in natural vegetation, as well as finding new and alternative energy sources such as biofuels. Climate and climate-related parameters such as temperature, precipitation, soil moisture, and sea level are changing at an unprecedented rate (IPCC, 2001). Changes in land use (predominantly in the tropical forests) accounted for the most significant part of anthropogenic carbon dioxide release during the 19<sup>th</sup> century and it was not until about 1950 that fossil fuel emissions became significantly larger than the source from land-use change (e.g. converting forest land into agricultural land). Present day emissions due to anthropogenic land-use change still amount to around 1 Gt C per year. Since 1900, [CO<sub>2</sub>] has risen from 290 μmol mol<sup>-1</sup> to 377 μmol mol<sup>-1</sup> in 2004 (Keeling & Whorf, 2005), and is currently increasing at a rate of 1.5 (0.9-2.8) μmol mol<sup>-1</sup> a<sup>-1</sup>, which is mainly due to fossil fuel combustion and deforestation (IPCC, 2001). The prediction is that [CO<sub>2</sub>] will rise to over 500 μmol mol<sup>-1</sup> by 2050 and to almost 700 μmol mol<sup>-1</sup> by 2100, which will result in an increase in global average temperature of approximately 2.5 – 3.5 °C (IPCC, 2001). Furthermore, since CO<sub>2</sub> has a potentially long atmospheric residence time (5-200 years depending on the rate of uptake by different removal processes), it can have a radiative forcing effect over decades, centuries or millennia before natural processes can remove quantities that are emitted (IPCC, 2001). So even if CO<sub>2</sub> emissions stabilised today, it would still take a very long time before atmospheric CO<sub>2</sub> concentrations would stabilise.

Predicted increases in mean surface temperatures are in the order of 2-6 °C (Burroughs, 2001), concomitant with this rise in global [CO<sub>2</sub>]. An increase in global air temperature has already been observed and deviations from the global mean temperature (*i.e.* anomaly) have been steadily increasing since 1970 (Fig. 2). In the past 11 years (1995-2005), ten of the warmest years in the entire series since 1856 occurred. It has been estimated that global mean temperature increased by 0.3 to 0.6 °C during the last century. This warming has not been consistent throughout the world, nor it is consistent through time (*cf.* Jones *et al.*, 1999; Jones & Moberg, 2003). The greatest warming as a result of increasing [CO<sub>2</sub>] is expected to be at northern high latitudes (IPCC, 2001). The number of “hot spots” has increased dramatically in the Northern Hemisphere in the last century compared to the past 1200 years (Osborn & Briffa, 2006). The response of forest ecosystems at these latitudes to climate change may be pivotal in determining the future extent to which forests act as net global sinks for atmospheric carbon.

Based on land and ocean surface temperatures, the 1990s was the warmest decade of the millennium (Jones *et al.*, 1999; Jones & Moberg, 2003) and it has recently been reported that the 20<sup>th</sup> century stands out as having unusually widespread warmth, compared to all of the natural warming and cooling episodes during the past 1200 years (Osborn & Briffa, 2006).

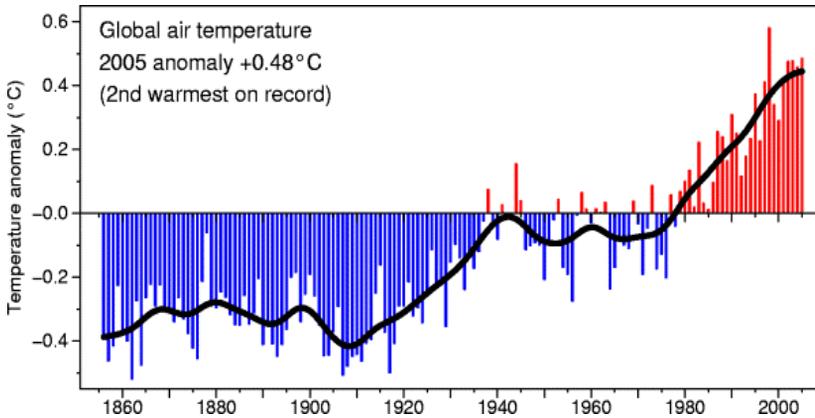


Figure 2. Change in global air temperature from 1856 to 2005, based on land and ocean surface temperatures. The key references for this time series are Jones *et al.*, 1999 and Jones & Moberg, 2003. Taken from <http://www.cru.uea.ac.uk/cru/info/warming>.

As trees account for two-thirds of terrestrial CO<sub>2</sub> fixation (Waring & Schlesinger, 1985), increases in temperature and carbon uptake could significantly alter the global carbon budget (Harmon, Ferrel & Franklin, 1990; Vitousek, 1991). The present rates and trends of this carbon uptake in terrestrial ecosystems are however, uncertain.

According to model predictions for northern Sweden, the annual mean temperature is predicted to increase between 2 and 5 °C within the next 100 years (Christensen *et al.*, 2001). Temperature changes have an important role in the processes involving CO<sub>2</sub> assimilation and carbon sequestration. Many of these processes, however, can partially or fully acclimate to long-term changes in temperature (*cf.* Strömberg & Linder, 2002). The mechanisms involved in CO<sub>2</sub> and temperature acclimation are complex and involve many structural and biochemical changes. Increases in temperature may lead to increases in photosynthesis; however, it may also increase autotrophic and heterotrophic respiration rates, which could reduce the size of the terrestrial carbon stock. In some regions, the changes in climate can also reduce plant photosynthesis and reduce the ability of vegetation to capture carbon due to increased drought or temperature stress.

The timing and rate of photosynthetic recovery after winter frosts governs the potential annual carbon uptake by boreal forests and the extent to which climate change will alter the timing of recovery each spring has important implications. Accurate measurements of tree photosynthesis, photosynthetic recovery, and commencement of recovery under the predicted future climate are invaluable for understanding and modelling forest ecosystem carbon exchange and storage.

## Methods used to study plant responses to climate change

Over the past few decades, there has been a mounting interest to determine the effects of climatic change on forests. Scientists are confronted with the challenges of measuring overall plant responses and the influence of elevated temperature and [CO<sub>2</sub>] in forest ecosystems. Despite the striking successes that have been achieved in elucidating the mechanisms that underlie the processes of growth and morphogenesis under controlled laboratory conditions, there is still too little known about these processes in natural surroundings (Larcher, 2003).

In recent decades, several methods have been devised to study the effects of elevated [CO<sub>2</sub>] and temperature on tree and plant physiological responses, which has resulted in the development of a range of systems to expose tree species to elevated [CO<sub>2</sub>] and/or temperature (*cf.* Schulze & Mooney, 1994; Pontaiiler *et al.*, 1998; Saxe, Ellsworth & Heath, 1998). Besides the indoor controlled environments, there are a number of methods, which have been developed for studies in the field. Approaches such as open-top chambers (*e.g.* Whitehead *et al.*, 1995; Jach & Ceulemans, 1999; Murray *et al.*, 2000), open-sided chambers (Liozon *et al.*, 2000), branch bags (*e.g.* Barton, Lee & Jarvis, 1993; Kellomäki & Wang, 1997; Saugier *et al.*, 1997; Roberntz, 1999), closed-top chambers (Kellomäki, Wang & Lemettinen, 2000; Sigurdsson, Thorgeirsson & Linder, 2001; Paper I), glass domes with adjustable windows (Urban *et al.*, 2001), and free air CO<sub>2</sub> enrichment (FACE) systems (*e.g.* Hendrey *et al.*, 1999; Hamilton, Thomas & DeLucia, 2001; Herrick & Thomas, 2001; Körner *et al.*, 2005), have been used to measure plant responses to elevated [CO<sub>2</sub>] and/or temperature. Most of these methods are, however, best suited to the study of seedlings or saplings (*cf.* Ceulemans, Janssens & Jach, 1999; Norby & Lou, 2004). Hence, most available information on the effects of elevated [CO<sub>2</sub>] and increased temperature on tree species comes from single factor experiments on seedlings or saplings (*cf.* Badeck *et al.*, 2004; Norby & Lou, 2004) and the results may not be directly applicable to mature trees or forest stands (*cf.* Ceulemans, Janssens & Jach, 1999; Norby & Lou, 2004). It is therefore obvious that there is a strong need for multifactor experiments, which at the tree and stand level, can become both a technical and financial challenge (*cf.* Beier, 2004).

To the best of our knowledge there have only been three experiments, with mature field-grown conifers where the impacts of both elevated [CO<sub>2</sub>] and temperature were controlled. The first was a large-scale enclosure, constructed in a small catchment in a Norwegian Scots pine stand (*cf.* Wright, 1998; Rasmussen, Beier & Bergstedt, 2002), a second was using whole-tree chambers (WTC) with temperature and [CO<sub>2</sub>] control, installed around individual Scots pine trees in Finland (*cf.* Kellomäki, Wang & Lemettinen, 2000), and a third was in a 40-year-old stand of Norway spruce in northern Sweden, where WTCs were used to study the interaction between elevated [CO<sub>2</sub>], temperature and nutrient availability (*cf.* Fransson *et al.*, 2001; Wallin *et al.*, 2001; Kostianen *et al.*, 2004; Comstedt *et al.*, 2006).

# Impacts of elevated temperature and [CO<sub>2</sub>] on boreal Norway spruce during spring

In the boreal zone, there are strong seasonal changes in climate. Physiological and structural traits of Norway spruce are crucial to withstand these variations in climate. At the end of the summer and early in the autumn, when temperatures decrease and days become shorter, Norway spruce trees enter a phase of transition where they develop cold hardiness to prepare themselves for the coming winter. At this point, new leaf and flower buds are produced and gradually move into a state of inhibited growth. This transition is characterised by a number of physiological and structural changes from the level of the cell to the whole-tree and the origins of the mechanisms underlying these processes are unclear (Linkosalo, 2000a). They serve the purpose of keeping vegetative buds dormant while frost resistance increases and photosynthetic activity slows down. During the winter, the trees remain 'dormant' until growing conditions become more favourable in the spring.

So why is spring so important? It is the period when physiological processes 'awaken', ontogenetic development begins, buds start to develop and burst, growth commences, photosynthetic activity increases and the synchrony of all these processes with temperature determines the fate of the actual production of the trees. In the spring, physiological activity of Norway spruce is restored under the influence of phytohormones (*i.e.* endogenous factors at the molecular and cellular level that coordinate the metabolic and developmental processes of the whole organism), in response to warmer temperatures and/or photoperiod (*e.g.* Lavender & Silim, 1987). The progress of spring photosynthetic recovery leads to ontogenetic development (*i.e.* bud development, bud burst, followed by shoot elongation). The timing of these events is crucial in order to maximise the photosynthetic production during the relatively short growing season, while avoiding the risk of spring frost events (Linkosalo, 2000a). The ability of boreal Norway spruce to successfully survive winter and recover from winter dormancy is one of the most important characteristics of spruce ecophysiology. This thesis focuses on the recovery of physiological processes in Norway spruce during spring and examines the possible outcome resulting from climatic warming and increased [CO<sub>2</sub>].

## Spring phenology

Phenology is the study of the timing of recurring phenomena of plant (and animal) life history in relation to climate. The onset and duration of phenological events varies from year to year, depending on prevailing weather conditions. Phenology is one of the most responsive and easily observable traits that changes in response to climate (*e.g.* Badeck *et al.*, 2004). The main factor determining the timing of bud burst is the occurrence of warm temperatures in spring (*cf.* Hänninen 1995a; Leinonen, Repo, & Hänninen, 1997; Leinonen & Kramer, 2002), but there are also results indicating that the autumn temperatures may affect the timing of bud break

the following spring (Heide, 2003). The duration of phenological events can be long in the boreal zone (Linkosalo, 2000a) and since the phenology of boreal forests is mainly driven by temperature, this affects the start time of the growing season and hence its duration (Kramer, Leinonen & Loustau, 2000). While the pattern and timing of phenological events in spring is quite regular from year to year, phenological observations (e.g. Menzel & Fabian, 1999; Ahas *et al.*, 2002) indicate that, in boreal and northern temperate regions, there has already over the last decades been an increase in the length of the growing season. Henceforth, it is crucial to understand the phenological development of boreal forest species so to be able to predict the likely impact on their structure and function under future climatic changes.

Most studies on the effects of elevated [CO<sub>2</sub>] have been on the growth response in terms of biomass and only a few have reported on the spring phenology, *i.e.* bud break and shoot elongation. The reported results do not provide a clear answer to the impact of elevated [CO<sub>2</sub>] on spring phenology, which may be a result of the differences between the studied species (Jach, Ceulemans & Murray, 2001) as well as differences in experimental conditions (*cf.* Linder & Murray, 1998; Murray & Ceulemans, 1998; Ceulemans, Janssens & Jach, 1999). An earlier bud burst was observed for seedlings of Scots pine (*Pinus sylvestris* L.) grown at elevated [CO<sub>2</sub>], (Jach & Ceulemans, 1999), but Murray *et al.* (1994) reported delayed bud burst of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) when grown in elevated [CO<sub>2</sub>]. Within the ECO-CRAFT project (*cf.* Jarvis, 1998) branch bags were used to expose branches of mature trees to elevated [CO<sub>2</sub>]. Bud burst occurred a few days earlier in branch bags, than on non-bagged control branches, which was the effect of increased temperature in the branch bags not elevated [CO<sub>2</sub>] (*cf.* Lee, Overdieck & Jarvis, 1998; Roberntz, 1999).

Ecological simulation models, using present-day climate with data gathered in natural conditions, are rather accurate in predicting phenology and growth of boreal forests, but are not sufficiently robust for predicting changes under future climatic conditions and [CO<sub>2</sub>] (*cf.* Hänninen, 1995b; Paper III) since our current understanding of the processes controlling spring phenology is an insufficient basis for reliable forecasts (Kramer, Leinonen & Loustau, 2000; Linkosalo *et al.*, 2000; Linkosalo, Häkkinen & Hänninen, 2006). The rate of ontogenetic development towards bud burst increases with increasing air temperature, *i.e.* the time required for bud burst decreases with increasing temperature (e.g. Sarvas, 1972, 1974; Fuchigami *et al.*, 1982; Cannell & Smith, 1983; Hänninen, 1990a). Several models have been developed to simulate ontogenetic development towards bud burst using arbitrary high temperature units (day degrees, forcing units, *etc.*) or arbitrary chilling units, either from a fixed date or when a certain amount of chilling or temperature units have accumulated. For model development and testing of the ability to predict changes under climatic warming, actual measurements of bud burst under future climatic conditions should be used.

## Spring photosynthetic recovery

To estimate the carbon balance of boreal forests, the timing of photosynthetic recovery and seasonal variation in photosynthetic capacity must be considered (*cf.* Linder & Lohammar, 1981). The commencement and rate of spring photosynthetic recovery are two of the most important factors governing the potential annual carbon uptake by boreal forests (*cf.* Jarvis & Linder, 2000). Photosynthetic recovery in the spring is strongly related to air temperature (*e.g.* Troeng & Linder, 1982; Strand & Lundmark, 1995; Lundmark *et al.*, 1998), but an earlier and faster recovery of the photosynthetic apparatus is also influenced by soil temperature and timing of soil thawing (*cf.* Bergh & Linder, 1999; Strand *et al.*, 2002). Two potentially important environmental limitations to photosynthesis during spring and summer are freezing nights and low minimum air temperatures. When low temperatures inhibit photosynthesis, a parallel decrease in stomatal conductance can be found (DeLucia, 1987). A modelling analysis of Norway spruce annual productivity by Bergh, McMurtrie & Linder (1998) using the process-based model BIOMASS (McMurtrie, Rook & Kelliher, 1990) highlighted the importance of considering the influence of variation in spring temperatures on photosynthetic recovery. They estimated that about 40% of the potential gross primary production (GPP) may be lost each year because of winter damages to the photosynthetic apparatus and slow spring recovery. The opportunity to capture this potential photosynthetic production when temperatures are warmer in the spring and the growing season is longer will inevitably be in the early spring months of March, April and May.

The effect of elevated CO<sub>2</sub> concentration on photosynthetic recovery of boreal conifers is not clear. Wiemken, Kossatz & Incichen (1996) showed that the ability of Norway spruce to resist frost damage was not affected by elevated [CO<sub>2</sub>]. Simulations of photosynthesis of boreal Scots pine showed an enhanced photosynthetic capacity during the growing season with elevated [CO<sub>2</sub>], but no change in the photosynthetic capacity during the spring or autumn (Kellomäki & Wang, 1998).

## Techniques for studying spring photosynthetic recovery

The photosynthetic apparatus function and recovery can be measured in several ways, but those which were used in Papers I and IV, include tree gas exchange, shoot gas exchange, the daily maximum rate of CO<sub>2</sub> assimilation ( $A_{max}$ ) of shoots, light-saturated photosynthetic capacity ( $A_{sat}$ ), apparent quantum yield ( $\phi$ , mol CO<sub>2</sub> mol photons<sup>-1</sup> - defined as the slope of the photosynthetic light response at a low irradiance), and chlorophyll fluorescence. The strong links between the recovery of photosynthetic capacity and air temperature in the spring for boreal Norway spruce foliage (Lundmark *et al.*, 1998) and light-saturated photosynthesis ( $A_{max}$ ) and photochemical efficiency of photosystem II (PSII;  $F_V/F_M$ ) means that the commencement of photosynthetic recovery in warm temperatures should be evident in the photochemical measurements of chlorophyll fluorescence.

As early as 1874 N.J.C. Müller, observed chlorophyll fluorescence with his bare eyes using a suitable combination of coloured glasses (Müller, 1874 as cited by Schreiber, 1983). Although Müller, already at that time, recognised the correlation between chlorophyll fluorescence and assimilation, a systematic means to study this correlation took half a century longer to develop (Schreiber, 1983). In the 1930's, H. Kautsky and his colleagues developed light sensitive devices with signal recorders for measuring the amount of re-emitted light that had been absorbed by green plants. Since photosynthetic processes in plants are so responsive to environmental conditions, measurements of chlorophyll fluorescence are today widely used for assessing plant physiological status and to assess the effects of environmental stress on plants (*cf.* Öquist, 1987; Krause & Weis, 1984). The amount and kinetics of chlorophyll fluorescence emitted from needles, or leaves, upon an actinic irradiation are an intrinsic fluorescent probe into the primary photochemistry of photosynthesis (Krause & Weis, 1991). Since the recovery of the photochemical efficiency of PSII is strongly temperature dependant (Lundmark, Hällgren & Hedén, 1988) and photosynthesis depends on temperature, it should be possible to use photochemical activity ( $F_V/F_M$ ) as a measure of photosynthetic capacity.

## Objectives

The main aim of this thesis was to elucidate the impacts of elevated temperature and [CO<sub>2</sub>] on spring phenology and the photosynthetic recovery of boreal Norway spruce in a whole-tree chamber experiment where temperature and [CO<sub>2</sub>] could be controlled. The whole-tree chamber system was considered the best approach to examine physiological responses (Paper I), as it was possible to examine the effects of temperature and [CO<sub>2</sub>] as single factors or in combination. More specifically, I wanted to determine the effect of elevated [CO<sub>2</sub>] and increased temperature on bud and shoot phenology of mature Norway spruce trees (II) in order to see if elevated temperature caused buds to develop earlier, thus prolonging the growing season, and whether or not elevated [CO<sub>2</sub>] affected the bud burst and growth of the shoots. This could have implications on the CO<sub>2</sub> uptake and thus, the amount of carbon that can be potentially sequestered. Furthermore, testing the accuracy of existing phenological models and their ability to predict bud burst under future climatic conditions (III) could give us important insight into how we can improve models so that they can more accurately forecast tree responses to changing climatic conditions.

In the boreal region, there are periods when gas exchange is difficult to measure because of low rates of net photosynthesis and/or freezing conditions. It was therefore, important to determine if chlorophyll fluorescence could be used as a proxy for the spring recovery of photosynthetic capacity of Norway spruce trees, and whether the relationship between chlorophyll fluorescence and photosynthetic capacity during the recovery phase is independent of temperature and [CO<sub>2</sub>] (IV). Since the future warming will affect the length of the photosynthetically active period, it is crucial to determine how great the influence of elevated temperature and [CO<sub>2</sub>] is on the timing, rate, and pattern of photosynthetic recovery (V).

# Materials and Methods

## Study site

All experiments referred to in this thesis were performed at the Flakaliden experimental research site in Northern Sweden (64°07' N, 19°27' E). Flakaliden was chosen in 1986, as a site for a nutrient optimisation experiment, which commenced in 1987. For further details about the treatments and site descriptions see Linder & Flower-Ellis (1992), Linder (1995) and Bergh *et al.* (1999).

The site consists of a Norway spruce stand planted in 1963 with four-year-old seedlings of a local provenance with an initial stand density of about 2500 trees ha<sup>-1</sup>. The climate is characterised by long, cold winters with short day lengths (3-5 hrs) and short, cool summers with day lengths lasting 20 to 22 hours. The annual mean temperature at the site is 2.3 °C and the monthly mean air temperature varies from -7.3 °C in January to 14.6 °C in July (mean for the period 1990 - 2004). The length of the growing season, *i.e.* the period with a daily mean air temperature above +5 °C, is *ca.* 140 days, with a mean air temperature of 11.6 °C (Fig. 3). Mean annual rainfall is 600 mm with approximately one-third falling as snow, which usually covers the frozen ground from mid-October to early May (Bergh *et al.*, 1999).

The soil at Flakaliden is a podzolic, glacial, loamy till with an average depth of approximately 1.2 m and a mean humus layer depth of 30-40 mm (Bergh & Linder, 1999). In winter the soil usually freezes, and often does not thaw until May. Understorey vegetation is of the low-herb type of dwarf shrubs mainly bilberry (*Vaccinium myrtillus* L.) and cowberry (*Vaccinium vitis-idaea* L.) (Flower-Ellis, 1993). The site is at an altitude of 310 m.a.s.l with minimal slope (< 4 %).

The temperature conditions, during the three years of the study, had a large variation within and between the years. Compared to the long-term annual mean air temperature, the mean annual air temperature in 2000 and 2002 was 1.1 °C warmer. The mean annual air temperature for eight of the 15 years from 1990 to 2004 was above the 15-year mean annual air temperature of 2.3 °C (Fig. 3) with 2000 and 2002 being the warmest years during this period.

The spring and summer of 2002 were exceptionally warm and had the warmest April, May, June, and August during the period 1990-2004 (Fig. 4). Notably, it also was the year with the coldest November and December mean temperatures. The year 2003 had the coldest January, and March and April were milder than normal, this was followed by a cool early summer with the warmest July temperatures recorded in the 15-year period of 1990-2004. The spring and early summer of 2004 were mild, but colder than the two previous years and had the same mean annual air temperature as the 15-year mean (*cf.* Figs. 3 & 4).

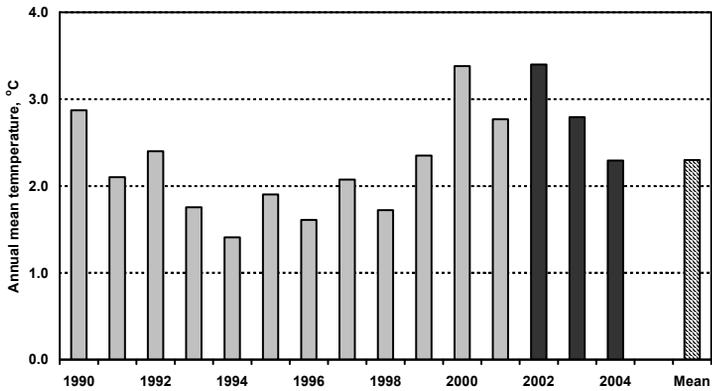


Figure 3. Annual mean temperature for each year from 1990 to 2004 and the 15-year mean temperature. Black bars indicate the period in which this experiment was carried out and the stripped bar denotes the 15-year annual mean temperature.

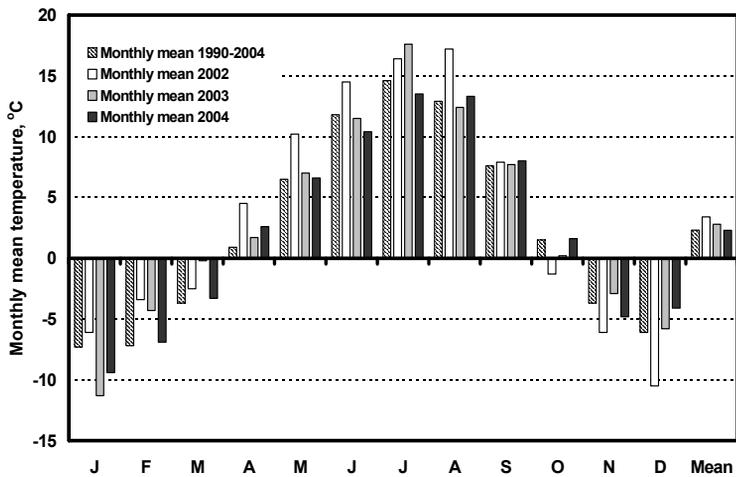


Figure 4. The monthly mean air temperature at the Flakaliden site for each year during the study (2002 to 2004) and the long-term annual mean temperatures (1990 to 2004).

## The whole-tree chamber system

From the long-term nutrient optimisation experiment that began in 1987, one control plot was selected on which to conduct the present experiment. During the spring and summer of 2001, twelve whole-tree chambers (WTC) were installed around twelve individual trees. The enclosed trees were chosen to represent the average tree size, and had when the treatments commenced in mid-August, 2001, an average height of 5.6 m. Three reference trees (R) without chambers were also selected and used as non-chambered control trees. The WTCs were used to examine the long-term physiological responses of field-grown Norway spruce to ambient (A) and elevated (E) atmospheric carbon dioxide (C) concentration and air temperatures (T). The C and T treatments were randomly assigned to each

chamber and in total there were three replicates of four treatments ( $T_A C_A$ ,  $T_E C_A$ ,  $T_A C_E$ ,  $T_E C_E$ ). The  $[CO_2]$  inside the  $C_A$  and  $C_E$  chambers was maintained at 365 and 700  $\mu\text{mol mol}^{-1}$ , respectively. The increase in air temperature inside the elevated temperature chambers was altered on a monthly time-step according to estimates made by the Swedish Regional Climate Modelling Programme, SWECLIM, (*cf.* Christensen *et al.*, 2001; Räisänen, Rummukainen & Ullerstig, 2001). SWECLIM predicted that an  $[CO_2]$  of 700  $\mu\text{mol mol}^{-1}$  will lead to climate forcing in the Flakaliden area of +2.8 and +5.6 °C during summer and winter, respectively (Fig. 5, Paper I).

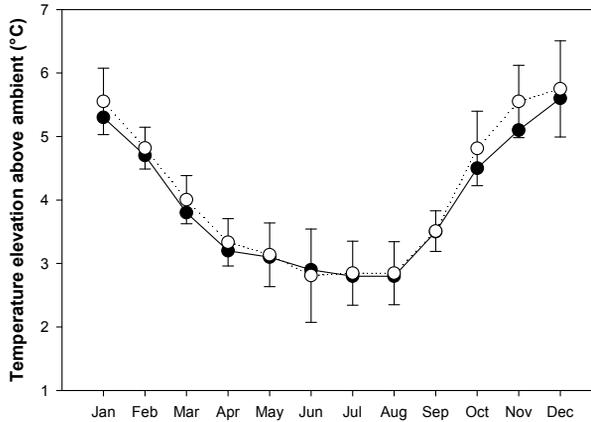


Figure 5. The target monthly temperature elevations used at Flakaliden for elevated temperature WTCs (filled circles). Actual mean monthly temperature elevations for the elevated temperature WTCs (open circles) are shown for the year 2002. Means were calculated by pooling all elevated temperature data for each month. Error bars show  $\pm$  one standard deviation ( $n=6$ ).

### Chamber design

The WTCs were modular in design and consisted of three main sections; the chamber base (soil compartment), the tree chamber (aboveground compartment), and the cooling unit. The circular frame ( $\varnothing$  3.25 m) of the base and the tree chamber was constructed from aluminium. The walls of the base section and tree chamber were sealed with 0.4 mm transparent PVC film. The base section was approximately 0.4 m in height and the lower part of the PVC-film was covered with soil to provide a seal between the base and the ground. The top of the chamber base was sealed with a combination of the PVC-film and transparent Perspex sheets and sealed around the tree stem to prevent air exchange between the soil compartment and the tree chamber. To allow any soil disturbances to subside, the base sections were installed in September 2000, eight months before the tree chambers were installed. Two micro-sprinklers were installed under the chamber floor so that the trees could be irrigated with the same amount of water that was measured by rain gauges outside the WTCs.

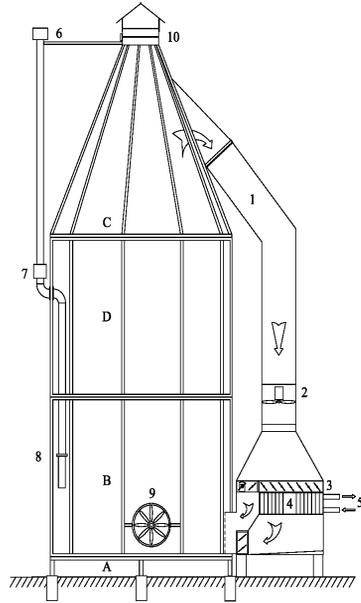


Figure 6. Picture and schematic diagram of the whole-tree chambers. (For a detailed description of the whole-tree chamber system showing the scale and describing all components outlined in the diagram, see Paper I, Fig. 1).

Each chamber was composed of two sections. The bottom section had a height of 2.5 m and the top section of the tree chamber was conical in shape and had a height of 3.0 m (Fig. 6). The WTC system is described in detail in Paper I.

### *Temperature and [CO<sub>2</sub>] control*

Air from the top of the WTC was drawn over a heat exchanger inside the cooling unit by a powerful fan at a rate of approximately 8000 m<sup>3</sup> h<sup>-1</sup>. Circulating glycol cooled to dew point temperature by a 150 kW refrigeration unit controlled the temperature of the heat exchanger. Conditioned air was then returned from the cooling unit at the base of the tree chamber. A series of dampers controlled the volume of circulating air that passed over the heat exchanger and the volume that bypassed the heat exchanger. Elevated temperature regulation was achieved by a combination of reducing the amount of air passing over the heat exchanger, and the use of two heating elements installed in the circulating air pathway in each WTC (Paper I). The air temperature inside each WTC was measured using a shielded and ventilated thermistor at a height of approximately five metres. Ambient outside air temperature was also measured with a shielded and ventilated thermistor. Air temperature inside and outside the WTCs was measured at three-minute intervals and the average was logged at 42-minute intervals. Daily mean air temperature was calculated for the duration of the experiment as well as the daily temperature sums, using a threshold of 0 and 5 °C (Papers I-V).

Fresh air was continuously added to the WTCs at approximately  $54 \text{ m}^3 \text{ h}^{-1}$ , a rate which was close to the internal chamber volume of  $56.3 \text{ m}^3$  (including air circulation pipe and cooling unit). This means that the volume of air in the chamber was exchanged every hour. During the winter months, the volumetric fresh airflow into the chambers was reduced to approximately  $42 \text{ m}^3 \text{ h}^{-1}$ , and hence the cost for  $\text{CO}_2$  and heating required to maintain treatment levels during the 'dormant' season was reduced. This was achieved by placing perforated caps over the fresh air inlet during each winter and removing them in early spring.

An infra-red  $\text{CO}_2$  gas analyser in each chamber was used to measure chamber  $[\text{CO}_2]$  at 90-second intervals. To maintain the set target  $[\text{CO}_2]$ , pure  $\text{CO}_2$  was injected into the circulating chamber air through a magnetic valve. The pure  $\text{CO}_2$  was supplied from a set of tanks containing liquefied  $\text{CO}_2$ . A pressure regulator on the  $\text{CO}_2$  line from the tanks maintained a constant  $\text{CO}_2$  pressure to the WTCs (Paper I).

Sample air was drawn continuously from each of the WTCs and from a 12 m high mast (reference air) in close proximity to the WTCs. The concentration of  $\text{CO}_2$  and  $\text{H}_2\text{O}$  in the sample air was measured in sequence by means of a differential  $\text{CO}_2/\text{H}_2\text{O}$  infra-red gas analyser.

#### *Additional measurements*

Incident photosynthetic photon flux density (PPFD,  $\mu\text{mol m}^{-2} \text{ s}^{-1}$ ) was measured using a quantum sensor (LI-190SA, Li-Cor, Lincoln, Nebraska, USA) mounted on an 11 m mast in close proximity to the WTCs and was measured at three-minute intervals and averaged for the measurement cycle of each WTC (approximately 42 min). Intercepted PPFD was calculated using the Lambert-Beer law and assuming an extinction coefficient ( $k$ ) of 0.46 (Lagergren *et al.*, 2005) and LAIs based on allometric relationships derived from previous tree harvests in Flakaliden. At the shoot level, PPFD was measured with a levelled cosine-corrected quantum sensor attached to each cuvette.

Pre-dawn needle water potential ( $\psi_{pd}$ ) measurements were made on all WTC trees and three adjacent trees not enclosed within a WTC. For each tree, one-year-old shoots were sampled from the ninth whorl and the  $\psi_{pd}$  of individual needles was immediately measured using a pressure chamber (Papers I, IV & V). These measurements were made periodically throughout the spring and summer during the experiment.

A single thermistor was installed under each WTC at a soil depth of 10 cm to provide continuous soil temperature measurements. The temperature of non-WTC (and WTC) air was measured using shielded and ventilated thermistors at a height of approximately five metres.

## Spring phenology

On each of the twelve WTC trees and the three non-chambered trees, branch terminal buds were monitored from mid-April to mid-June, each year (2002-2004). Bud development was monitored on first order shoots of two south-facing branches on the fifth and six whorls from the top of the tree. Bud development monitoring was conducted on two or three occasions per week. Development was recorded according to a six-class classification system (Fig.7, Paper II). The classification of bud development was based on a classic method by Langlet (1960) that was later used in a somewhat modified form by Krutzsch (1973) and Hannerz (1999).

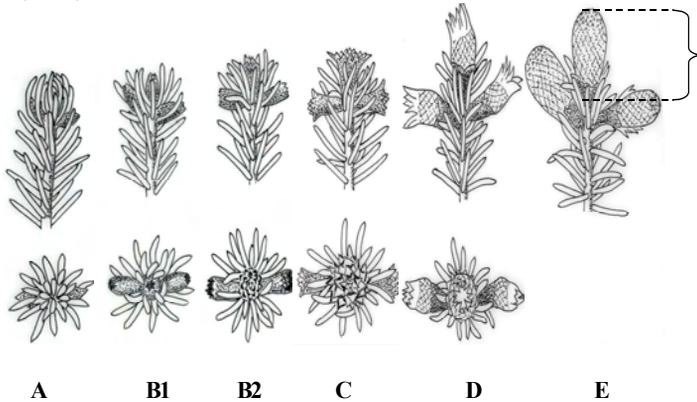


Figure 7. A side and top-view of Bud Development Classes. The shoot length was measured from the centre of the previous years needles, which covered the bud, to the top of the new needles (indicated by the bracket for class E). For a description of bud development classes, see Paper II, Fig. 2. Illustration by Torkel Welander.

Once bud scales had fallen and shoots were measurable (~2 mm of fresh green needles), shoot growth was recorded until the length did not change for three consecutive weeks. Shoots were measured, using a digital calliper, from the middle of the uppermost needles of previous year shoots (see Fig. 7), which are aligned parallel at the base of the new shoots and were once covering the buds (*cf.* Romell, 1925; Krutzsch, 1973).

## Phenological models

Models of bud burst are useful tools for assessing the probable effects of the predicted climatic warming on the timing of bud burst (Cannell, 1985; Cannell & Smith, 1986; Murray, Cannell & Smith, 1989; Hänninen, 1990b, 1991; Kramer, 1994b; Murray *et al.*, 1994; Linkosalo *et al.*, 2000). Using models of bud burst Murray *et al.* (1989) found that depending on the local climate and the requirement of the trees, climatic warming may either hasten or delay bud burst, or cause no change in the timing. In the boreal conditions climatic warming has been both predicted (Hänninen, 1990a, 1991; Linkosalo *et al.*, 2000) and found to hasten bud burst of the trees (Hänninen, 1995b).

For development and testing of the models, three major approaches were applied (*i.e.* *Fixed Day Model*, the *Sarvas Chilling Model* and the *Landsberg Chilling Model*). Actual observations of bud development and bud burst were used (Paper II) to test the three different modelling approaches in order to determine which was sufficiently robust in predicting the observed date of bud burst under [CO<sub>2</sub>] and temperature conditions predicted for the end this century. For a description of these different approaches, see Paper III.

## Spring photosynthetic recovery

The impact of climate on the pattern and extent of seasonal photosynthetic recovery was studied by using the whole-tree chamber system (Paper I), the automated measurements of canopy and shoot gas exchange, combined with measurements of chlorophyll fluorescence (*cf.* Lamontagne, Bigras & Margolis, 2000; Lundmark, *et al.*, 1998; Strand & Lundmark, 1995) and repeated sampling of foliage for analysis of needle chemistry (mineral nutrients and carbohydrates). This enabled spring recovery to be linked from the photosynthetic biochemical level (Paper IV) to the level of shoot (Papers IV & V), and canopy photosynthesis (Paper V).

### *Chlorophyll fluorescence*

Maximum photochemical efficiency of one-year-old shoots was measured on several occasions from March to June 2002, using a pulse amplitude modulated fluorometer. Measuring photochemical efficiency of chlorophyll fluorescence *i.e.* maximum photochemical efficiency of photosystem II (PSII) was considered an appropriate index of PSII activity as it minimises the effects on chlorophyll fluorescence yield caused by differences in excited needle area and chlorophyll content (Lundmark, Hällgren & Hedén, 1988). Fully exposed, second order one-year-old shoots were collected from each WTC (from the sixth to eighth whorl). Shoots were sampled from the northern and southern aspects of each tree and were dark acclimated at 0 °C for one hour. Chlorophyll fluorescence was measured at room temperature, in complete darkness by attaching each shoot to a bundle of optic fibres. To obtain minimum dark fluorescence yield ( $F_0$ ), each sample was exposed to a weak measuring beam from a pulsed light-emitting diode. Maximum fluorescence yield ( $F_M$ ) was determined by exposing the dark-adapted samples to a 1-s saturating pulse of white light (about 8000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) from a modified Schott fibre illuminator, closing all reaction centres. The ratio of variable fluorescence ( $F_V = F_M - F_0$ ) to  $F_M$  is a relative measure of the magnitude of the maximum variable fluorescence emitted from PSII and was used as the index of PSII function (*cf.* Strand & Lundmark, 1995; Lundmark *et al.*, 1998).

### *Photosynthesis*

The WTC and non-WTC ambient air was analysed for [CO<sub>2</sub>] in a continuous sequence by a CO<sub>2</sub>/H<sub>2</sub>O infra-red gas analyser (Paper V). The net CO<sub>2</sub> flux of each tree in the WTCs was calculated using the volumetric flow and [CO<sub>2</sub>] of air entering the WTC via the fresh air inlet and the amount of pure CO<sub>2</sub> injected into

the WTC to maintain the WTC air at the set  $[\text{CO}_2]$ . Tree net  $\text{CO}_2$  assimilation rates were expressed on a projected needle area basis using an allometric relationship to estimate total tree needle area (S. Linder, unpublished data).

Light-saturated photosynthetic rate of  $\text{CO}_2$  assimilation ( $A_{\text{sat}}$ ) and daily maximum net shoot  $\text{CO}_2$  exchange ( $A_{\text{max}}$ ) were respectively measured, on a single one-year-old shoot on the fifth or sixth whorl from the top of each tree enclosed within the WTCs from January 1 to June 30 (Papers IV & V). A 55-mm portion of each shoot was enclosed in a temperature-controlled, 0.15-litre cuvette fitted with a transparent Perspex (Plexiglas) top. Air from the shoot cuvette and a parallel cuvette containing reference air was passed through an infra-red gas analyser running in open mode with 36 parallel channels, of which 15 were used in the present studies. Airflow rates were regulated with mass flow controllers. The  $\text{CO}_2$  exchange of each shoot was measured for 30 s at 30-min intervals and expressed on a projected needle area basis. Projected needle area was measured from scanned images of the needles, using the WinSEEDLE Pro 5.1a software. Daily maximum net  $\text{CO}_2$  fluxes were used as an index of the photosynthetic capacity of shoots (*cf.* Wallin *et al.*, 2001).

Daily mean apparent quantum yield was calculated from the initial slope of the light response curve of net shoot assimilation rate, thus eliminating the effect of variations in irradiance within the trees and thus enabling comparisons of photosynthetic efficiency of different shoots in different treatments (Troeng & Linder, 1982). In Papers IV and V daily mean apparent quantum yield ( $\phi_{\text{shoot}}$ , mol  $\text{CO}_2$  mol<sup>-1</sup> photons) was calculated using shoot net  $\text{CO}_2$  assimilation rates measured at light intensities between 0.5 and 80  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , from January 1 to June 30 2002. Tree apparent quantum yield ( $\phi_{\text{tree}}$ , mol  $\text{CO}_2$  mol<sup>-1</sup> photons) was calculated using net tree  $\text{CO}_2$  assimilations rates when intercepted photosynthetic photon flux density (PPFD) levels were between 10 and 80  $\mu\text{mol m}^{-2} \text{s}^{-1}$ .

#### *Additional measurements*

Shoots were destructively sampled for measurement of starch, sugar, carbon and nitrogen concentrations on nine occasions throughout the spring and early summer 2002 (Paper V). Current and one-year-old shoots (previous year) were taken from the seventh whorl from the top of each tree and immediately immersed in liquid nitrogen. Samples were taken from all 12 WTC trees. Samples were then stored at -18 °C until they were dried at 70 °C for 48 hours and then processed in the laboratory. The carbon and nitrogen analyses were conducted using an elemental combustion analyser (Carlo Erba, NA 1500, Carlo Erba Strumentazione, Milan, Italy). Starch and sugars were analysed enzymatically, according to Steen & Larsson (1986), slightly modified (50 mg sample extracted 60 min at 90 °C, Termamyl 300 L) (*cf.* Bergh & Linder (1999)). To avoid dilution effects caused by seasonal changes in needle carbohydrates, nutrient concentrations were expressed on a 'structural mass' basis. Structural mass was calculated by removing the mass of starch, glucose and fructose (*cf.* Linder, 1995).

# Results and Discussion

## Climatic conditions in Flakaliden

The mean monthly temperature conditions during the three years of the study had a large variation within and between years (Figs. 3, 4, 8). The climate at the Flakaliden study site is typical of what characterises a boreal climate, *i.e.* there is more than one month, but less than four months, with a mean temperature above 10 °C (*cf.* Walter, 1973). According to the current definition, Flakaliden will no longer be defined as boreal if the predicted climate for 2100 prevails, as there will be more than three months with mean monthly temperatures above 10 °C (Fig. 8). Instead, Flakaliden will have a northern temperate climate.

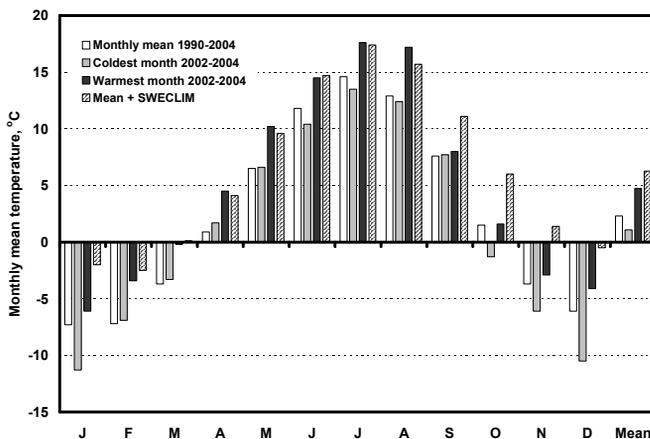


Figure 8. The coldest and warmest monthly mean temperature during the period in which this study occurred (2002 to 2004) compared to the monthly mean temperature from 1990 to 2004 and annual mean temperature for the 15-year period, and the SWECLIM projection of monthly temperatures in 2100.

## Whole-tree chamber performance

The whole-tree chamber system enabled a very accurate control of temperature (Table 2). Shown from the performance tests (Paper I), the temperature regulation in the  $T_E$ -WTCs was within  $\pm 0.5$  °C of the target temperature for 99% of the time during the test periods. During the winter period, when the cooling capacity was reduced, the accuracy of the temperature regulation in  $T_A$ -WTCs decreased ( $\pm 0.5$  °C of ambient temperature for 54%), but when full cooling capacity was reinstated in the spring, the temperature was within  $\pm 0.5$  °C of ambient temperature 89% of the time. The good performance of the WTCs can also be seen in the small differences in temperature sums between ambient air and WTCs operated at ambient air temperatures (Paper II, Table 1; Paper IV, Fig. 2; Paper V, Table 1), and the similar maximum net shoot  $\text{CO}_2$  exchange ( $A_{max}$ ) rates obtained in the  $T_{AC_A}$  trees as compared with non-chambered trees (Paper V, Fig. 2).

Strict temperature control, as demonstrated by the WTCs, enables important insights into the interactive effects of [CO<sub>2</sub>] and temperature on tree functioning while avoiding misleading conclusions that can result from experiments with confounding effects of temperature on tree responses to the primary variable of interest, *e.g.* [CO<sub>2</sub>], which can be a problem with systems that lack temperature control (*cf.* Saxe, Ellsworth & Heath, 1998).

Table 2. Whole-tree chamber (WTC) temperature control by temperature treatment. Note that during February the temperature control was deliberately reduced to minimise power consumption over the winter months. Temperatures measured on a 42-min cycle from each WTC were pooled by temperature treatment (n=6). T<sub>A</sub> is ambient temperature and T<sub>E</sub> is elevated temperature.

Treatment	Target temperature elevation (°C)	Difference from target (°C)	Standard Deviation	10 <sup>th</sup> percentile (°C)	90 <sup>th</sup> percentile (°C)
<i>14—28 February 2002</i>					
C <sub>A</sub>	0	+0.6	±0.6	0.0	+1.3
C <sub>E</sub>	+4.7	+4.8	±0.2	+4.6	+5.0
<i>3—17 May 2002</i>					
C <sub>A</sub>	0	0.0	±0.4	-0.3	+0.3
C <sub>E</sub>	+3.1	+3.1	±0.2	+3.0	+3.4

The whole-tree chamber system enabled very accurate [CO<sub>2</sub>] control (Paper I, Table 3) in C<sub>E</sub>-WTCs. The injection of CO<sub>2</sub> directly into the circulating air stream from the cooling unit facilitated good mixing of the CO<sub>2</sub> and prevented vertical [CO<sub>2</sub>] gradients from developing within the WTCs and the high frequency of [CO<sub>2</sub>] measurements (every 90 seconds), enabled the system to maintain a tight control over the [CO<sub>2</sub>].

The [CO<sub>2</sub>] in the C<sub>E</sub>-WTCs was between 690 and 710 μmol mol<sup>-1</sup> for 98% and 93% of the time for the winter and spring periods, respectively (Paper I). During the winter period, the target [CO<sub>2</sub>] of 365 μmol mol<sup>-1</sup> was not accurately achieved in C<sub>A</sub>-WTCs; and was between ± 10 μmol mol<sup>-1</sup> for only 5% of the time. During the spring period, the [CO<sub>2</sub>] was between ± 10 μmol mol<sup>-1</sup> for 51% of the time. The lower accuracy of the target [CO<sub>2</sub>] in C<sub>A</sub>-WTCs during winter can be explained by the naturally high [CO<sub>2</sub>] of the ambient air that entered the chambers. The [CO<sub>2</sub>] of C<sub>A</sub>-WTCs during the winter test period was approximately +20 μmol mol<sup>-1</sup> above the target [CO<sub>2</sub>] of 365 μmol mol<sup>-1</sup> (Table 3), but this deviation (<10%) above the target was not considered to have any physiological consequences for the trees since they were not active during that time of year. In retrospect the target [CO<sub>2</sub>] should have been 20 μmol mol<sup>-1</sup> higher than the ambient target [CO<sub>2</sub>] of 365 μmol mol<sup>-1</sup>, to encompass the annual fluctuation of [CO<sub>2</sub>] in high northern latitudes (Keeling, Chin & Whorf, 1996). A higher target [CO<sub>2</sub>] would have enabled a better control of [CO<sub>2</sub>] during the winter and been able to compensate for night-time respiration during summer.

Table 3. Regulation of [CO<sub>2</sub>] in the whole-tree chambers (WTC) by [CO<sub>2</sub>] treatment. The [CO<sub>2</sub>] values measured by the central IRGA on a 42-min cycle from each WTC were pooled by [CO<sub>2</sub>] treatment. C<sub>A</sub> is ambient [CO<sub>2</sub>] and C<sub>E</sub> is elevated [CO<sub>2</sub>], n=6.

Treatment	Target [CO <sub>2</sub> ] ( $\mu\text{mol mol}^{-1}$ )	Difference from target ( $\mu\text{mol mol}^{-1}$ )	Standard Deviation	10 <sup>th</sup> percentile ( $\mu\text{mol mol}^{-1}$ )	90 <sup>th</sup> percentile ( $\mu\text{mol mol}^{-1}$ )
<i>14–28 February 2002</i>					
C <sub>A</sub>	365	+20.1	±5.0	+15.3	+23.5
C <sub>E</sub>	700	+2.4	±3.3	-0.8	+5.3
<i>3–17 May 2002</i>					
C <sub>A</sub>	365	+8.9	±14.9	-6.5	+25.0
C <sub>E</sub>	700	-1.6	±5.8	-7.4	+3.5

To illustrate the ability of the WTC system to continuously measure physiological performance of mature trees in response to climate change, the maximum day-time rate of net CO<sub>2</sub> uptake and night-time respiration for one tree in an ambient temperature and [CO<sub>2</sub>] chamber (T<sub>A</sub>C<sub>A</sub>) during one year (2003) is shown (Fig. 9). The maximum tree net CO<sub>2</sub> uptake rate was 6.1  $\mu\text{mol m}^{-2} \text{s}^{-1}$  which was measured during August. The maximum respiration rate during June and July was 1.2  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and the cumulative respiration for the entire year was 17% of the cumulative photosynthesis. The T<sub>A</sub>C<sub>A</sub> tree showed a late spring recovery of photosynthetic activity during March to April and an early decline in photosynthetic activity during October to November (Paper I, Fig. 8a).

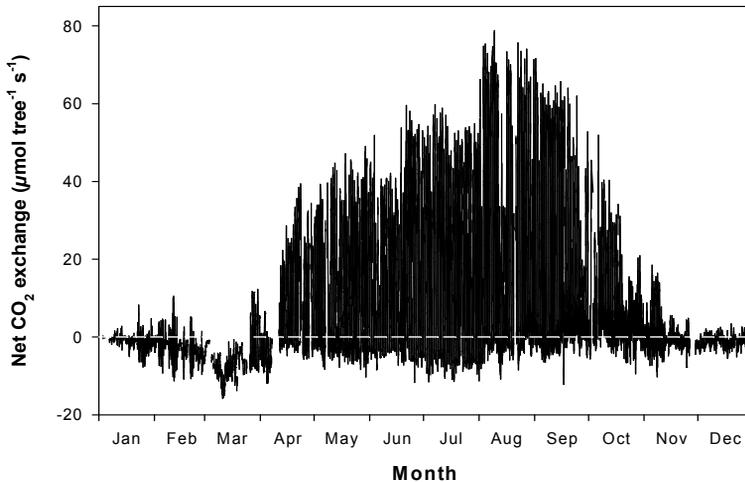


Figure 9. Annual course of net CO<sub>2</sub> exchange of a tree exposed to ambient temperature and [CO<sub>2</sub>], T<sub>A</sub>C<sub>A</sub> throughout 2003. Values shown are the maximum rates of day-time net CO<sub>2</sub> uptake and night-time respiration, respectively. In July 2003 the stem diameter at breast height (1.3 m) was 84 mm. Tree surface needle area was estimated using allometric relationships derived from an earlier destructive harvest at the Flakaliden site (B. Sigurdsson, unpublished data). The estimated needle area was 13.0 m<sup>2</sup>.

## Phenology and phenological models

The large variation in spring temperatures, between and within the years of the study (Figs. 3, 4 & 8), resulted in pronounced effects on bud development and timing of bud burst (Paper II, Fig. 5). Elevated temperature significantly accelerated bud development and was significantly faster than in  $T_A$  and non-chamber reference trees (Paper II). Buds on the  $T_A$ -WTCs trees took a longer time to develop compared to those in the  $T_E$ -WTCs, with new shoots emerging 10 to 20 days later (Paper II, Fig. 5). Since bud development and timing of burst were similar for  $C_A$  and  $C_E$ -WTCs, this indicated that an elevated  $[CO_2]$  of 700  $\mu\text{mol mol}^{-1}$  had no significant effect on development nor was there any significant interaction effect of  $[CO_2]$  and temperature (Paper II, Tables 2 & 3). The ranking of bud burst between treatments was consistent every year despite the 6 to 10 day difference of when it occurred and there was little between-tree variation in the day of year when bud burst occurred between treatments. Trees in  $T_E$ -WTCs broke bud approximately two weeks earlier than those in  $T_A$ -WTCs, which were 4 to 6 days earlier than non-chamber trees. When using temperature sum ( $T_{\text{sum}}$ ) to determine how many day degrees were required for bud burst across all treatments and years with starting points from January 1<sup>st</sup> or April 1<sup>st</sup>, thresholds  $\geq 0$ ,  $\geq 1$ ,  $\geq 2$ ,  $\geq 3$ ,  $\geq 4$  and  $\geq 5$  °C gave more variation than using the same thresholds from April 1<sup>st</sup>. The smallest variation within and between treatments was obtained with  $\geq 0$  °C from April 1<sup>st</sup>. In a study using black cottonwood, Sigurdsson (2001) found the best  $T_{\text{sum}}$  when accumulating day degrees ( $>0$  °C) from March 1<sup>st</sup> rather than January 1<sup>st</sup>.

Given the exceptionally early and warm spring in 2002, the fastest relative shoot growth was observed in that year (Paper II). While the pattern and duration of shoot growth varied between years and treatments (Fig. 10) the duration of the shoot growth period was not affected by  $[CO_2]$  or temperature. The time it took for shoots to reach 90% of their final length after bud burst occurred was different between years, but similar between treatments. In the early spring of 2002, it took two weeks or less for shoots to reach 90% of their final length after bud burst while

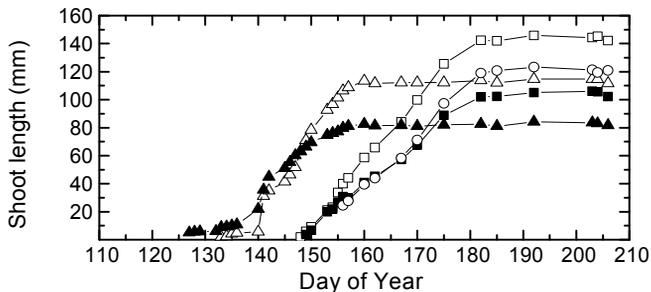


Figure 10. Shoot length for all trees in each treatment during 2003. Each point is an average of six shoots (three trees and two shoots per tree). Symbols and abbreviations: open square ( $T_A C_A$ ) = ambient temperature and ambient  $[CO_2]$ ; filled square ( $T_A C_E$ ) = ambient temperature and elevated  $[CO_2]$ ; open triangle ( $T_E C_A$ ) = elevated temperature and ambient  $[CO_2]$ ; filled triangle ( $T_E C_E$ ) = elevated temperature and elevated  $[CO_2]$ , and open circle (R) = non-chambered reference trees.

in 2003 and 2004, an extra 10 days were required to reach the same stage of shoot development (Paper II, Table 3). Although there were differences in the final shoot length of all trees between years and treatments, the effect of  $[\text{CO}_2]$  and temperature was not significant on final shoot length either year (Paper II, Table 3).

When comparing the relative growth rate of shoots between treatments after bud burst, the differences between treatments were obvious (Fig. 11, left). Although non-chambered trees were the last to break bud (Fig. 10), they appeared to have the highest relative growth rate. Since these shoots had no  $\text{CO}_2$  enrichment or temperature elevation, the growth rate reflected the temperature conditions during the shoot development period. When these growth rates were plotted against  $T_{\text{sum}} (\geq 5^\circ\text{C})$ , the differences between treatments disappeared (Fig. 11, right; Paper II, Fig. 7). Hence the prevailing temperature in spring was predominantly responsible for the commencement and rate of shoot development during each year since the differences were not explained in relation to  $T_{\text{sum}}$ .

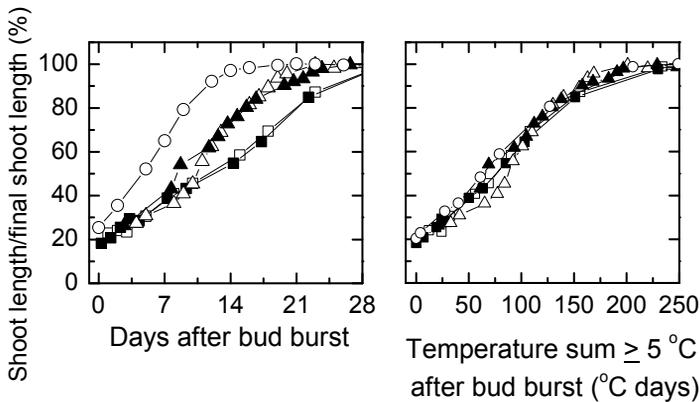


Figure 11. Shoot length in per cent of final length vs. days after bud burst (left) and temperature sum after bud burst (right) in 2003. Symbols and abbreviations: open square ( $T_{AC_A}$ ) = ambient temperature and ambient  $[\text{CO}_2]$ ; filled square ( $T_{AC_E}$ ) = ambient temperature and elevated  $[\text{CO}_2]$ ; open triangle ( $T_{EC_A}$ ) = elevated temperature and ambient  $[\text{CO}_2]$ ; filled triangle ( $T_{EC_E}$ ) = elevated temperature and elevated  $[\text{CO}_2]$ , and open circle (R) = non-chambered reference trees.

Temperature sums cannot be used to predict bud burst accurately, at the tree or stand level, because of large within-tree variations, between-tree variations (e.g. Romell, 1925; Žumer, 1968, 1969; Roberntz, 1999), and provenances (e.g. Langlet, 1960; Hannerz, 1999), and the unpredictable temperature climate from year to year. Although the results (Paper I) paint a clear picture for the 6<sup>th</sup> - 7<sup>th</sup> whorl (from the top), there are still certain variations that exist. Since bud development and bud break in conifers is not synchronous within the canopy and proceeds from the bottom and moves upward (cf. Kozłowski & Ward, 1961), one cannot expect to find a single temperature sum that could predict bud burst at the whole-tree level.

Elevated [CO<sub>2</sub>] did not alter the commencement or the rate of bud development, nor did it have any effect on time of bud break, the final length of shoots or the duration of the growth period. Similar results, from experiments using mature trees, have been reported for Norway spruce (Roberntz, 1999) and Scots pine (Kilpeläinen *et al.*, 2006). The fact that all differences in shoot growth across treatments and between years disappeared when relative shoot growth was normalized and compared to T<sub>sum</sub> indicated that the duration of shoot growth in boreal Norway spruce was determined by the time taken to accumulate a certain T<sub>sum</sub> to reach final length. Further to that, the smallest deviation from the mean T<sub>sum</sub> between years and across treatments was obtained when a threshold value of  $\geq 0$  °C was used and calculated from April 1<sup>st</sup>, which agreed with the model analysis using the same data (Paper III).

Several simulation models have been developed for describing the effects of air temperature on timing of bud burst in the temperate and boreal trees (for reviews, see Hänninen 1990a, 1995a, Hunter & Lechowicz 1992; Kramer 1994a,b, 1996; Chuine, Cour & Rousseau, 1998, 1999; Häkkinen, 1999a; Linkosalo, 2000a; Chuine, 2000; Chuine, Kramer & Hänninen, 2003). Three such models were tested (Paper III), where the *Fixed Day Model* had the highest accuracy of the tested models, while inconsistent results were obtained for the temperature response of the ontogenetic development (Paper III, Fig. 4a). This suggests that high air temperatures were not physiologically effective until late in the spring. From the models used in this study, it was not possible to estimate the proper air temperature response with the optimisation method. One of the reasons for the difficulties in using this approach to predict ontogenetic development is owing to the fact that the simulation models only use the timing of the end point (*i.e.* bud burst) to describe a physiological process that lasts several months. Another shortcoming lies in the fact that some of the sub-models underlying phenological processes in the models are fixed on the basis of results from previous studies (*e.g.* Sarvas, 1972, 1974; Häkkinen, Linkosalo & Hari, 1998; Häkkinen, 1999b; Linkosalo, 2000b; Linkosalo *et al.*, 2000) often with different tree species than those being used for model development and testing (*cf.* Hänninen, 1995a; Linkosalo, 2000b). This was the case in our study (Paper III) for models that could accurately predict the timing of growth onset (Hänninen, 1995a) or frost hardiness of Scots pine (Repo, Hänninen & Kellomäki, 1996; Hänninen *et al.*, 1996) under present climatic conditions, but failed completely in their predictions when tested using data gathered in T<sub>E</sub> WTCs (Paper II) with expected future climatic conditions. Although these models were not able to predict the regulation of the timing of bud burst in Norway spruce trees, they did provide a clear indication that rest completion in Norway spruce trees growing in northern Sweden takes place near the spring equinox. The mechanisms involved in rest completion and the risk of frost damage in boreal trees, under climatic warming are, however, not yet sufficiently understood and a more detailed understanding of the mechanisms driving the control of phenological events would greatly improve the accuracy of the models (Linkosalo, 2000a).

## Chlorophyll fluorescence and photosynthetic capacity

During early spring (*i.e.* March and April), the recovery of the photosynthetic apparatus was faster in T<sub>E</sub> than in T<sub>A</sub> treatments, as seen in variable chlorophyll fluorescence ( $F_v/F_M$ ), apparent quantum yield ( $\phi$ ) and light saturated photosynthesis ( $A_{sat}$ ), but elevated [CO<sub>2</sub>] had no effect during this period. Elevated temperature increased  $A_{sat}$  in early spring, but as spring progressed an increase in  $A_{sat}$  was also the result of elevated [CO<sub>2</sub>] (Table 4). It is also worth mentioning that since the measurements were taken from shoots in the top of the canopy, there was no pronounced difference in light conditions between north and south facing shoots (data not shown).

Table 4. Mean monthly apparent quantum yield ( $\phi$ ), light saturated photosynthesis ( $A_{sat}$ ) and variable fluorescence ( $F_v/F_M$ ) for all treatments during spring 2002. Values shown are mean  $\pm$  standard deviation.

$\phi$ (mol CO <sub>2</sub> mol <sup>-1</sup> photons)				
	March	April	May	June
T <sub>A</sub> C <sub>A</sub>	0.004 $\pm$ 0.002	0.037 $\pm$ 0.004	0.047 $\pm$ 0.005	0.050 $\pm$ 0.001
T <sub>E</sub> C <sub>A</sub>	0.022 $\pm$ 0.002	0.050 $\pm$ 0.008	0.052 $\pm$ 0.006	0.049 $\pm$ 0.006
T <sub>A</sub> C <sub>E</sub>	0.008 $\pm$ 0.004	0.042 $\pm$ 0.006	0.051 $\pm$ 0.004	0.055 $\pm$ 0.005
T <sub>E</sub> C <sub>E</sub>	0.024 $\pm$ 0.004	0.055 $\pm$ 0.001	0.049 $\pm$ 0.001	0.054 $\pm$ 0.004
<b>R</b>	0.015 $\pm$ 0.001	0.036 $\pm$ 0.004	0.048 $\pm$ 0.001	0.053 $\pm$ 0.003
$A_{sat}$ ( $\mu$ mol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )				
T <sub>A</sub> C <sub>A</sub>	0.8 $\pm$ 0.53	8.0 $\pm$ 0.65	10.3 $\pm$ 0.76	10.7 $\pm$ 0.75
T <sub>E</sub> C <sub>A</sub>	3.0 $\pm$ 0.23	10.2 $\pm$ 1.27	10.6 $\pm$ 0.74	10.8 $\pm$ 0.73
T <sub>A</sub> C <sub>E</sub>	1.0 $\pm$ 0.62	10.8 $\pm$ 1.13	14.8 $\pm$ 0.61	14.6 $\pm$ 1.50
T <sub>E</sub> C <sub>E</sub>	4.2 $\pm$ 0.43	14.0 $\pm$ 0.81	14.1 $\pm$ 0.40	14.2 $\pm$ 1.25
<b>R</b>	1.6 $\pm$ 0.34	7.2 $\pm$ 1.30	10.5 $\pm$ 0.88	11.3 $\pm$ 0.56
$F_v/F_M$				
T <sub>A</sub> C <sub>A</sub>	0.39 $\pm$ 0.099	0.73 $\pm$ 0.090	0.80 $\pm$ 0.021	0.84 $\pm$ 0.006
T <sub>E</sub> C <sub>A</sub>	0.54 $\pm$ 0.074	0.84 $\pm$ 0.032	0.82 $\pm$ 0.010	0.81 $\pm$ 0.008
T <sub>A</sub> C <sub>E</sub>	0.44 $\pm$ 0.065	0.77 $\pm$ 0.066	0.81 $\pm$ 0.026	0.84 $\pm$ 0.007
T <sub>E</sub> C <sub>E</sub>	0.54 $\pm$ 0.017	0.84 $\pm$ 0.033	0.80 $\pm$ 0.021	0.83 $\pm$ 0.010
<b>R</b>	0.41 $\pm$ 0.031	0.68 $\pm$ 0.116	0.79 $\pm$ 0.035	0.83 $\pm$ 0.005

Overnight frost events had significant negative effects on the  $\phi$  (Fig. 12). The number of frost events were, however, too few to be able to identify a temperature threshold that caused a setback in the recovery. Lundmark *et al.* (1998) found that spring frosts retarded photosynthetic recovery, but after temperatures increased and remained above 0 °C, recovery commenced and  $F_v/F_M$  recovery almost completely within two weeks. When modelling the recovery of photosynthetic

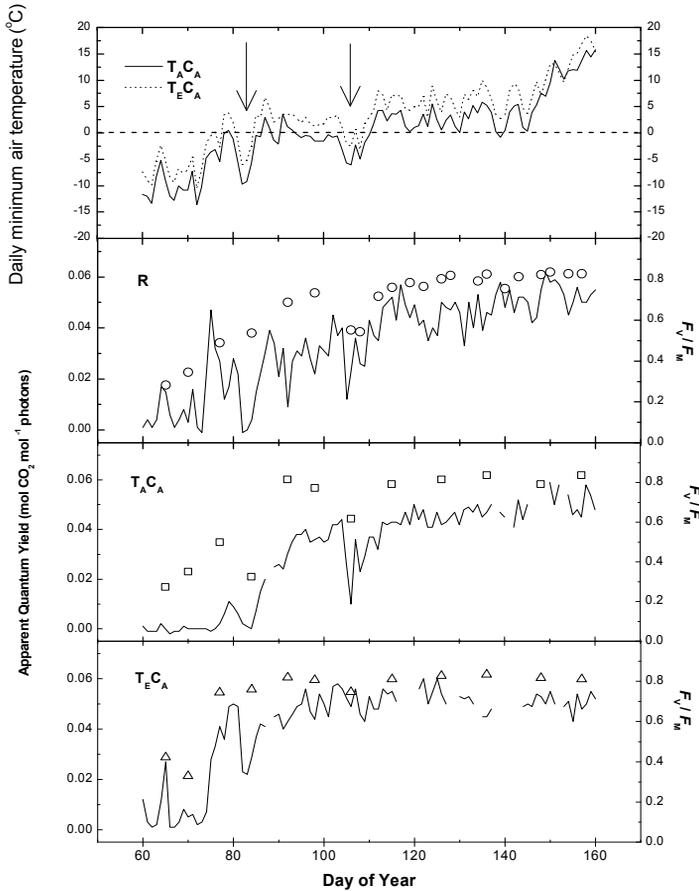


Figure 12. Daily mean minimum air temperature, apparent quantum yield ( $\phi$ ) and chlorophyll fluorescence ( $F_V/F_M$ ) for non-chambered reference trees (R), ambient and elevated temperature treatments ( $T_A C_A$  and  $T_E C_A$ ) during spring 2002.

capacity of boreal Norway spruce, Bergh *et al.* (1998) introduced a function where the rate of recovery was reduced by mild frosts ( $-3$  to  $0$  °C) for two days and a severe overnight frost (below  $-3$  °C) reduced photosynthetic capacity and halted photosynthetic recovery for two days. During the mild spring of 2002, however, only two such frost events occurred after the spring equinox (March 21<sup>st</sup>, *cf.* Day of Year 80 in Fig. 12).

Apparent quantum yield varied with changes in daily minimum air temperature, but chlorophyll fluorescence did not always reflect the same pattern (Fig. 13). The frost events (indicated by arrows in Fig. 12) that occurred in the end of March and the middle of April (Day of Year 85 and 105, respectively), clearly coincided with a decrease in apparent quantum yield in R,  $T_A C_A$ , and  $T_E C_A$  trees.  $F_V/F_M$  was,

however, not measured on the days directly after the first frost event and thus a corresponding decrease in  $F_V/F_M$  could not be confirmed. A decrease in the  $F_V/F_M$  was, however, observed when measured the day after the second frost, while not as large a decrease as in apparent quantum yield ( $\phi$ ).

There was a linear relationship ( $R^2 = 0.75$ ) between  $F_V/F_M$  and apparent quantum yield and the relationship was irrespective of temperature or  $[CO_2]$  (Fig. 13, left), when averaged across treatments the relationship had an  $R^2$  of 0.95. Before shoots had regained full photochemical efficiency ( $F_V/F_M < 0.8$ ), there was a small difference in light saturated photosynthetic rates between treatments (Fig. 13, right), but when  $F_V/F_M$  indicated full recovery ( $\sim 0.80$ ), there were clear differences between ambient and elevated  $[CO_2]$  treatments in terms of  $A_{sat}$  (Table 4 & Fig. 13, right). After full recovery had been reached both  $A_{sat}$  and  $\phi$  seemed to be stable and the day-to-day variations may have been caused by other climatic factors. Some of the scatter seen in Fig. 13 may have been based on the limited number of measured shoots and/or by shading of individual shoot cuvettes or light sensors.

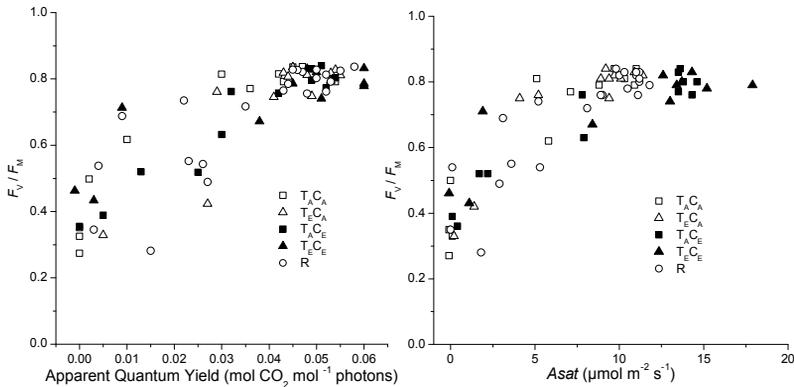


Figure 13.  $F_V/F_M$  in relation to apparent quantum yield and  $A_{sat}$  for all treatments during spring 2002.  $T_A C_A$  = ambient temperature and ambient  $[CO_2]$ ;  $T_A C_E$  = ambient temperature and elevated  $[CO_2]$ ;  $T_E C_A$  = elevated temperature and ambient  $[CO_2]$ ;  $T_E C_E$  = elevated temperature and elevated  $[CO_2]$ , and R = non-chambered reference trees.

Chlorophyll fluorescence measurements were a reasonable measure of the recovery of the photosynthetic apparatus, but cannot be used as a proxy for gas exchange. While measurements of chlorophyll fluorescence were reasonable indicators of photosynthetic capacity, they cannot be used as an alternative for photosynthesis measurements since net photosynthesis is so variable and responsive to temperature and  $[CO_2]$ . Frost events seemed to have affected photosynthesis ( $\phi$ ), but this was not reflected in chlorophyll fluorescence. This could be the result of the relatively low frequency of fluorescence measurements and the fact that  $F_V/F_M$  only indicates the status of the electron transport system. The recovery of photosynthetic capacity in the  $T_E$  treatment commenced approximately 10 days earlier than in  $T_A$  treatments.

The present study supports earlier studies from Flakaliden on the usefulness of chlorophyll fluorescence to follow spring recovery (Lundmark, *et al.* 1998), effect of soil warming (Bergh & Linder, 1999), effect of mineral nutrition (Strand, 1997), and autumn decline in photosynthetic efficiency (Strand & Lundmark, 1995; Lundmark *et al.*, 1998). Measurements of fluorescence is a useful proxy during periods when differences in gas exchange are difficult to measure because of low rates of net photosynthesis and/or freezing conditions.

## Photosynthesis at shoot and tree level

Rising air temperatures will lead to earlier spring photosynthetic recovery of boreal Norway spruce forests (Papers IV & V). The general pattern of photosynthetic recovery, at the shoot- and tree-level, was in good agreement, but there were some differences in its pattern and magnitude (Figs. 14 & 15). Within each treatment the commencement of significant recovery started at the same dates at the shoot and tree level. There were, however, some differences in timing and magnitude later during the recovery period (Paper V, Figs. 14 and 15). In the  $T_E C_A$  treatment, the recovery period was shorter at the shoot-level (6<sup>th</sup> whorl) than at the tree-level (41 and 56 days, respectively). Decreases in  $A_{max}$ , due to frost events, were more pronounced at the shoot than the whole-tree level (Paper V). This difference might be due to a situation where the less exposed shoots in the tree crown experience less frost exposures and thus get less damaged. The faster recovery might be caused by a combination of this effect and a more favourable light interception at the tree level early in the season. When the sun elevation is low, the incoming radiation is to a large extent perpendicular to the largest spatial distribution of the crown. Despite the observed differences, it is clear that shoot-level measurements of  $CO_2$  uptake are a valid and useful means of assessing whole-tree performance (*cf.* Wallin *et al.*, 2001).

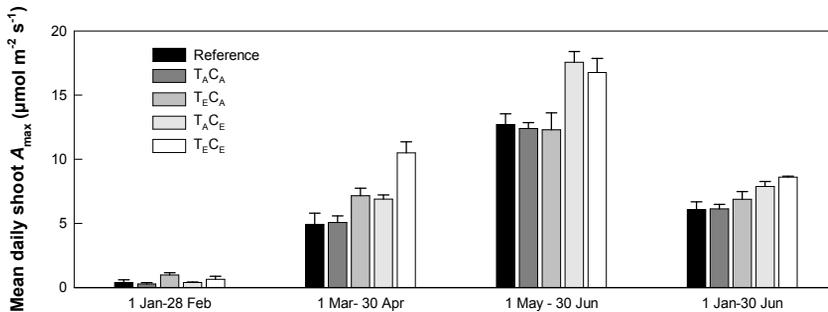


Figure 14. Mean maximum shoot photosynthetic rate ( $A_{max}$ ) of Norway spruce by treatment for the period 1 January to 30 June 2002. Values shown are means of three shoots per treatment.

Elevated air temperature produced an earlier onset of  $A_{max}$  while elevated  $[\text{CO}_2]$  did not alter the timing of recovery. Spring photosynthetic recovery commenced in elevated temperature treatments during warm spells already in early January and February, while the main recovery, in 2002, commenced in mid-March. This was nearly two weeks earlier in  $T_E$ -WTCs than in  $T_A$ -WTCs (Paper V, Fig. 3 for shoot-level  $A_{max}$ ). Elevated  $[\text{CO}_2]$  did not alter the starting date of photosynthetic recovery, but rather produced greater photosynthetic capacity once recovery had commenced. This is in agreement with many others who have found increases in photosynthetic capacity, as a result of elevated  $[\text{CO}_2]$ , for a range of tree species (e.g. Teskey, 1997; Roberntz, 2001; Bernacchi *et al.*, 2003).

In May to June, the  $[\text{CO}_2]$  enrichment increased on average the  $A_{max}$  by 35% (Paper V). The maximum value of  $A_{max}$  during that period was identical for both  $C_A$ -WTC treatments (i.e.  $T_EC_A$  and  $T_AC_A=15.1 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) as well as for  $C_E$ -WTC treatments (i.e.  $T_EC_E$ , and  $T_EC_E=21.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ ).

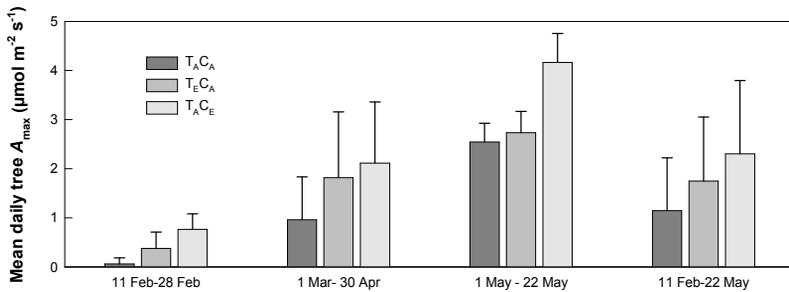


Figure 15. Mean maximum tree photosynthetic rate of Norway spruce by treatment for the period 1 January to 22 May 2002. Values shown are means of three shoots per treatment. Due to technical problems, data from the  $T_EC_E$  treatment could not be included.

Combined temperature and  $[\text{CO}_2]$  elevation resulted in both earlier photosynthetic recovery and higher  $A_{max}$  values of shoots, but this was minimal on days of diffuse radiation (Paper V). Similar to shoots, the increase in tree-level  $A_{max}$  as a result of elevated temperature and  $[\text{CO}_2]$  was greatly diminished on days of predominantly diffuse radiation. The apparent decrease in  $A_{max}$  in elevated  $[\text{CO}_2]$ , at low light levels, is indicating that the limitation of photosynthesis at low light is more related to light-use efficiency than to the carboxylation efficiency. This reduction in  $A_{max}$  suggests that treatment-driven changes in  $A_{max}$  at the top of the canopy make an important contribution to the tree-level net  $\text{CO}_2$  uptake, as it is mainly the upper canopy that is exposed to high radiation (cf. Lewis *et al.*, 1999).

Shoot apparent quantum yield ( $\phi_{\text{shoot}}$ ) was greater in T<sub>E</sub>-WTCs compared to T<sub>A</sub>-WTCs (Paper V, Fig. 5a), but elevated [CO<sub>2</sub>] did not have any effect on the pattern of  $\phi_{\text{shoot}}$  (Paper V, Figs. 5b,c). The mean  $\phi_{\text{shoot}}$  values for the period 1 March to 30 June 2002 were 0.033, 0.043, 0.038, and 0.043 mol CO<sub>2</sub> mol<sup>-1</sup> photons for the T<sub>A</sub>C<sub>A</sub>, T<sub>E</sub>C<sub>A</sub>, T<sub>A</sub>C<sub>E</sub> and T<sub>E</sub>C<sub>E</sub> shoots, respectively. Maximum  $\phi_{\text{shoot}}$  values reached during the measurement period were 0.056, 0.060, 0.066, and 0.066 mol CO<sub>2</sub> mol<sup>-1</sup> photons for the T<sub>A</sub>C<sub>A</sub> (30 May), T<sub>E</sub>C<sub>A</sub> (2 May), T<sub>A</sub>C<sub>E</sub> (20 June), and T<sub>E</sub>C<sub>E</sub> (6 April) shoots, respectively (Paper V). Tree apparent quantum yield ( $\phi_{\text{tree}}$ ) was also greater in T<sub>E</sub>-WTCs compared to T<sub>A</sub>-WTCs in early spring, but differences between treatments had largely disappeared by the end of March (Paper V, Fig. 6). During the period of significant photosynthetic activity mean  $\phi_{\text{tree}}$  values were 0.0088 and 0.0085 mol CO<sub>2</sub> mol<sup>-1</sup> photons for the T<sub>A</sub>C<sub>A</sub> (25 March-23 May) and T<sub>E</sub>C<sub>A</sub> (15 March-23 May) treatments, respectively. Maximum daily  $\phi_{\text{tree}}$  values were 0.0189 and 0.0193 mol CO<sub>2</sub> mol<sup>-1</sup> photons for the T<sub>A</sub>C<sub>A</sub> and T<sub>E</sub>C<sub>A</sub> treatments, respectively (Paper V).

The strong influence of air temperature on the timing of photosynthetic recovery in Norway spruce agrees with findings from previous studies of boreal tree species (*e.g.* Strand & Lundmark, 1995; Lundmark *et al.*, 1998; Strand *et al.*, 2002; Suni *et al.*, 2003; Ensminger *et al.*, 2004; Mäkelä, 2004).

The starch accumulation during March and April reflected the earlier recovery of photosynthesis in T<sub>E</sub> treatments (Paper V, Table 2), but the ranking shifted in advantage of the C<sub>E</sub> treatment in May when full recovery had taken place in all treatments (*cf.* Fig. 14) and elevated [CO<sub>2</sub>] had a positive effect on  $A_{\text{sat}}$  (*cf.* Fig. 13). The peak values in needle starch concentrations were similar to what has been reported earlier from the Flakaliden site (*cf.* Linder, 1995). The decrease in needle nitrogen concentration during early summer was most pronounced in the T<sub>E</sub> treatments (Paper V) and reflected the earlier bud break and shoot development, which under nutrient limited conditions results in re-translocation of nutrients from older foliage to the new developing shoots (*cf.* Linder, 1995; Roberntz & Stockfors, 1998).

## Conclusions

The whole-tree chamber system worked well for measuring physiological responses of individual trees to elevated  $[\text{CO}_2]$  and temperature for examining the effects of climate change on tree performance. The precision of temperature and  $[\text{CO}_2]$  control in the WTC system gives confidence in the ability of the system to reproduce natural conditions as well as providing the conditions for a realistic assessment of tree responses to climate change. Tree-level measurements obtained from WTCs provided an integration of measurements that incorporate all shoots, with varying crown position and age class, along with respiratory  $\text{CO}_2$  released from woody tissue, thus avoiding over- or underestimations when scaling measurements from shoots to the level of trees and forest stands.

The present studies show that climate warming will result in earlier bud burst in boreal Norway spruce trees and that this is strongly determined by the temperature sum accumulated after the spring equinox. Elevated  $[\text{CO}_2]$  did not affect the timing of bud burst or shoot development, but in a warmer climate a longer growing season can be expected, with an earlier and faster photosynthetic recovery in spring as well as earlier shoot development leading to an increase in the potential photosynthetic production.

The inability to accurately predict the timing of bud burst by using an accumulation of temperature sums or ecophysiological simulations models, suggests that in adult trees there are additional environmental cues that affect rest completion and the commencement of ontogenetic development (*cf.* Partanen, Hänninen & Häkkinen, 2005). To improve our ability to predict the likely impacts of climate change on the structure and function of forest ecosystems, there is an urgent need to establish more long-term, multifactor experiments in mature forest stands (*cf.* Saxe *et al.*, 2001; Beier, 2004; Norby & Lou, 2004).

From the studies, presented in the present thesis, it can be concluded that both elevated air temperature and  $[\text{CO}_2]$  have the potential to increase the carbon uptake of boreal Norway spruce forests, but whether or not this will change translate into increased carbon gain in a future climate, remains to be seen. In most boreal forests, growth stimulation can only be expected if the increased carbon uptake can be met by an increased nitrogen uptake. Little or no long-term growth response to elevated temperatures and/or  $[\text{CO}_2]$  can be expected in forests where the availability of nitrogen, rather than carbon, is the main growth-limiting factor (*cf.* Tamm, 1991; Linder, 1995; Körner, 2003).

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To thank all the people who have made this thesis a reality deserves an appendix of its own. I'm not sure 'Acknowledgements' is the correct heading for this section but since I can't think of a single English word that better expresses my gratitude, I might as well follow tradition. You have all made this a very enjoyable experience and if I had arms long enough, I would like to give you all a GREAT BIG group hug! I will try to be concise (which, anyone who knows me can confirm, is a problem for me) but please be patient and read carefully as parts of this section will be written in several languages (my mother tongue, Newfoundland English, Swedish and Swenglish). While this might be the longest acknowledgments section in the history of Thesis', I really can't see any way around it, since I have carried out this work over four and a half years in three different parts of Sweden (from the north to the south) and was fortunate to encounter so many great people that truly deserve a proper thanks.

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