EFFECTS OF ELEVATED ATMOSPHERIC CARBON DIOXIDE AND NUTRIENTS ON THE GROWTH, PHENOLOGY AND PHYSIOLOGY OF SITKA SPRUCE [*PICEA SITCHENSIS* (BONG.) CARR.]

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A thesis submitted for the degree of Doctor of Philosophy
to the
University of Edinburgh
1997

To my family

..... the earth, gentle and indulgent, ever subservient to the wants of man, spreads his walks with flowers, and his table with plenty; returns with interest, every good committed to her care.

Pliny the Elder

Acknowledgements

I would like to express my thanks and appreciation to the many people who have given me their support and encouragement throughout the duration of this thesis.

Firstly , I would like to thank my supervisor Professor Paul G. Jarvis for his support, encouragement and particular patience in editing this thesis. I would also like to take this opportunity to thank ITE management for allowing me the opportunity to combine the work presented in this thesis with my work at ITE.

I am also indebted to a great many people who have given freely of their time to encourage me when I faltered. I would particularly like to mention Dr. Jan Dick, she will never know just how much her constant enthusiasm for the science and determination that I should complete this thesis has meant to me. She has truly been a superb supervisor but without any of the credit, thank you Jan. Thanks also go to Derek Eamus who encouraged and supported me at the start of this thesis. I would also like to thank Ron Smith for all his statistical advice and great patience in teaching me the various techniques employed in this thesis. I would like to extend my sincere thanks to the following people all of whom have help in so many ways they are too numerous to mention, Frank Harvey, Alan Crossley, Lucy Sheppard, John McCormack and Ian Leith. A special big thank you should also go to Marjorie Ferguson for her help in formatting this thesis, she will be remembered most for her phrase "no problem" irrespective of the problems or task ahead.

My thanks also goes to the analytical chemistry division at ITE, Merlwood, Grange-over-sands who carried out all the chemical analyses and who's data have been invaluable to this study. Thanks also to Ron Wilson for the superb line drawing.

In Edinburgh University, I would like to thank a very special person Ana Rey Simó, who always found time to help me no matter how busy she was. Her superb thesis gave me the inspiration I needed to complete mine. Thanks also to Craig Barton who designed and installed the computer based CO_2 recording system.

And finally, I must thank the two very special men in my life, David and Christopher. Davids patience, help and trust in me has enabled the completion of this thesis and Christopher's love and needs have also allowed me to balance the importance of science with life at home.

This study was jointly funded by the European Community through the Environment R&D Programme (EPOCH and ECOCRAFT, Contract No. EV5V-CT92-0127) and the Natural Environmental Research Council, (NERC).

CONTENTS

ABSTRACT

The concentration of carbon dioxide [CO_2] in the atmosphere is increasing globally and has risen 30 % since pre-industrial times. Its concentration is predicted to double by the end of the next century. Because the current atmospheric [CO_2] limits C_3 photosynthesis, and CO_2 is the primary substrate for photosynthesis, any increase in its concentration will have a direct effect on terrestrial vegetation. The impact of rising global [CO_2] is of particular importance to forest species, as their longevity makes them particularly susceptible to long term changes in [CO_2] and they themselves play a vital role in the global carbon cycle.

The aim of this thesis was to investigate the effects of elevated [CO_2] and varying nutrient supply rates on the growth, phenology and physiology of Sitka spruce (*Picea sitchensis* (Bong.) Carr.). Two approaches were taken both using open top chamber facilities to fumigate seedling with either ambient (355 μmol mol^{-1} CO_2) or elevated (700 μmol mol^{-1} CO_2). Firstly, a long term study on the direct impact of elevated [CO_2] was undertaken, this experiment ran for three consecutive years. Comparisons between responses obtained after one and three years exposure to elevated [CO_2] were made. In addition, the impact of growing seedlings in OTCs *per se* was also investigated. Secondly, a study was conducted into the interaction between [CO_2] and nutrient supply rate on growth, phenological and physiological responses. The effect of OTCs were also included in this experiment.

Biomass accumulation was always enhanced by elevated [CO_2] when nutrients were not limiting. In the second experiment biomass stimulation as a result of elevated [CO_2] was 16 and 37 % for seedlings with a foliar [N] of 1.9 and 2.4 %, respectively. However, there was no enhancement at a foliar [N] of 0.9 %. A similar result was also observed in the first long term study experiment. There was no significant chamber effect on biomass but seedlings growing inside OTCs were 25 % taller.

Biomass allocation was affected by [CO_2], nutrient supply rate, growth inside OTCs and experimental duration. The amount of biomass allocated to roots was increased by elevated [CO_2] and decreasing nutrient supply rates. With those seedlings receiving elevated [CO_2] and low-N supply rates having the highest R/S ratios. Growth inside OTCs reduced the amount of biomass allocated to roots. Seedlings receiving elevated [CO_2] and low nutrient supply rates had smaller amounts of biomass allocated to stems. Total leaf area and mass were both increased by elevated [CO_2] and increasing nutrient supply rate but there was no significant effect of elevated [CO_2] on specific leaf area, leaf area ratio or leaf mass ratio.

Elevated [CO_2] significantly affected bud phenology, delaying budburst and advancing budset, thereby reducing the growing season of seedlings with foliar [N] of 2.0 % or below. Increasing nutrient supply rates lengthened the growing season *per se*.

Phenology of seedlings with a foliar [N] of 2.4% were not affected by elevated [CO_2]. There was a large clonal variation in the phenological response to [CO_2] and the effect of OTC was bigger than that of [CO_2]. The effect of elevated [CO_2] and climatic warming on spring frost damage was modelled and future risks of damage were predicted to decrease.

Elevated [CO_2] had a positive effect on the net photosynthetic rate of shoots irrespective of experimental duration or nutrient supply rate. Acclimation of photosynthesis as a response to growth in elevated [CO_2] was only observed in nutrient limited seedlings, where both V_{cmax} and J_{max} were lower. Elevated [CO_2] always increased dark respiration rates, but had no effect on stomatal conductance.

In summary, under nutrient limited conditions despite increased photosynthetic rates elevated [CO_2] did not increase seedling biomass. Stem wood production was decreased as a result of enhanced biomass allocation to the roots. Therefore, it is likely that unless elevated [CO_2] indirectly stimulates the soil mineral N pool, young Sitka spruce grown on nutrient poor sites will have a reduced aboveground net primary productivity (ANPP). However, under nutrient rich conditions elevated [CO_2] will stimulate photosynthesis and biomass production, ultimately resulting in increased ANPP.

Symbols

Symbol	Discription	Units
α	Initial slope of *A*/*I* response curve	mol CO_2 mol^{-1} quanta
θ	Convexity of *A*/*I* response curve	-
Γ^*	CO_2 compensation concentration in absence of mitochondrial respiration	μmol mol^{-1}
A	Net photosynthetic rate	μmol m^{-2} s^{-1}
A_{max}	Model maximum net photosynthesis	μmol m^{-2} s^{-1}
A_{mmax}	Measured maximum net photosynthesis	μmol m^{-2} s^{-1}
A_{RuBP}	RuBP regeneration-limited net photosynthesis	μmol m^{-2} s^{-1}
A_{carb}	RuBP regeneration-saturated net photosynthesis	μmol m^{-2} s^{-1}
b	mean time to bud burst	days
C_a	Atmospheric CO_2 concentration	μmol mol^{-1}
C_d	Number of chill days to bud burst	days
C_i	Intercellular CO_2 concentration	μmol mol^{-1}
[C]	Total carbon concentration	% C g^{-1} dry mass
D	Root collar diameter	cm
D_d	Thermal requirement to bud burst	Σ °C
E	Transpiration rate	mol m^{-2} s^{-1}
F	Cumulative distribution function	-
g_m	Mesophyll conductance	mol m^{-2} s^{-1}
g_s	Stomatal conductance	mol m^{-2} s^{-1}
G_{season}	Length of growing season	days
H	Stem height	cm
I	Incident PPFD	μmol m^{-2} s^{-1}
J_{max}	Maximum electron transport capacity	μmol m^{-2} s^{-1}
k_c	Michaelis constant for carboxylation of Rubisco	μmol CO_2 mol^{-1}

k_o	Michaelis constant for oxygenation of Rubisco	μmol O_2 mol^{-1}
[K]	Potassium concentration	% K g^{-1} dry mass
L	Leader length	mm
L_a	Projected needle area	cm^2
L_m	Needle dry mass	g
M	Total seedling dry mass	g
N	Nitrogen content of plant	g N g^{-1} dry mass
[N]	Nitrogen concentration	% N g^{-1} dry mass
p	Probability level	-
[P]	Phosphorus concentration	% P g^{-1} dry mass
R	Model dark respiration rate	μmol m^{-2} s^{-1}
R_d	Day respiration	μmol m^{-2} s^{-1}
R_m	Measured dark respiration rate	μmol m^{-2} s^{-1}
R_{dm}	Annual relative growth rate	g g^{-1} day^{-1}
R_{sm}	Relative growth rate for G_{season}	g g^{-1} day^{-1}
R_w	Weekly relative extension rate	mm mm^{-1} day^{-1}
R_{aw}	Annual relative extension rate	mm mm^{-1} day^{-1}
T	Time	day
V_{cmax}	Maximum carboxylation rate of Rubisco	μmol m^{-2} s^{-1}
VPD	Vapour pressure deficit	mb

Abbreviations

Abbreviation	Description
a/b	Ratio of chlorophyll *a* to *b*
ANOVA	Analysis of variance using Genstat 5
ANPP	Aboveground net primary productivity
Ambient [CO_2]	Chamber grown 355 μmol CO_2 mol^{-1} treatment
A/C_i	Internal CO_2 concentration response curve
A/I	PPFD response curve
C_i/C_a	Ratio of intercellular to atmospheric CO_2 concentration
C/N	Carbon to nitrogen ratio
[CO_2]	Atmospheric CO_2 concentration
DMF	Dimethylformamide
Elevated [CO_2]	Chamber grown 700 μmol CO_2 mol^{-1} treatment
FACE	Free air carbon dioxide enrichment
High-N	Nutrients supplied at 2.0 x optimum rate
IRGA	Infrared gas analyser
LAR	Leaf area ratio
LMR	Leaf mass ratio
Low-N	Nutrients supplied at 0.1 x optimum rate
LSD	Fisher's least squared difference test
Medium-N	Nutrients supplied at 0.5 x optimum rate
NPP	Net primary productivity
NUE	Nutrient use efficiency
Outside	Outside control plot at 355 μmol CO_2 mol^{-1} treatment
OTCs	Open-top chambers
PAR	Photosynthetically active radiation

PCR	Photosynthetic carbon reduction cycle
PPFD	Photosynthetic photon flux density
RGR	Relative growth rate
R/S	Root to shoot mass ratio
Rubisco	Ribulose, 1, 5-bisphosphate carboxylase/oxygenase
RuBP	Ribulose-1, 5-bisphosphate
SEM	One standard error of the mean
SLA	Specific leaf area
TLA	Total leaf area per seedling
TLM	Total leaf mass per seedling
WUE	Water use efficiency

CHAPTER 1

Introduction and aims

1.1 Changing atmospheric CO_2 concentrations

The concentration of carbon dioxide (CO_2) in the atmosphere is increasing globally at an alarming rate, haven risen some 30% since pre-industrial times (i.e., 1750), (IPCC, 1995). The primary cause of this trend is largely attributable to anthropogenic activities (i.e.,'us') through the utilisation of fossil fuels with significant contributions also coming from agriculture and land-use change, e.g. tropical forest deforestation, (Bazzaz, 1990). If carbon dioxide emissions were maintained at 1994 levels, they would lead to a nearly constant rate of increase in atmospheric concentrations for at least two centuries, reaching about 500 μmol mol^{-1} CO_2 (approaching double the pre-industrial value of 280 μmol mol^{-1} CO_2) by the end of the 21 st century. Indeed a range of carbon cycle models indicate that stabilisation of atmospheric CO_2 concentrations at 450, 650 or 1000 μmol mol^{-1} CO_2 could only be achieved if global anthropogenic CO_2 emissions drop to the 1990 amount 40, 140 or 240 years from now, respectively, and thereafter drop substantially below 1990 amounts (IPCC, 1995).

1.1.1 The "Greenhouse effect"

Carbon dioxide has become widely known as the "greenhouse gas" because of its absorptive properties for longwave infrared radiation and abundance in the atmosphere. It is responsible, together with other greenhouse gases for absorption of the long wavelength radiation, reradiated back to space by the earths surface. This naturally occurring "greenhouse phenomenon" accounts for the earths current mean

temperature of ~15 °C. Other "greenhouse gases" include methane, nitrous oxide, hydroflurocarbons, perfluorocarbons and sulphur hexafluoride. However these other "greenhouse gases", despite having higher absorptive properties on a molecule for molecule basis, occur at much lower atmospheric concentrations than CO_2 and are generally considered less important on a global scale. In addition to the above, ozone formed in the troposphere from reactions involving man-made pollutants such as nitrogen oxides, volatile organic compounds and carbon monoxide, is also a greenhouse gas. Since both elevated [CO_2] and [O_3] are known to affect plant physiology directly and the entry point for both gases is via stomata, the recent increase in the number of publications reporting the impact of elevated atmospheric CO_2 concentrations in combination with enhanced ozone concentrations is not surprising (Barnes *et al.,* 1995; Lippert *et al.,* 1996 and 1997). Studies combining the impact of atmospheric pollutants and elevated [CO_2] are necessary in order to address the serious question of just how vegetation will respond to CO_2 fertilisation in combination with future pollution levels. This being a more realistic scenario, as increasing pollution and rising CO_2 concentrations are a thing of the present and likely to become an ever increasing problem for future generations.

Governments throughout the world are now seriously concerned about the anthropogenic enhancement of atmospheric CO_2 concentrations and the consequent impact this will have as a result of its greenhouse properties on future global climates. The British government aims to preserve and enhance sinks and reservoirs of carbon (forests and soils) and to secure an annual increase in the total stock of sinks (Climate Change, 1997). Tree cover in the UK has doubled this century to 2.4 million hectares (ha). This is largely the result of a range of incentive schemes for new planting which are currently increasing tree cover by 20,000 ha per annum. These include the farm woodland premium scheme, set out to encourage farmers to convert productive agricultural land to woodland and the woodland grant scheme,

operated by the Forestry Commission offering establishment grants for new woodland and management grants for existing woodlands.

Although such measures are essential and to be commended, much of our knowledge as to how future atmospheric CO_2 concentrations will directly affect woodland tree species and thus our potential carbon 'sinks and reservoirs' is uncertain. In a review of land-use and the carbon cycle, Houghton (1995) reported an uncertainty in the estimation of carbon fluxes between terrestrial ecosystems and the atmosphere, with figures ranging from a net source of 1.8 PgC y^{-1} to a net sink of 0.5 PgC y^{-1}. Because terrestrial ecosystems, atmospheric CO_2 concentrations and climate are closely coupled, any change in either the climate or [CO_2] of the atmosphere will result in changes in the structure and function of terrestrial ecosystems and thus their capacity to act as carbon stores or sinks.

Clearly the likely biological consequences for forests, both commercial and natural, and indeed for individual trees are two fold: firstly, the direct effect of rising CO_2 concentrations *per se*, i.e., the CO_2 fertilisation effect, and secondly the indirect effect via changes in regional climates, as a result of its longwave absorptive properties, i.e. climatic warming. Because of the central role of CO_2 in the physiology of plants (Eamus 1992) much attention has focused upon determining the responses of trees to [CO_2] enrichment. Trees are of particular importance because of their role in the global carbon economy, because of their importance as determinants of local climate and because of the economic, conservation, aesthetic and social importance of forests. The work presented here is focused primarily on the effects of changing atmospheric CO_2 concentrations on the phenology, growth and physiology of an important tree crop species in Britain, Sitka spruce (*Picea sitchensis* (Bong.) Carr.).

1.2 Fumigation Techniques

Since the early 1980's when the scientific community began seriously to focus on elevated [CO_2] issues, an ever increasing and sophisticated range of [CO_2] fumigation techniques using a host of facilities have developed. Much of the early CO_2 work on trees utilised facilities developed for horticultural or crop research, these being the areas in which prior research into [CO_2] fumigation had focused. Because of the complex form, longevity and sheer size of trees in comparison with crops, it is not surprising that some of these techniques have proved less than satisfactory. This stimulated the development and production of new fumigation techniques. The most common of these are outlined below along with their relative merits and drawbacks.

1.2.1 Growth chambers

This range of techniques developed to supply elevated [CO_2] to young trees includes glasshouses, poly tunnels both within and outside glasshouses and controlled growth cabinets. These facilities allow the researcher to fumigate with CO_2 under easily controlled conditions and at relatively low cost. In addition, in the case of controlled growth cabinets, all environmental conditions can be directly controlled i.e., light, temperature, humidity etc. thus allowing more precise measurement of direct [CO_2] effects and interpretation of experimental results. Such enclosure techniques also allow for the simultaneous combination of [CO_2] fumigation and gas flux measurements; that is the chambers can be used as large system cuvettes studying whole plant and soil exchanges. The most obvious disadvantages of these systems are their restricted size, lack of replication as cabinets are expensive to purchase, and the fact that plants are grown in pots within an artificial environment. In particular, growth cabinets are only suitable for short-term experiments, the reduced light environment being their biggest drawback, in terms of plant growth. Glasshouses

offer a potentially more spacious growth environment but tend to have much higher than ambient air temperatures and, to a somewhat lesser extent, poor lighting as well. These problems can be ameliorated by installation of expensive cooling systems and supplemental lighting.

1.2.2 Open top chambers

Open-top chambers, having been used for many years in the pollution field to study the impacts of rising SO_2, ozone and acid precipitation on crops and individual plants, were readily adapted for fumigation with elevated [CO_2]. Currently, this is the most widely used approach for 'long term' (i.e. years rather than months) CO_2 studies (Ceulemans and Mousseau, 1994). In general, environmental variables inside OTCs are similar to those of the surrounding area, with minimal interuption to solar radiation, rainfall and ambient atmospheric pollutants. Fowler *et al.* (1989) gave a value of 15 % for the interception of the short-wave solar radiation by the OTC frame and walls and a reduction in relative humidity between 5 and 13 %. In addition, despite large flow-rates, typically 2-6 complete volume air changes per minute, there can be a heating effect on hot sunny days resulting from the adsorptive properties of the wall structure and the chamber fan units, and this in turn influences water vapour pressure deficit. It has also been demonstrated that rainfall within the OTC may average only 45 % of that outside (Mandl and Kohut, 1990). It is therefore, essential when using such methods to establish an outside control plot, thus allowing determination of those responses attributable to the OTC *per se*. The CO_2 consumption rate of OTCs is high necessitating the need for bulky and expensive storage facilities. This in itself limits the use of OTCs to well-funded projects within fairly accessible regions. There is no question, however, that this method of [CO_2] fumigation is more preferable to those described above for long term studies. Their use within the CO_2 field has proven to be highly flexible with studies ranging from young pot grown-tree seedlings through to natural forest stands

planted or regenerated directly in the soil (Murray *et al.,* 1994; Durrant *et al.,* 1993; Laitat *et al.,* 1993).

1.2.3 Branch bags

The driving force toward the development of branch bags was principally that the phenology, physiology and morphology of seedlings differ compared to mature trees (Cregg *et al.,* 1989). This is the primary limitation when extrapolating results obtained using the techniques described so far which involve the use of young trees, because mature trees are by their very nature large and space within OTCs, glasshouses and growth cabinets is restricted. Branch bags have been used in three main [CO_2] studies (Teskey, *et al.,* 1991; Barton, *et al.,* 1993 and Dufrene *et al.,* 1993), essentially they consist of a solid framework completely enclosed with heavy duty polyethylene or acrylic plastic, through which CO_2 is injected. A complete branch on a mature tree growing in the field is completely enclosed within the bag. CO_2 control of this system is both simple and cheap allowing for readily affordable replication. The 'greenhouse' effect on temperature within such bags is similar to those experienced inside OTCs, being high on hot sunny days and falling to around zero on cooler / cloudy days and at night. They have proven a useful tool to investigate the response of trees to elevated [CO_2] where sinks are unlimited. The central assumption regarding the validity of the 'branch bag' technique is that each branch on a mature tree is autonomous with respect to carbon and water fluxes. This assumption is far from being accepted within the scientific community especially in the case of deciduous trees. In addition, to what extent do the responses of individual tree branches to elevated [CO_2] tell us about whole tree responses? Indeed are we any closer to understanding the impact of elevated [CO_2] on mature trees using branch responses as opposed to seedlings or young trees? These questions inevitably lead toward the next stage of facility development, free air carbon dioxide enrichment (FACE).

1.2.4 FACE systems

Because of their longevity and size, it has rarely been possible to study large mature trees and most investigations of tree responses to [CO_2] enrichment have up until now used small, young tree seedlings. However the FACE fumigation technique offers the opportunity to alter this trend by exposing entire tree stands at the ecosystem scale to elevated atmospheric CO_2 concentrations, without modifying their surrounding environment (Ellsworth *et al.* 1995). It involves a circular array of computer controlled / monitored CO_2 injection outlets positioned within the crop or tree stand (Lewin *et al.* 1994). The primary disadvantage of such a fumigation technique is its complexity and cost, both in terms of development and operation. However, as it represents the 'purest' method of investigating true responses of vegetation to elevated [CO_2], it certainly appears to be the way forward. Indeed, Eamus (1996) stated that on a ground area basis the costs incurred in running a FACE system is if anything lower than those of OTCs. However, in his comparison with OTCs Eamus (1996) did not take into account the true cost of running a replicated FACE experiment, replication of more than 2 FACE rings would be prohibitively expensive. As an indication of how rapidly this area of research has advanced it is interesting to observe comments made by Jarvis (1989), a mere eight years ago, when it was perceived as "impractical to expose areas of forests experimentally to increases in CO_2 concentration and if it were possible, there might well be ethical objections".

Although there is a great deal of support within the scientific community for FACE studies (Ceulemans and Mousseau, 1994; Eamus, 1996), this technique does have its limitations. Because of the inevitable costs incurred in such studies the number of locations where this type of facility can be established is limited. This in turn restricts the range of ecosystem types on which research into elevated [CO_2] impacts can be conducted. Therefore, research using a range of techniques is essential in

order to test as wide a range of scenarios and hypotheses as possible. No one experiment or experimental technique will elucidate the complex interaction between responses at the cellular, plant and ecosystem level to changes in global atmospheric CO_2 concentrations.

1.3 CO_2 effects

Up until fairly recently much of the 'CO_2' work was concentrated around improving crop yields inside greenhouses (Enoch and Kimball, 1986) or investigating the short-term impacts of transient changes in atmospheric CO_2 concentrations (Ludlow and Jarvis, 1971; Beadle *et al.*, 1979). Although results from these studies provided some insight into the possible responses of plants to future elevated CO_2 concentrations, clearly more specific longer term studies were essential. Since the early 1980s research in this field has mushroomed, culminating in more than 70 reviews, reporting on results obtained from many hundreds of individual studies conducted on and using almost as many different species and experimental protocols. To an extent this vast and diverse array of information has led to our current problems when trying to synthesise results and extract general trends. Despite these problems, of the many CO_2 reviews several have attempted to do just this, these include Kimball (1983*a*,*b*), Cure and Acock (1986), Mortensen (1987), Eamus and Jarvis (1989), Luxmoore *et al.* (1993), Mousseau and Saugier (1993), Poorter (1993), Idso and Idso (1994), and this is by no means a comprehensive list.

1.3.1 Growth responses

At its current atmospheric concentration, CO_2 limits the ability of C_3 species to fix carbon, therefore any increase in its concentration will tend to enhance the rate of assimilation, thus potentially stimulating plant growth and productivity. Early work investigating the effect of CO_2 concentration on field grown crop species clearly

established that elevated CO_2 concentrations almost always enhanced growth and hence plant production (Kramer, 1981; Kimball, 1983*b*). It has even been suggested that woody plant responses will be even more responsive to rising $[CO_2]$ levels compared with those found in herbaceous plants (Idso and Idso, 1994). Comprehensive reviews conducted by Idso (1992) and Idso and Idso (1994) reported results on dry mass accumulation which support this conclusion obtained from 342 peer-reviewed scientific journal papers. From experiments in which C_3 woody plants were exposed to elevated CO_2 concentrations for reasonable periods, it appears that photosynthesis, growth rate and biomass were all increased, e.g. *Fagus sylvatica* (Heath and Kersteins, 1997), *Liriodendron tulipifera* (Norby and O'Neill, 1991), *Quercus alba* (Norby *et al.,* 1986; Gunderson *et al.,* 1993), *Picea sitchensis* (Canham and McCavish, 1981; Townend, 1993), *Pinus contorta* (Higginbotham *et al.,* 1985), *Pinus taeda* (Rogers *et al.,* 1983), *Pinus radiata* (Hollinger, 1987; Conroy *et al.,* 1988). As always there are exceptions to this general rule with little or no growth stimulation being found for *Liquidamber styraciflua* and *Pinus taeda* (Tolley and Strain, 1984). However for the vast majority of C_3 woody species plant growth is estimated to be stimulated by some 41%, in comparison the estimation for C_4 and CAM species is 22% and 15%, respectively (Poorter, 1993).

Shifts in biomass partitioning have also been reported in a number of elevated $[CO_2]$ studies, showing that the proportion of carbon allocated to the roots was generally increased (Higginbotham *et al.,* 1985; Sionit *et al.,* 1985; Norby and O'Neill, 1991; Norby *et al.,* 1992).

1.3.2 Physiological responses

As observed in the many published response curves relating CO_2 assimilation (A) to internal CO_2 concentration (A/C_i) (Pettersson and McDonald, 1994), current CO_2 concentrations limit net photosynthetic rates. Because the principle enzyme involved

in C_3 photosynthesis is ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco) and both carbon dioxide and oxygen compete for sites, one might assume that increasing CO_2 concentration will favour carbon fixation over photorespiration. This would, therefore, result in a stimulation of net carbon fixation and hence net assimilation rate. Once again, although this is the case for the vast majority of studies (see review Ceulemans & Mousseau, 1994), this is not necessarily true in all cases or for all species (see recent review covering 41 tree species by Curtis, (1996)). Many studies reporting results from A/C_i analysis show a decline in maximum carboxylation capacity (V_{max}) of Rubisco after fumigation with elevated CO_2 concentrations (see reviews by Pettersson and McDonald, 1994 and Kerstiens *et al.,* 1995). This would indicate a decline in either, all or a combination of the amount, activity or kinetic properties of Rubisco (Atkinson, 1996). On average, the photosynthetic rate of tree species has been shown to be stimulated by 44% by a doubling of atmospheric CO_2 concentration (Gunderson and Wullschleger, 1994). However, it is still unclear as to whether this stimulation will be sustained after long term exposure to elevated [CO_2]. Once again central to this result is the enzyme Rubisco; declines in its activity after longer term exposure to elevated [CO_2] has been reported (Besford *et al.,* 1990; Bowes, 1991; Van Oosten *et al.,* 1992), but as yet there is no clear consensus on species specificity, timing, quantity or mechanism of the response of Rubisco to long term exposure.

Typically between one- and two thirds of the carbon fixed in photosynthesis is lost through utilisation via respiratory pathways (Amthor, 1991). Therefore, any changes in respiration rates may be as important as photosynthesis *per se,* in terms of the entire carbon budget of a woody plant growing in elevated [CO_2]. Reviews conducted by Poorter *et al.* (1992) and Amthor (1991) on 10 tree species show no clear impact of elevated [CO_2] on respiration. Respiration is usually divided into two components: growth respiration associated with the synthesis of new biomass and

maintenance respiration associated with the maintenance of the existing plant material (Amthor, 1984). In contrast to photorespiration, which occurs in the chloroplasts and peroxisomes, mitochondrial respiration (or 'dark respiration') provides energy for both growth and maintenance purposes through the oxidation of organic compounds. The discrepancies observed in the current literature, which show both enhancements (Hrubec *et al.*, 1985; Townend, 1993) and reductions (Bunce, 1990; Drake *et al.*, 1997) in dark respiration rates in response to elevated [CO_2], may be related to a poor understanding of growth and maintenance costs in woody species.

Any decline in stomatal conductance in elevated CO_2 concentration will reduce the rate at which water vapour is lost through transpiration of a leaf. This effect, along with any stimulatory influence of [CO_2] on net photosynthesis, has the potential to produce a beneficial increase in water-use efficiency (WUE) (Eamus and Jarvis, 1989; Jarvis, 1989). However, despite such optimistic predictions for future WUE, our understanding of the magnitude and effect, if any of elevated [CO_2] on stomatal conductance, plus the mechanisms involved is still very limited. Although it has generally been concluded that stomata will close to some degree in most species exposed to elevated [CO_2], with predicted reductions in stomatal conductance ranging between 30 and 40% (Atkinson, 1996), many exceptions occur. These exceptions tend to be prevalent in northern temperate coniferous tree species (Samuelson and Seiler, 1993; Thomas *et al.*, 1994; Eamus, 1996; Lippert *et al.*, 1996). However, stomatal insensitivity and in some cases increases in conductance in response to elevated [CO_2] have also been reported for deciduous species e.g. in *Liriodendron tulipifera* (Norby and O'Neill, 1991) and *Fagus sylvatica* (Heath, Kersteins, 1997; Dick, *et al.*, pers comm).

1.4 Nutrient interactions

Just as CO_2 concentration is limiting and important to plant productivity, so to is nutrient availability. Much of our current forestry is situated on inferior land both in terms of cultivation and climate. The dominant soils in such areas generally tend to be poor in mineral nutrients, with the predominate soil type being a peaty gley. Since the extent to which plants are able to respond to increasing CO_2 concentrations is as much a function of their ability to capture and utilise mineral resources as the CO_2 concentration *per se,* nutrient availability is a key issue in [CO_2] response studies.

According to the 'limiting factor' concept, the productivity of plants may be so closely tied to the availability of nutrients that elevated CO_2 concentrations may have only a minimal direct impact on plant growth (Kramer, 1981; Sinclair, 1992). The majority of studies on the effect of elevated [CO_2] on growth, biomass and photosynthesis have to-date been conducted on plants receiving adequate if not luxuriant nutritional supplies, in particular nitrogen (Townend, 1993; Kerstiens *et al.,* 1995; Heath and Kersteins, 1997, see review by Eamus, 1996). Despite the speculation that the potential of CO_2 concentration to enhance tree growth will be diminished by limited nutrient supplies, there is non the less evidence to the contrary. Available data indicate that the relative increase in tree growth brought about by elevated CO_2 concentration is about the same with and without nitrogen deficiency (see reviews Idso, 1992; Idso and Idso, 1994; Wullschleger *et al.,* 1995).

The common observation of a shift in dry mass allocation from shoots to roots often attributed to elevated [CO_2], is more likely a result of decreased tissue nitrogen concentrations in plants exposed to elevated [CO_2]. A reduction in foliar nitrogen concentrations in plants fumigated with elevated [CO_2] compared with ambient

$[CO_2]$ is an almost universal observation (see review by Mousseau and Saugier, 1993). Even in a study using steady-state nutrition in culture solution, total plant nitrogen (% dry mass) of young *Betula pendula* seedlings was lower in elevated $[CO_2]$ compared with ambient $[CO_2]$ treated plants (Pettersson *et al.*, 1993). These results have led to speculation that nutrient use efficiency (NUE) will increase in future higher atmospheric CO_2 concentrations. However, not all mineral nutrients will necessarily be diluted by increased growth in this way. It has been shown, for example, that phosphorus is usually taken up in proportion to plant growth (Conroy *et al.*, 1988; Thomas *et al.*, 1994; Conroy, 1992). Therefore, it is likely that interactions between CO_2 concentration, biomass production and nutrient concentration in plants will differ across the range of nutrients.

Identifying the way in which elevated $[CO_2]$, in conjunction with a range of nutrient regimes and mineral deficiencies, will affect overall plant growth is one of the principle challenges today.

1.5 Sitka spruce (*Picea sitchensis* (Bong.) Carr)

Since forests (i) possess the ability to sequester large quantities of carbon and therefore act as potential future sinks for increasing atmospheric CO_2 concentrations, and (ii) account for some 30% of the Earth's land surface and approximately 70% of terrestrial carbon assimilation, information concerning the response of trees and forests to elevated $[CO_2]$ is clearly of paramount importance.

This study was carried out on Sitka spruce because it is the most widely planted tree species in the UK (Forestry Commission, 1984), and also has one of the highest yields (Ford, 1982), and is therefore of primary importance to the forest industry. It has a typical C_3 photosynthetic pathway. As it is frequently grown on nutrient

poor upland soils a major factor currently limiting its productivity is insufficient amounts of one or more mineral nutrients (Chandler and Dale, 1993). However, in the future this may change, as increased government funding encourages the planting of forests on what was previously much richer agricultural land (Climate Change, 1997). If, as hypothesised, future increases in atmospheric CO_2 concentrations will interact with (i) the mineralisation rate (and thus the availability) of nutrients (Gifford, 1992), (ii) partitioning within and uptake rates of nutrients into trees and (iii) ultimately alter NUE, the results of experiments combining this species, elevated [CO_2] and nutrient availability will be of importance to both the scientific community and foresters alike.

Because of the massive investment required to investigate the growth of mature trees under elevated [CO_2] in the field (see 1.2.4 FACE systems) and the specificity of this study to one forest plantation crop, a smaller scale open-top chamber study was initiated (Plate 1.1). Juvenile Sitka spruce trees were used in all the experiments. Although this poses a limitation when trying to extrapolate observed responses to mature forest stands, it does provide valuable information as to the likely performance of young trees in the field in future elevated CO_2 concentrations. Since much of the cost incurred in plantation forestry occurs within the first five years of planting, e.g. in ground preparation, planting, weed and pest control, any changes in such procedures driven by [CO_2] responses will have important economic consequences, regardless of whether or not they are carried on into maturity.

1.6 Aims of the study

To test whether a doubling of present day atmospheric CO_2 concentration will be beneficial to the growth of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) in the future.

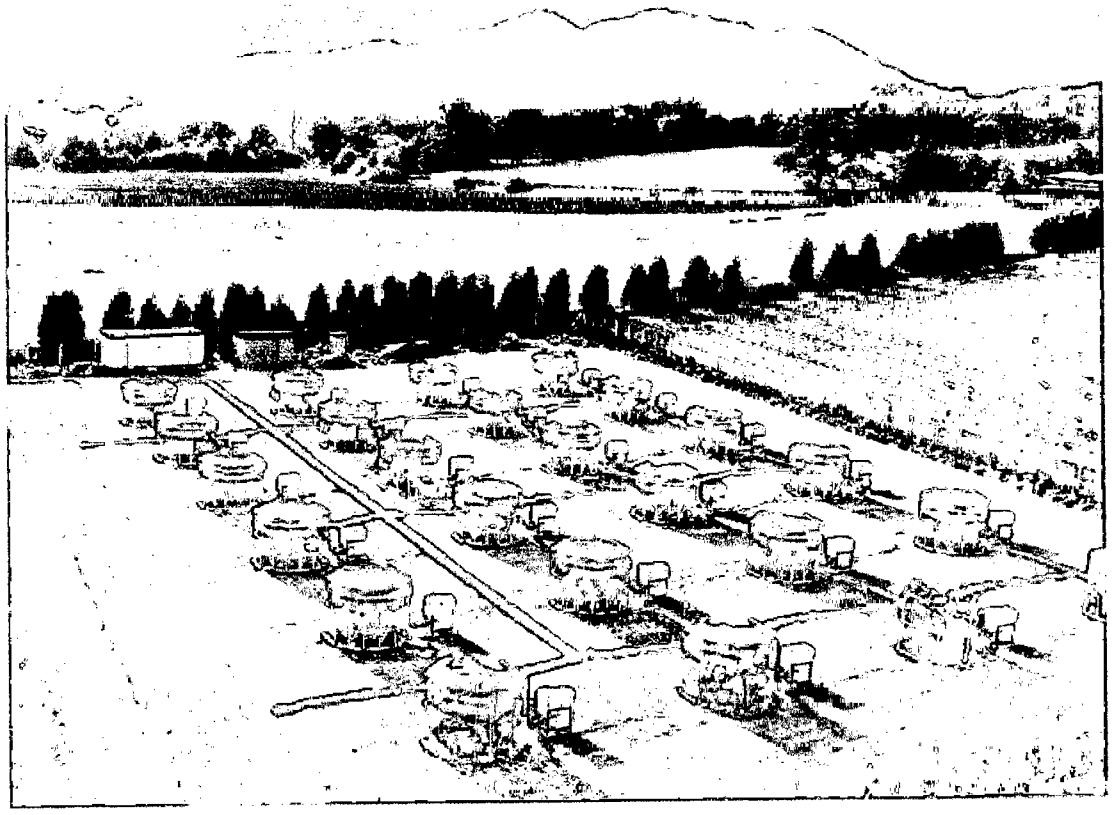

Plate 1.1: OTCs at the ITE, Bush field site.

1.6.1 Specific objectives

More specifically this study has the following aims:

(i) to study the impact of elevated [CO_2] on physiology, growth and biomass allocation of Sitka spruce and identify potential problems or exploitable opportunities for plantation forestry in the UK;

(ii) to identify differences between short and long-term impacts of exposure to elevated [CO_2] on growth and physiology;

(iii) to evaluated the effect of open-top chambers *per se* on physiology, growth and biomass allocation of Sitka spruce;

(iv) to study the relative importance of nutrient availability on growth and physiological impacts of elevated [CO_2]; and

(v) to study the impact of elevated [CO_2], plant nutrition and genetic variability on phenology of Sitka spruce and model the likely consequences of global climatic warming on budburst and frost damage.

1.6.2 Outline of the thesis

This thesis consists of seven chapters each of which covers a different aspect of Sitka spruces response to elevated atmospheric CO_2 concentration [CO_2] or its interaction with nutrient supply rate.

Chapter 1. Introduction

This chapter introduces the need for work on elevated [CO_2]. The problem itself and the problems associated with the various techniques currently available for elevated [CO_2] exposure. It shows the OTC facility used in this study and gives a brief introduction to Sitka spruce. It also outlines the main objectives of the study.

Chapter 2. The effect of long term CO_2 enrichment on the growth, biomass partitioning and mineral nutrition of Sitka spruce (*Picea sitchensis* (Bong.) Carr.)

This chapter presents the effects of elevated [CO_2] on growth, biomass and seedling nutrition over the course of a three year exposure experiment. It focuses on contrasting results obtained after various intervals of the experimental period. A comparison between chamber-grown seedlings and those grown outside in a control plot is also presented. This chapter has been published in *Trees* (1996) **10**, 393-402, see Appendix C.

Chapter 3. Assimilation and stomatal conductance responses of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) to elevated [CO_2] exposure

This chapter focuses on physiological responses to elevated [CO_2]. It presents the effects of elevated [CO_2] on photosynthesis, stomatal conductance and dark respiration after one and two years exposure. Effects of OTCs on seedling physiology are also presented.

Chapter 4. Effect of elevated [CO_2] and varying nutrient supply rate on the growth, biomass allocation and foliar mineral nutrition of Sitka spruce (*Picea sitchensis* (Bong.) Carr.)

This chapter presents the results of varying nutrient supply rates and elevated [CO_2] on seedling growth and biomass allocation after two years exposure to elevated [CO_2] and 12 months treatment with one of three nutrient supply rates. The effect of each nutrient treatment on foliar mineral contents and total carbon content are also presented.

Chapter 5. Effect of elevated [CO_2] and varying nutrient supply rates on gas exchange of Sitka spruce (*Picea sitchensis* (Bong.) Carr.)

This chapter presents physiological responses to elevated [CO_2] of seedlings at two contrasting nutrient supply rates. It focuses on photosynthetic acclimation in response to nutrient availability and the response of light-saturated net photosynthesis, stomatal conductance and chlorophyll content to foliar nitrogen concentrations.

Chapter 6. Effects of elevated [CO_2], nutrition and climatic warming on bud phenology in Sitka spruce (*Picea sitchensis*) and their impact on the risk of frost damage

This chapter focuses on phenological responses to elevated [CO_2]. It examines the impact of nutrient supply rate, experimental duration, OTCs and clonal variation on

the phenological responses of budburst and budset of Sitka spruce to elevated [CO_2]. The effects of elevated [CO_2] plus a 0, 2 or 4 °C climatic warming on the timing of bud burst and the subsequent risk of frost damage are assessed using a simulation model and meterological data. This chapter is published in *Tree Physiology* (1994) **14**, 691-706, see appendix B.

Chapter 7. Synthesis and conclusions

This chapter brings together the results from the long-term study and the nutrient experiment and summarises the main conclusions drawn from the experiments concerning seedling , growth, biomass allocation, phenology and physiology. It also attempts to draw conclusions in terms of Sitka spruces productivity in the future under nutrient poor and rich conditions.

CHAPTER 2

The effect of long term CO_2 enrichment on the growth, biomass partitioning and mineral nutrition of Sitka spruce [*Picea sitchensis* (Bong.) Carr.]

Abstract

Sitka spruce (*Picea sitchensis* (Bong.) Carr.) seedlings were grown for 3 years in an outside control plot or in ambient (~355 μmol mol^{-1}) or elevated (ambient + 355 μmol mol^{-1}) atmospheric [CO_2] environments, within open top chambers (OTCs) at the Institute of Terrestrial Ecology, Edinburgh.

Sequential harvests were carried out at the end of each growing season and throughout the 1991 growing season, five in all. Plants grown in elevated [CO_2] had, (i) 35 and 10% larger root/shoot ratios at the end of the first and third season, respectively; (ii) significantly higher summer leader extension relative growth rates, which declined more rapidly in early autumn than ambient grown plants; (iii) after three growing seasons a significantly increased mean annual relative growth rate; (iv) consistently lower foliar nutrient concentrations, and (v) after two growing seasons smaller total projected needle areas.

Plants grown inside OTCs were taller, heavier and had a smaller root/shoot ratio than those grown outside the chambers.

There was no effect of CO_2 concentration on Sitka spruce leaf characteristics, although leaf area ratio, specific leaf area and leaf weight ratio all fell throughout the course of the 3 year experiment.

Keywords: Elevated [CO_2], Sitka spruce, Growth, Allocation, Nutrients

2.1 Introduction

Carbon dioxide is the most abundant greenhouse gas currently being released into the atmosphere through anthropogenic processes. It is widely accepted that the atmospheric level of CO_2 has risen from a pre-industrial value around 280 μmol mol^{-1} in 1900, to a present day level of around 355 μmol mol^{-1} and is increasing at a rate of about 1.6 μmol mol^{-1} per annum (Keeling, 1993). Combustion of fossil fuels and the destruction of major terrestrial carbon pools, such as tropical rain forests, have been the major cause of this dramatic increase (Holdgate, 1993). Such activities are unlikely to cease in the foreseeable future and indeed, are more likely to continue at an increasing rate well into the next century, despite recent attempts to introduce policies directed at reducing both CO_2 emissions and the destruction of tropical rain forests. The consequence of this is an estimated increase in the global-mean atmospheric CO_2 concentration of roughly 150 μmol mol^{-1} by the end of the twenty-first century (Gates *et al.*, 1992). Although discrepancies exist between estimates of the rates at which [CO_2] is predicted to increase, there is little doubt that overall global-mean atmospheric CO_2 concentrations will increase dramatically throughout the next century and beyond.

Because of direct effects of atmospheric [CO_2] on plant photosynthesis and stomatal conductance, any rise in the atmospheric CO_2 concentration will directly affect the rate at which organic matter and plant nutrients are assimilated and internally cycled. Indirect effects of increased CO_2 concentrations will also affect plant competitiveness and survival, through altered photosynthate concentration, composition and translocation, growth rate, assimilate partitioning, growth form, reproduction, plant water status and plant tolerance to gaseous atmospheric pollutants (Acock and Allen, 1985). This is especially true for plants with the C_3 photosynthetic pathway; a recent review by Poorter (1993) found that for C_3 species,

plant growth was increased by 41%, compared to 22% for C_4 and 15% for CAM. In addition, C_3 species constitute 95% of the earth's plant species, and will therefore be an important component in the earth's carbon cycle (Idso and Idso, 1994).

The majority of published studies assessing the impact of rising CO_2 concentrations have been carried out on cereals, annual crop species or in the case of woody perennial species, over one or less than one growing season (that is on non-acclimated plants). Acclimation is especially important for a coniferous species such as Sitka spruce [*Picea sitchensis* (Bong.) Carr.], because its needle primordia are initiated during the previous growing season. In the present study, Sitka spruce seedlings were exposed to elevated [CO_2] for 3 years. Luxmoore *et al.* (1993), published a detailed evaluation of experimental results of elevated [CO_2] effects on forest tree species.

Tree crop species are an important group of C_3 plants to study, in terms of their economic value and the significant role they play in the global carbon balance (Ceulemans and Mousseau, 1994). Another important aspect of trees is their longevity, this inevitably increases their susceptibility to changing CO_2 concentrations. In addition, trees will only experience a few breeding cycles within the time scale predicted for such change. This will reduce their ability to adapt genetically to the rapidly changing CO_2 environment.

Sitka spruce is the most widely planted timber producing species on upland sites in the UK, yielding approximately 12 m^3 ha^{-1} $year^{-1}$(Milne, *et al.*, 1997). Despite this, it has received little attention within the CO_2 scientific community. Consequently, the effect of future CO_2 concentrations on Sitka spruce is unknown.

Currently Sitka spruce tree breeders, selecting for wood yield, pick trees with a rapidly growing habit. This has generally established a lasting benefit on total yield per hectare and hence productivity of harvestable timber. However changes in allocation of biomass between plant components in elevated [CO_2] is frequently reported and appears to be highly species specific (Brown and Higginbotham, 1986; Norby and O'Neill, 1991; Idso and Idso, 1994; Rouhier *et al.*, 1994). Therefore, in order to understand and predict the possible effect of future CO_2 concentrations on productivity, possible changes in selection criteria, and thus ultimate economic value of this important timber species, it is necessary to evaluate the long term impact of elevated [CO_2] on Sitka spruce directly.

A long term study to evaluate the likely impact of a doubling of atmospheric [CO_2] on the biomass and nutrient partitioning of Sitka spruce, over several growing seasons, was initiated in open top chambers (OTCs) at the Institute of Terrestrial Ecology, Edinburgh.

2.2 Materials and methods

2.2.1 Plant material

In June 1990, 2000 unflushed 1+1 bare-rooted Sitka spruce seedlings [Forestry Commission identity number 83(2015)S LOT2, provenance 20, origin Queen Charlotte Island] were taken from a cold store and potted into 2.0 dm^3 pots using a composite soil. The soil consisted of sphagnum peat, 5 mm quartz and sterilised loam in the ratio 13:4:3 by volume. Vitax Q_4 fertiliser (N:P:K 5.3:7.5:10) was added at 4 g dm^3 of compost to the soil and thoroughly mixed.

The plants were then randomised, 250 selected per chamber, and evenly distributed between 10 randomised blocks within each of eight OTCs (2000 seedlings in total).

In March 1991, to avoid the plants becoming pot bound, 30 from each of the eight chambers were repotted into 4.5 dm^3 pots using the above composite mix. An additional 30 plants were randomly selected from each of the [CO_2] treatments, repotted and placed in two additional chambers, increasing the replicate number of chambers per treatment to five. At the start of the growing season the plants were top dressed with 5.5 g of a slow release fertiliser (Osmocote mini, 5-6 months formulation; composition: 18% N, 6% P_2O_5, 11% K_2O, 2% MgO, and trace elements; Grace-Sierra, Nottingham, UK). In March 1992, plants from each of the 10 chambers were repotted into 18.0 dm^3 pots using the same composite soil as before, top dressed with 22 g of Osmocote mini, and returned to their respective chambers. The plants were watered by capillary matting during 1990 and 1991, and because of increased pot size and hence soil volume, by trickle irrigation in 1992.

2.2.2 OTCs and [CO_2] treatment

Eight octagonal OTCs (Waytogrow Greenhouses Ltd., Essex, UK), were used in 1990. Four of the OTCs received ambient [CO_2] (~355 μmol mol^{-1}) and four received elevated [CO_2] (~700 μmol mol^{-1}). In March 1991, the number of OTCs was increased to ten, giving five replicates per [CO_2] treatment during 1991 and 1992. Each chamber was 2.7 m high with a floor area of 7.0 m^2, constructed from an octagonal aluminium frame with standard 3 mm horticultural glass side panels. For a more detailed description of the chambers and their properties see Fowler *et al.* (1989).

Ambient air was supplied to each chamber by individual fan units (EK31, Radial and Axial, Herts, UK). Prior to injection into all chambers, the ambient air was passed through a series of ten impregnated, activated charcoal filters to remove ozone, sulphur dioxide and nitrogen dioxide (Emcel filters, Machine control, Sussex, UK). The ambient [CO_2] chambers then received this air directly via a polyethylene

manifold (400 mm layflat tubing, McKinnon and Hay, Edinburgh, UK), 1.5 m above ground level. The CO_2 concentration in these chambers fluctuated diurnally around a mean daily value of 355 μmol mol^{-1}. The elevated [CO_2] chambers received air which was supplemented with pure CO_2 to raise the ambient concentration by 355 μmol mol^{-1}, i.e. to double the average present day concentration. The pure, liquid CO_2 was stored on site in a 6 ton tank (Distillers MG, Lanarkshire, UK). The CO_2 was vaporised and passed through individual mass flow controllers [FC28, Tylan General (UK) Ltd., Wilts., UK] driven by an FC288 control box [Tylan General (UK) Ltd., Wilts., UK]. The vaporised CO_2 was fed directly into the ambient air stream within the chamber fan units at a pre-set flow rate, where it was mixed thoroughly before being released into the chambers. The CO_2 concentration inside the elevated chambers varied around 700 ± 80 μmol mol^{-1}, depending on the ambient concentration and external windspeeds which affected ambient air incursion through the open top.

2.2.3 [CO_2] monitoring system

The PC controlled monitoring system consisted of an interface card (ADC42, Blue Chip Technology), a relay box, 2-way solenoid valves, infra-red gas analyser and control software. A diaphragm pump (B100-DE, Charles Austen Pumps, Surrey, UK) drew air continuously from all of the elevated [CO_2] chambers and one of the ambient [CO_2] chambers, through 4-mm-internal diameter nylon sample lines (Phase Separations, Clwyd, UK) to the monitoring cabin. Each sample line contained a 2-way solenoid valve which allowed the air stream to be vented to waste, or when activated, diverted to an infra-red gas analyser (IRGA; SB-300, The Analytical Development Co., Hoddesdon, UK). The air sample was drawn through the IRGA at a constant rate by an internal pump. The software program cycled through the air samples from each chamber in turn, allowing a 60 s period of purging through the IRGA followed by a 60 s period of recording; the average recorded CO_2 concentration over this period was then stored on hard disk.

2.2.4 Growth analysis

At the start of the experiment (June 1990), 15 plants were randomly selected and destructively harvested. The dry mass was determined separately for roots, needles and shoots. In subsequent harvests the plant material was further subdivided into current and previous years stem and branch wood and needles. These harvests consisted of random samples of 30, 5, 5, 15 and 5 plants from each of the chambers in January 1991, June 1991, August 1991, February 1992 and February 1993, respectively. Outside control plants were included in the February 1992 and 1993 harvests. At each harvest, plant height, root collar diameter and leaf area were also measured. Projected needle area was determined using an image analysis system (IIR, Digithurst, Royston, UK). Needles were placed on a light box to increase edge definition, and black and white video images digitised at 512 x 512 pixel resolution. Threshold settings for binary imaging were determined prior to measurements, using calibration standards. Leaf area ratio (total projected needle area/total plant mass, $cm^2\ g^{-1}$), specific leaf area (projected needle area/needle mass, $cm^2\ g^{-1}$) and leaf mass ratio (leaf mass/total plant mass), were calculated for the June 1991, August 1991, February 1992 and February 1993 harvests.

Throughout the 1990 and 1992 growing seasons, measurements of weekly leader extension were made on all chamber grown plants, and in 1992 on the additional outside control plot. Weekly relative extension rates (R_w) were calculated from (Hunt, 1978):

$$R_w = \frac{lnL_2 - lnL_1}{T_2 - T_1} \qquad (1)$$

where L_1 and L_2 are leader length (mm) at times T_1 and T_2 (days), previous weeks and current weeks measurement, respectively. Since this form of sampling was not destructive, consecutive measurements were made on the same plants and pairing

was not necessary. At the start of the experiment plants of similar size were paired in order to provide statistical estimates of error in mean annual relative growth rates of dry mass (R_{dm}). R_{dm} was calculated for the time intervals between each end of season harvest, using Eq. 1, where L_1 and L_2 was substituted with M_1 and M_2 (total plant dry mass) at times t_1 and t_2.

2.2.5 Nutrient analysis

In August 1991, February 1992 and February 1993, amounts of nitrogen (N), phosphorus (P) and potassium (K) were measured in the roots and both current and previous years needles and wood removed from the plant stem and branches (i.e. nine tissue classes). In January 1991 and June 1991 samples from stem tissue were not taken, so that there were only five tissue classes. Each plant tissue class was individually bulked by chamber, sub-sampled then ground using a mill (Wiley - DCFH48, Glen Creston, Stanmore, UK) to less than 0.8 mm, in preparation for the above analysis. An Aliquot of the ground material was redried in an air-circulated oven at 105 °C for 3 h, and 350 mg of the oven-dry sample was digested by a modified Kjeldahl procedure in the presence of H_2O_2, with Li_2SO_4 to increase boiling point and Se as catalyst (Parkinson and Allen, 1975). Concentrations of N and P were measured by continuous flow colorimetry (Skalar Analytical) via indolephenol blue, and molybdenum blue respectively, and K was measured by flame emission spectrometry (Corning Flame photometer 430). Total carbon (C) was measured on all nine tissue types from the February 1993 harvest, using elemental analysis (Carlo Erba Strumentazione, Mod 1106, Fison Instruments, Sussex, UK). Samples were prepared for C analysis by initially grinding using a Wiley mill (type DCFH48, Glen Creston, Stanmore, UK) to less than 0.8 mm then ball milling to a fine particle size.

2.2.6 Statistical analyses

Differences in dry mass and nutrient content among treatments and harvests for each

tissue class were tested by analysis of variance. A randomised split plot design was used with chamber as the main plot and plants the subplots. Fumigation with or without additional CO_2 was the treatment. The analysis of variance was performed using Genstat 5 software (Rothamsted Experimental Station, Harpendon, Herts, UK). Regression analysis was performed on root and shoot data using Sigmaplot (Jandel Scientific, Germany).

2.3 Results

2.3.1 Effects of elevated [CO_2] on growth and biomass production

The initial (June 1990) and final (February 1993) values of growth parameters of Sitka spruce are given in Table 2.1. After 3 years there was no significant treatment difference between total dry mass, root collar or root mass/shoot mass ratio. However, plants grown inside the OTCs were significantly taller than those grown outside.

Table 2.1: Effects of CO_2 concentration and open top chamber on growth parameters of 5-year-old Sitka spruce [*Picea sitchensis* (Bong.) Carr.] after three growing seasons (June 1990 to February 1993). Values within each row followed by a different letter indicate a significant difference ($p < 0.05$, n=5). Initial harvest values are means ± 1 SEM, n=15.

Parameter	Initial harvest (June 1990)	Final harvest (February 1993)		
		OTC and ambient [CO_2]	OTC and elevated [CO_2]	Outside and ambient [CO_2]
Dry mass (g)	6.3 ± 1.1	792	828	718
Height (mm)	274 ± 11	1874a	1790a	1496b
Root collar diameter (mm)	4.0 ± 0.26	31.3	32.0	28.6
Root mass/shoot mass	0.40 ± 0.03	0.31	0.34	0.44

There was no significant effect of elevated [CO_2] on the total biomass of Sitka spruce plants raised in OTCs by the end of the 3 year period (Figure 2.1a). Total biomass differences observed between the two [CO_2] treatments in August 1991, may be attributed to variations in mean weekly relative growth rates (R_w), (Figure 2.1b). During the first experimental season (1990), the R_w was similar in both treatments (Figure 2.1b). This was probably because of the predetermined nature of Sitka's growth pattern and the early stage in the experiment. By mid-summer R_w peaked in both treatments, but was significantly ($p < 0.05$) higher in the elevated [CO_2]

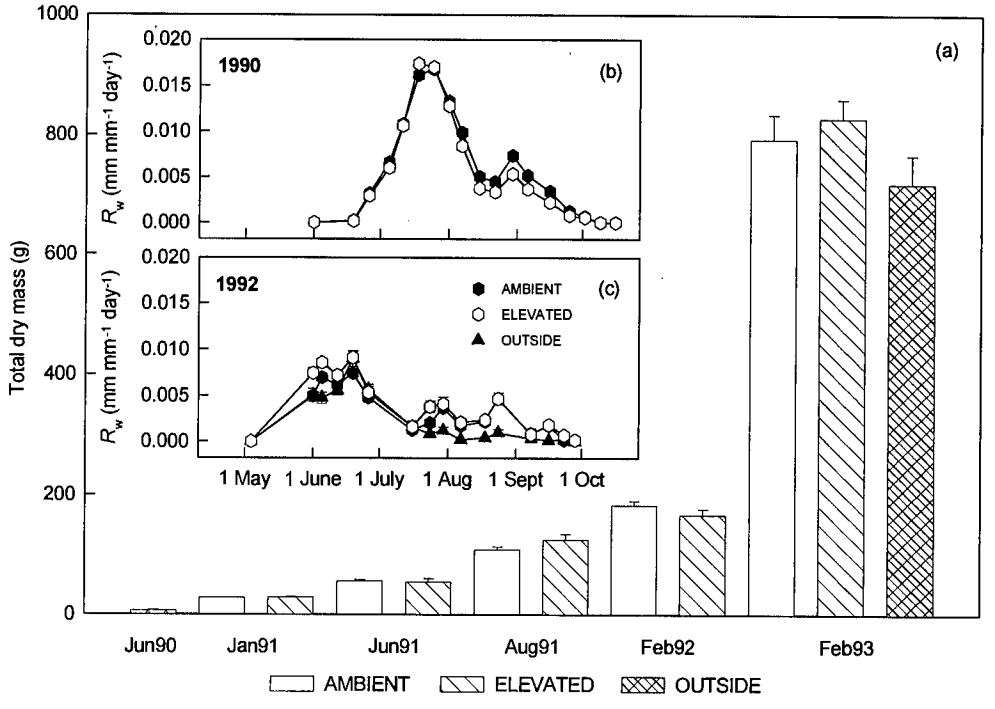

Figure 2.1: Effects of OTC and CO_2 concentration on (a) total biomass produced over three growing seasons, (b) weekly relative growth rates of leader extension (R_w) in 1990 and (c) 1992. For this and subsequent figures Ambient = chamber grown, ambient [CO_2] (~355 μmol mol^{-1}) treated plants, Elevated = chamber grown, elevated [CO_2] (~700 μmol mol^{-1}) treated plants, and Outside = plants growing outside chambers under ambient [CO_2] (~355 μmol mol^{-1}). Values are means ± 1 SEM, n=5).

treatment compared with the ambient [CO_2] chamber treatment. Thereafter the R_w of elevated [CO_2] treated plants declined more rapidly, becoming significantly lower during late summer and early autumn ($p < 0.01$). This accounts for the loss of growth enhancement found in the elevated [CO_2] plants between August 1991 and February 1992. On 6 June 1992, after two full growing seasons in their respective treatments, the elevated [CO_2] plants were growing significantly faster than either the ambient CO_2 chamber or outside control plants ($p < 0.01$ and 0.001 respectively).

Mean annual relative growth rates of woody biomass (R_{dm}) did not differ between treatments during the first 2 years but were significantly higher ($p < 0.05$) in plants

Table 2.2: The mean annual relative growth rate of dry mass (R_{dm}, % day^{-1}) for chamber grown ambient and elevated [CO_2] treated plants in 1990, 1991 and 1992 ± 1 SEM. Values within each column followed by a different letter indicate a significant difference ($p \leq 0.05$, n=5).

CO_2 treatment	1990	1991	1992
Ambient [CO_2]	0.47 ± 0.02	0.51 ± 0.02	0.42 ± 0.006a
Elevated [CO_2]	0.47 ± 0.02	0.49 ± 0.009	0.45 ± 0.01b

receiving elevated CO_2 compared to ambient CO_2 during the 3rd year, 1992 (Table 2.2). There was an initial increase in R_{dm} from 1990 to 1991 followed by a decrease in 1992 for all treatments.

2.3.2 Partitioning of plant biomass

In January 1991, after one growing season in either elevated or ambient CO_2, Sitka spruce seedlings showed significant treatment effects on plant biomass distribution (Figure 2.2a,b). Elevated CO_2 had no effect on shoot biomass (January 1991, 17.4 vs 18.1 g. $p < 0.4$), but significantly enhanced root biomass (January 1991, 11.2 vs 9.3 g, $p < 0.01$). Root dry mass was significantly larger in elevated CO_2 treated plants compared with ambient CO_2 treated plants, on all harvest dates. The effect of

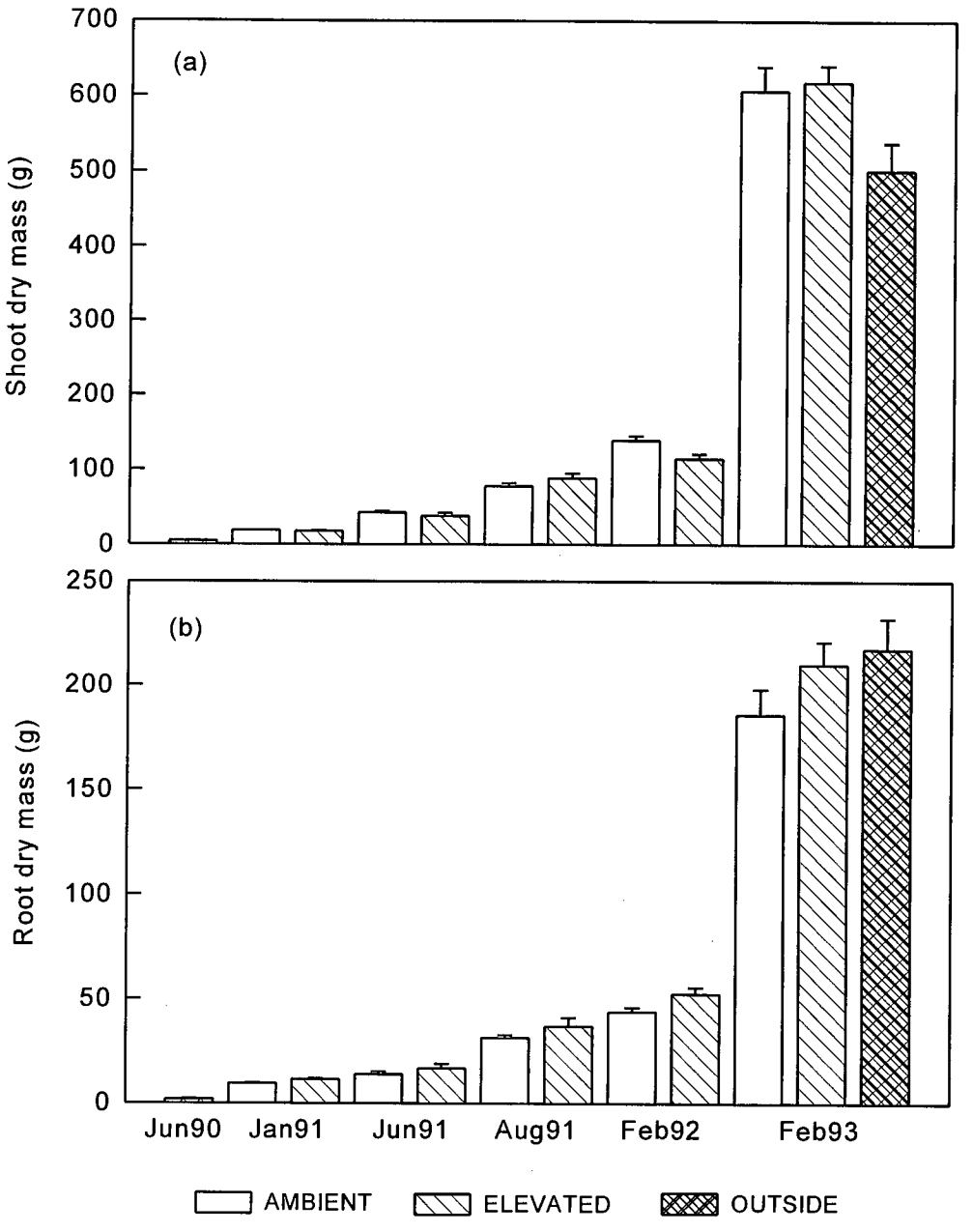

Figure 2.2: Effects of OTC and CO_2 concentration on (a) shoot total dry mass and (b) root total dry mass at each harvest, from June 1990 to February 1993 inclusive. Values are means ± 1 SEM, n=5.

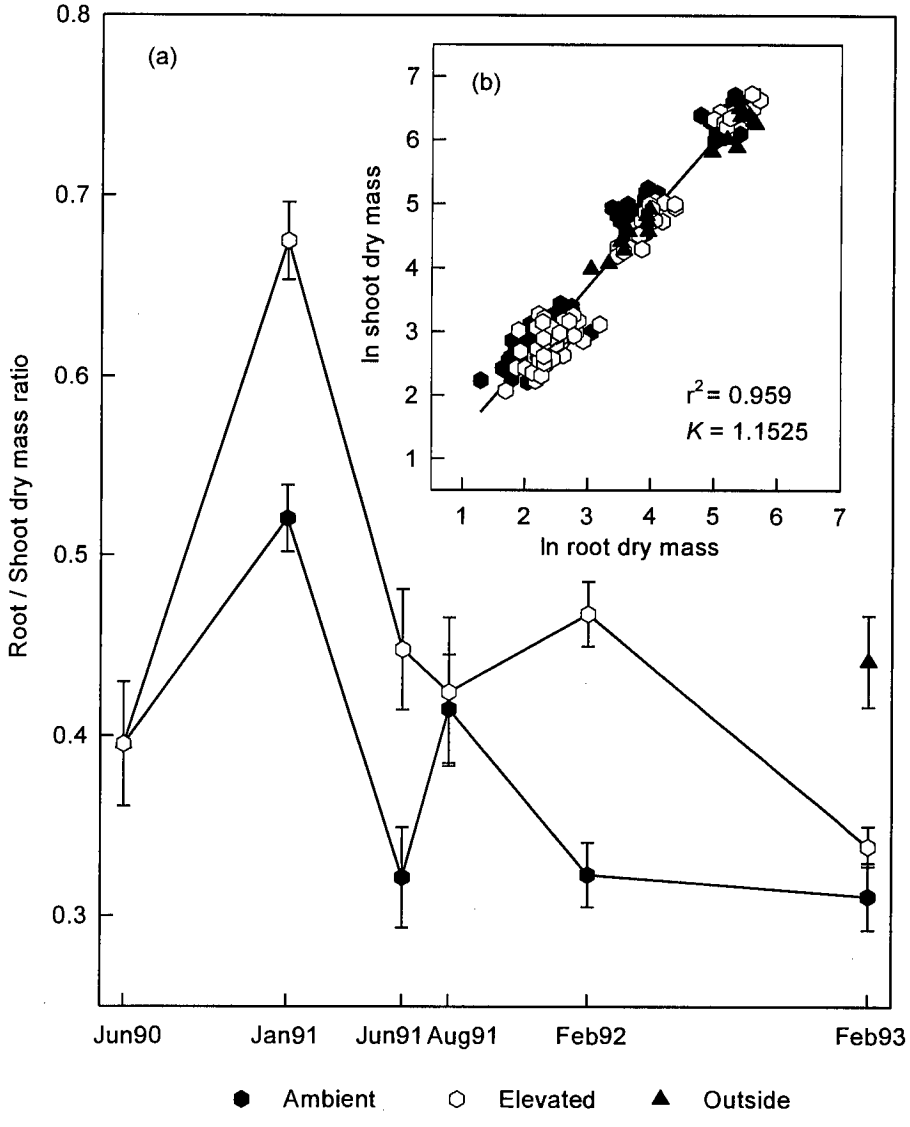

Figure 2.3: (a) Changes in the root/shoot dry mass ratio from June 1990 to February 1993, under ambient and elevated [CO_2]; values are means ± 1 SEM, n=5. Values for the outside control plot are included in 1993. (b) The linear relationship between ln root and ln shoot dry mass at each end-of-season harvest (January 1991, February 1992 and February 1993). (*K* = correlation coefficient).

Table 2.3: Effects of OTC and CO_2 concentration on total wood biomass and partitioning between stem and branch dry mass at each harvest, from June 1990 to February 1993, inclusive. (A = ambient $[CO_2]$, E = elevated $[CO]_2$ and O = outside control. Values are means ± 1 SEM. Values within each row followed by a different letter indicate a significant difference; ($p \leq 0.05$, n=5).

Harvest date	Jan 1991		Jun 1991		Aug 1991		Feb 1992		Feb 1993		
Treatment	A	E	A	E	A	E	A	E	A	E	O
Tissue Type (g)											
Wood dry mass	9.2±0.3	8.6±0.3	19.3±0.8	16.4±1.7	44±2.9	50±3.9	87±4.3a	67±3.7b	391±21a	409±19a	306±28b
Stem dry mass	6.3±0.2	5.8±0.2	13.0±0.5	11.6±1.1	30±1.8	34±3.0	62±2.8a	47±2.7b	246±14a	256±12a	187±19b
Branch dry mass	2.9±0.2	2.8±0.1	6.3±0.5	4.8±0.8	14±1.4	16±1.5	25±2.1a	20±1.6b	145±11a	154±10a	119±11b

[CO_2] on shoot partitioning was time dependent; the shoot dry mass of elevated [CO_2] plants was smaller than that of ambient [CO_2] plants in January 1991, June 1991 and February 1992, but larger in August 1991 and February 1993 ($p \leq 0.4$, 0.47, 0.011, 0.2 and 0.78, respectively).

Carbon allocated to the woody biomass was unaffected by elevated [CO_2] in the 1st and 3rd years, but was significantly decreased in the 2nd year ($p < 0.01$; Table 2.3). There was little or no effect of elevated [CO_2] on the allocation of wood between stem and branches. OTCs significantly increased the woody biomass of both stems and branches ($p < 0.05$). The overall root mass/shoot mass ratio (R/S) of plants raised in both ambient and elevated [CO_2] declined with time (Figure 2.3a). Initially R/S ratios were significantly increased by elevated [CO_2], but by the end of the third growing season, the effect of elevated [CO_2] on R/S had disappeared. The R/S of plants growing outside was significantly higher than either of the two chamber treatments.

The allometric relationship between root and shoot dry mass was not significantly affected by elevated [CO_2] throughout the 3 year experiment (Figure 2.3b). There was nevertheless, an effect of chamber (though not significant on root/shoot allometry; the slope of the relation between In shoot mass and In root mass (K) was increased for chamber grown plants ($K = 1.16$, $r^2 = 0.96$; $K = 1.20$, $r^2 = 0.97$ and $K = 0.98$, $r^2 = 0.97$, for ambient elevated and outside treatments, respectively).

A steady increase in the wood mass/needle mass ratio occurred throughout the experiment in all treatments. There was no overall effect of [CO_2] concentration on the wood/needle ratio, but outside control plants had a significantly lower ratio than chamber grown plants ($p < 0.01$), (Figure 2.4).

2.3.3 Effects of elevated [CO_2] on leaf characteristics

By the end of the second and third growing seasons total projected needle area was significantly smaller in Sitka spruce plants treated with elevated [CO_2] compared to plants grown in ambient [CO_2] ($p < 0.05$, Table 2.4). This decrease in total needle area was attributed to a reduction in the needle area of current year foliage (< 1-year-old) in elevated [CO_2] (Table 2.4). There was no significant effect of elevated [CO_2] on the area of previous years needles (> 1-year-old), (Table 2.4). Again, the effect of elevated [CO_2] on needle area was time dependent: samples taken in June 1991 showed no significant effect of treatment. There was also an effect of OTC on both current and previous year needles ($p < 0.05$ and n.s., respectively). Plants growing inside chambers had larger needle areas than those growing outside in the control plots.

Table 2.4: Leaf properties of Sitka spruce grown inside OTCs after 2 (FEB 1992) and 3 (FEB 1993) years of fumigation with ambient or elevated [CO_2], and grown outside in ambient air after two growing seasons. (Current = needles <1 year old, previous = needles >1 year old and total = current and previous years needles bulked. Values within each row followed by a different letter indicate a significant difference ($p \leq 0.05$, n=5).

	FEBRUARY 1992			FEBRUARY 1993		
	Ambient	Elevated	Outside	Ambient	Elevated	Outside
Needle area (m^2)						
Total	24.8 ± 1.1a	21.2 ± 1.8b	-	84.8 ± 4.7a	76.1 ± 3.2b	70.7 ± 5.2b
Current	18.9 ± 1.0a	14.6 ± 1.2b	-	66.9 ± 3.7a	58.6 ± 2.4b	54.9 ± 4.8b
Previous	5.9 ± 0.4	6.6 ± 0.6	-	17.9 ± 1.5	17.5 ± 1.8	15.8 ± 0.8
Specific leaf area						
($cm^2\ g^{-1}$)	48.9 ± 2.2a	46.0 ± 2.3a	56.0 ± 1.3b	39.8 ± 1.0	36.4 ± 0.7	36.1 ± 1.7
Total	46.4 ± 1.2a	41.3 ± 1.0b	55.6 ± 3.7c	40.9 ± 1.3a	36.8 ± 0.8b	37.3 ± 2.0c
Current	58.7 ± 3.2	61.1 ± 4.1	57.7 ± 2.5	35.7 ± 1.0	35.0 ± 1.1	32.6 ± 1.3
Previous						
Leaf area ratio						
($cm^2\ g^{-1}$)	13.8 ± 0.6a	12.6 ± 0.6a	17.7 ± 0.7b	10.8 ± 0.4	9.3 ± 0.4	9.8 ± 0.3
Leaf mass ratio	0.28 ± 0.01a	0.28 ± 0.01a	0.32 ± 0.01b	0.27 ± 0.01	0.25 ± 0.01	0.28 ± 0.01

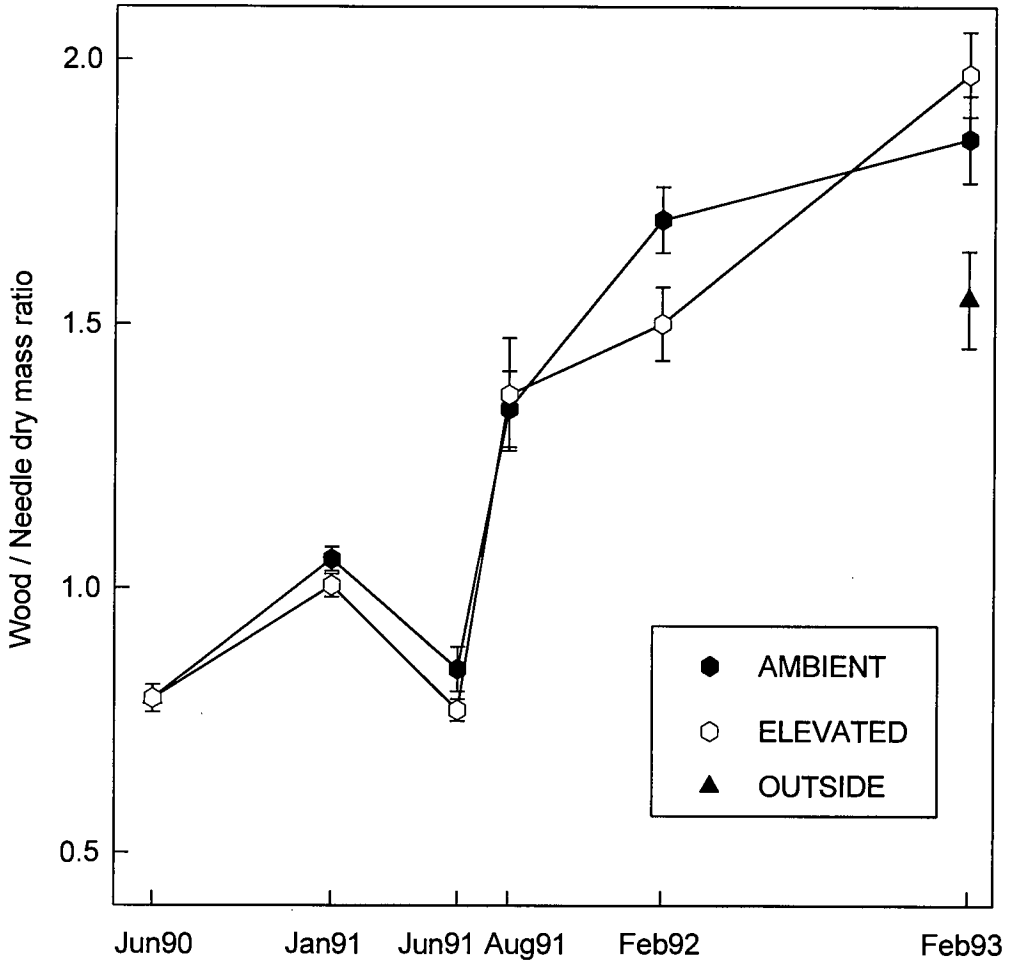

Figure 2.4: Changes in the wood/needle dry mass ratio over three growing seasons in either ambient or elevated [CO_2], or outside in control plot (last growing season only), (means ± 1 SEM, n=5).

Values of leaf area ratio (LAR), specific leaf area (SLA) and leaf mass ratio (LMR) fell significantly throughout the experiment (Table 2.4). Leaf area ratios and specific leaf areas of chamber grown plants subjected to elevated [CO_2] were slightly lower than those receiving ambient [CO_2] by the end of the 1991 and 1992 seasons, though not significantly so ($p > 0.05$; February 1992 and 1993 harvests, Table 2.4). Neither CO_2 concentration nor chamber affected the LMR of Sitka spruce (Table 2.4). By

the end of the experiment no chamber effect was found on LAR, SLA or LMR. When the SLA was examined separately for current (< 1-year-old needles) and previous (> 1-year-old needles) year foliage, an effect of elevated [CO_2] was found on current year needles in February 1992 and 1993 (Table 2.4). Plants receiving elevated [CO_2] had lower SLA than ambient [CO_2] treated plants ($p < 0.05$ and $p > 0.05$ for 1992 and 1993, respectively). There was no treatment effect on the characteristics of the previous years foliage.

2.3.4 Effects of elevated [CO_2] on plant nutrition

Foliar nitrogen concentration was consistently lower in the chamber grown, elevated CO_2 treated plants compared to chamber grown, ambient [CO_2] treated plants (Table 2.5). Current year branch foliar N concentrations were significantly lower in the January 1991 and February 1992 end of season harvests. However, there was no significant difference in the N content of any plant component by the end of the experiment (February 1993), (Table 2.5). Foliar N concentration was highest in needles from current-year branches and lowest in needles from previous-year stem and branch wood. There was no statistically significant chamber effect on N concentration after plants had been growing outside for two full seasons.

Neither CO_2 concentration nor OTC had much effect on either root, wood or foliar concentrations of phosphorus or potassium throughout the duration of the 3 year experiment. After three growing seasons (February 1993 harvest), foliar nutrient concentrations (% oven dried mass) of phosphorus and potassium were 0.19 and 0.77, 0.18 and 0.74, and 0.17 and 0.74, for ambient [CO_2], elevated [CO_2] and ambient [CO_2]-outside plants, respectively. The P concentration in wood tissue from current-year branches, was significantly lower in elevated [CO_2] plants than chamber grown ambient [CO_2] plants, during August 1991 and February 1993 (0.2 and 0.22,

respectively, $p < 0.05$). Despite this result there was no consistent pattern between CO_2 treatment and phosphorus values.

Table 2.5: Effects of CO_2 concentration and OTC on tissue nitrogen concentrations (% of dry mass) of Sitka spruce [*Picea sitchensis* (Bong.) Carr.] for each harvest. (A = ambient [CO_2], E = elevated [CO_2] and O = outside control. Values are means with significant differences indicated by a different letter within each row for each harvest date. $p \leq 0.05$, n=5)

Harvest date	January 1991		June 1991		August 1991		February 1992			February 1993		
Treatment	A	E	A	E	A	E	A	E	O	A	E	O
Tissue type (g)												
Root	1.1a	0.9b	1.2a	1.0b	1.1	1.0	1.5	1.2	1.9	1.6	1.4	1.2
Current Year												
Stem												
Needles					1.9a	1.4b	1.8a	1.5b	2.1a	1.6	1.4	1.5
Wood					0.9	0.6	1.0	0.9	1.3	1.3	1.1	1.1
Branch												
Needles	1.8a	1.4b	1.5	1.4	1.7	1.4	1.9a	1.5b	2.1a	1.7	1.6	1.6
Wood	0.9	0.8	1.1	1.3	0.9a	0.7b	1.2	1.0	1.5	1.2	1.1	1.1
Previous Year												
Stem												
Needles					1.5a	1.2b	1.5a	1.3b	0.8c	1.2	1.1	1.1
Wood					0.5	0.5	0.5	0.5	0.4	0.6	0.5	0.6
Branch												
Needles	1.6a	1.4b	1.1	1.2	1.4	1.4	1.6	1.4	1.0	1.4	1.3	1.2
Wood	0.5	0.5	0.9	0.7	0.6	0.6	0.7	0.7	0.5	0.7	0.6	0.7

The total amount of nitrogen present in elevated [CO_2] treated plants was consistently lower than chamber grown, ambient [CO_2] treated plants ($p < 0.05$, < 0.05 and > 0.05 for the January 1991, February 1992 and February 1993 harvests, respectively), (Figure 2.5). Plants growing outside in the control plots also had a lower total nitrogen content compared with chamber grown ambient [CO_2] treated plants, though this was the result of the smaller amount of biomass present and not the nitrogen concentration *per se*. By the end of the third growing season, over 30%

of total plant nitrogen was held in the foliage of branches less than 1 year old (Figure 2.6). At the end of each growing season there was a significantly smaller

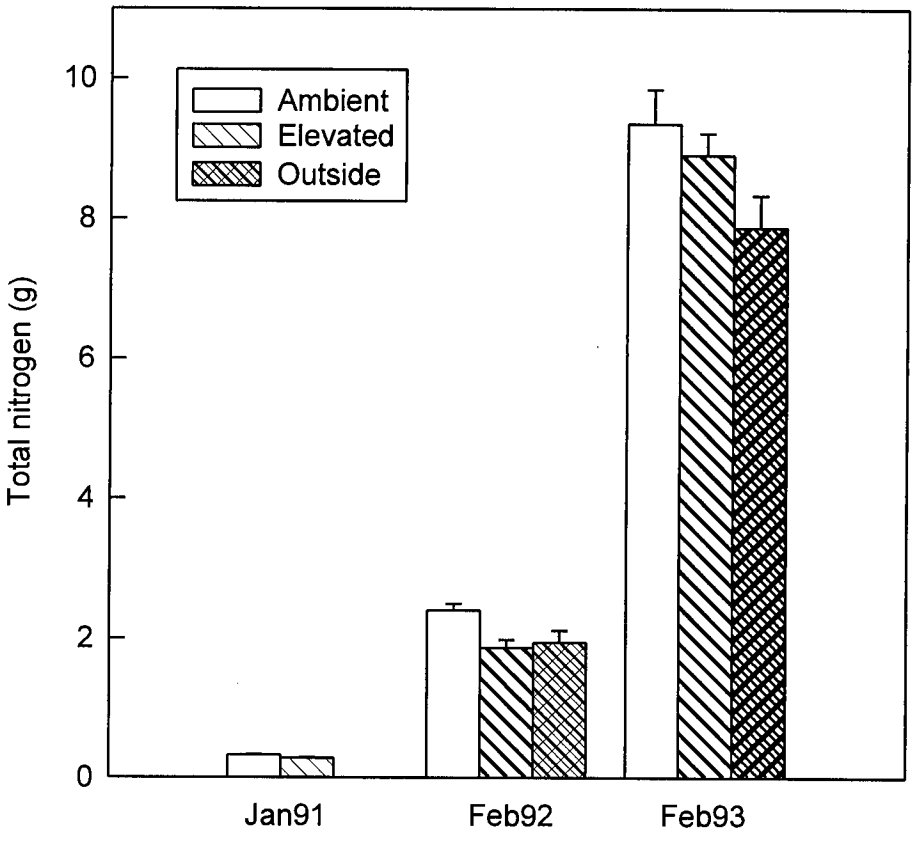

Figure 2.5: Effects of OTC and CO_2 concentration on the mean total amount of nitrogen present per plant ± 1 SEM, n=5, at the end of each growing season.

amount of nitrogen held within needles on current year branches in elevated CO_2 compared with ambient [CO_2] plants ($p < 0.05$). The second largest sink for nitrogen was stem wood (including bark). There was a significant reduction in the total

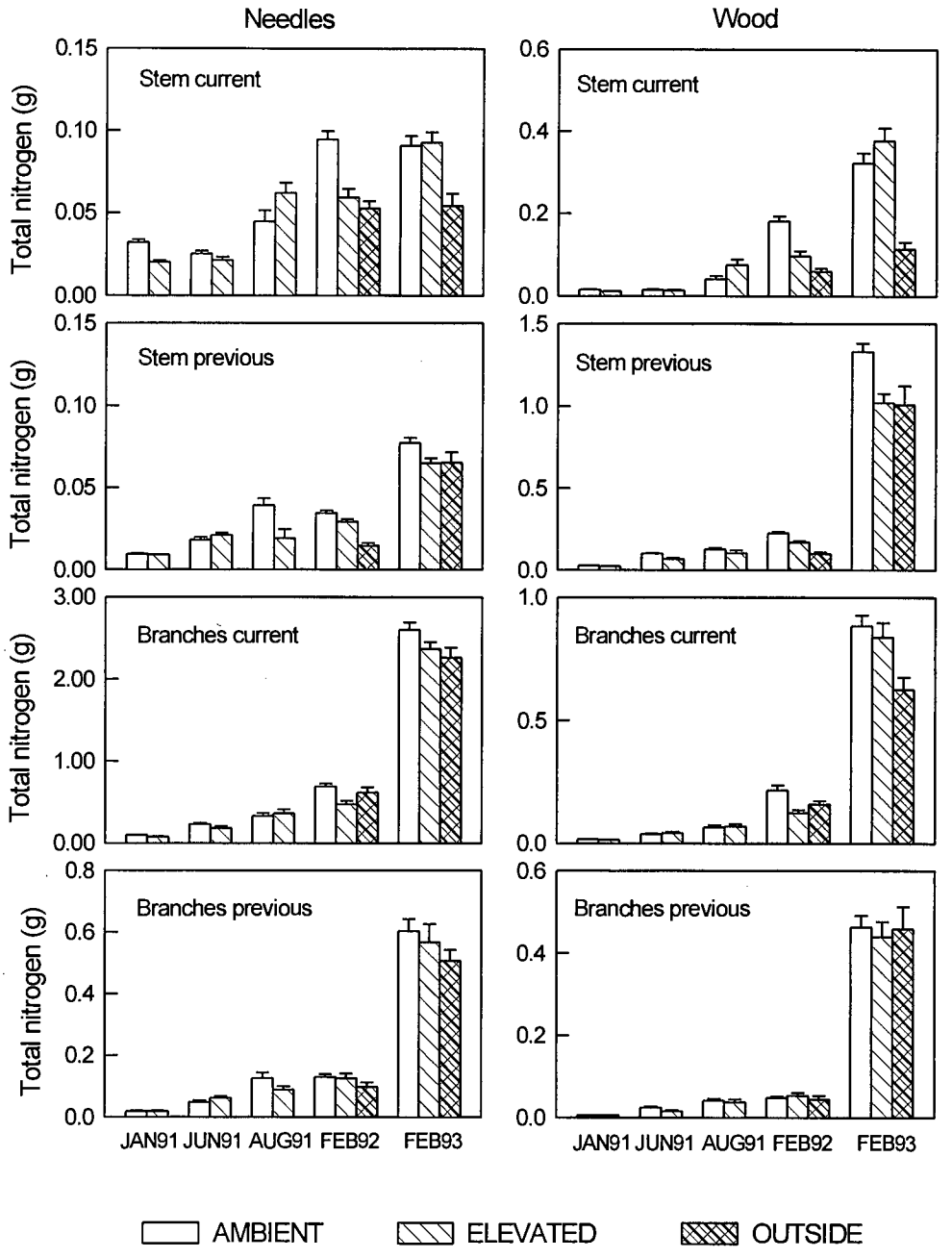

Figure 2.6: Total amount of nitrogen partitioned between the needles (*left hand column of graphs*) and wood (*right hand column of graphs*), of current (< 1 year old) and previous year (> 1 year old) stem and branches for each harvest. Values are means ± 1 SEM, n=5.

amount of nitrogen present in the stem wood (current + previous stem tissue) of elevated [CO_2] treated plants compared with ambient CO_2 treated plants ($p < 0.01$, Figure 2.6). Significant differences in the total amount of nitrogen held within tissue classes between chamber grown ambient [CO_2] plants and plants growing in the outside control plot were largely attributable to differences in plant biomass rather than to tissue nitrogen concentration. There was no significant effect of elevated [CO_2] or chamber on the partitioning of nitrogen between plant sinks.

The total carbon mass/nitrogen mass ratio at the end of the 3 year experiment was unaffected by elevated [CO_2]. It was reduced in current year foliage and roots of plants growing in OTCs compared with those growing outside in the control plot.

2.4 Discussion

2.4.1 Biomass accumulation

Changes in biomass accumulation and allocation are frequently reported for many C_3 species grown in elevated concentrations of atmospheric CO_2 (Tolley and Strain, 1984; Johnsen, 1992; Pettersson *et al.*, 1993; Silvola and Ahlholm, 1993). It is therefore logical to expect an increase in net primary productivity (NPP) of Sitka spruce under conditions of enhanced atmospheric [CO_2]. However after 3 years fumigation with 700 μmol mol^{-1} CO_2, no increase in total plant height or biomass (g, dry mass) was observed. Although elevated [CO_2] increased the root/shoot in this study by 35% in the first season, there was no significant change in the functional relationship between plant parts by the end of the third growing season. These results demonstrate that an enhanced level of CO_2 substrate may not necessarily result in an ultimate increase in NPP and hence greater timber yields for Sitka spruce.

2.4.2 Environmental influences

The concentration of atmospheric CO_2 is just one of many environmental variables that control plant partitioning and productivity, e.g. local climate, soil and plant nutritional levels, competition and solar radiation (Bazzaz and Miao, 1993; Rogers and Runion, 1994). The balance between above-ground (harvestable timber) and below-ground plant components is as likely to be a reflection on the limitations of such environmental variables as elevated atmospheric CO_2 concentrations. Under conditions where photosynthesis is the limiting process, then carbon is preferentially partitioned to the shoots, but if either nitrogen or water are limiting growth then carbon is preferentially partitioned to the roots (Robinson, 1986; Conroy, 1992; Proe and Millard, 1994).

2.4.3 Experimental protocol

Consequently in many cases, the experimental protocol and research techniques applied within the study, i.e. nutrient regimes, watering techniques and use of OTCs, directly affect plant growth and physiology. In fact, their impact may be as great if not greater than that of the [CO_2] treatment itself. Our results demonstrate a strong influence of OTC on the growth pattern of Sitka spruce. Both shoot dry mass and plant height increased significantly in response to chamber, with the result that plants grown inside chambers had a lower root/shoot ratio compared with those grown outside. This response was probably because air temperatures inside OTCs were higher on days with high solar radiation, (see Murray *et al.*, 1994 for a fuller explanation). Great care should therefore be taken when attempting to extrapolate elevated [CO_2] results for purposes such as model validation or future scenario predictions.

Differences that exist amongst experimental studies reporting responses of plants to elevated [CO_2] compared with those in ambient [CO_2], most probably result from

differences in nutrient and water availability, light quality (especially in experiments carried out in growth chambers) and pot size (Zak *et al.*, 1993). Arp (1991) suggested that restricted available sinks for carbohydrates, such as are found with pot-bound plants which have a restricted rooting volume, may contribute to changes in photosynthetic capacity and thus affect ultimate productivity. This variable alone may account for many of the conflicting experimental results, a suggestion supported by Townend (1993), who estimated that a rooting volume of 8 dm^3 was necessary for 3-year-old Sitka spruce seedlings, to ensure pot size was not confounding the experimental results. The plants in this study were repotted annually in order to avoid becoming pot bound. Though inadequate pot volumes can have a negative effect on growth stimulation of some tree species in elevated [CO_2], this may not be true of all species. Kerstiens and Hawes (1994) concluded from their study on young cherry saplings, that there was no evidence of rooting volume reducing the stimulation of growth in elevated [CO_2].

2.4.4 Interaction with N availability

Enhanced root production and smaller projected needle areas in elevated [CO_2] found in this and many other studies, may be the result of what is known as the "dilution or fertilisation" effect. Increased concentrations of atmospheric CO_2 may result in an increased demand by the trees for water and nutrients. Norby *et al.* (1992) found that increased photosynthesis was not accompanied by significant increases in leaf area or growth. Instead, the turnover of fine roots increased and leaf production decreased. Our findings are similar to these results and are typical responses of plants in which nutrition is limiting growth. Nutritional requirements are complex; increasing nutrient uptake via enhanced production and rapid turnover of fine roots may result in increased release and loss of nutrients from the soil rooting zone, a problem especially relevant for experiments carried out on potted plants. Zak *et al.* (1993) found a significantly larger pool of respired C in the

rhizosphere of *Populus grandidentata* plants grown in elevated atmospheric [CO_2]. This suggests that respired C increases in response to greater root growth in elevated [CO_2] and may result from enhanced root mortality, exudation, or cortical cell sloughing. The rate of nitrogen mineralisation was also shown to be higher in the elevated [CO_2] soil, probably because of enhanced microbial populations and activity in the rhizosphere.

Our results show a decline in the concentration of nitrogen, on a unit dry mass of tissue basis, in elevated [CO_2] plants compared with ambient [CO_2] plants (1.6 vs 1.7 % foliar dry mass, after 3 years fumigation). This result is consistent with a number of other studies in which nutrient conditions were considered adequate although not luxuriant, for ambient [CO_2]. Sitka spruce is the most commonly planted tree species on nutrient-poor upland soils in the UK (Chandler and Dale, 1990). We imposed a nutrient regime simulating likely field conditions to obtain results that could be extrapolated to the field. The importance of plant nutrition can be seen by comparing our results on Sitka spruce with those observed by Townend (1993). Where we found no significant increase in total biomass produced in elevated [CO_2]. Townend (1993) reported enhanced root, shoot and total growth in elevated [CO_2]. His plants were supplied with un-limiting nutrients, resulting in foliar N concentrations of 2.9%, a concentration unlikely ever to be achieved in field conditions. Nitrogen concentrations reported here are by no means symptoms of acute deficiency. Optimum N foliar concentrations in plantations are generally in the range 1.2-2.0% (Everard, 1973; Binns *et al.*, 1980).

2.4.5 Experimental duration

The results presented in this paper clearly demonstrate the importance of long term experimental studies, which allow plants to "acclimate" for several growing seasons. Had we presented our results after just 1 year of fumigation, elevated [CO_2] would

have been shown to significantly increase root/shoot ratios, decrease shoot biomass and have no effect on mean annual relative growth rates and needle areas. Similar results to these can be found in many recent publications (Brown and Higginbotham, 1986; Campagna and Margolis, 1989; El Kohen *et al.*, 1992; Zak *et al.*, 1993). However, after 3 years of fumigation the results are different. Root enhancement of elevated [CO_2] treated plants was reduced to 10%. There was no significant effect of elevated [CO_2] on the allometric relationship between roots and shoots. Also, plants growing in elevated [CO_2] had significantly higher mean relative growth rates and significantly reduced current-year needle areas compared to the ambient [CO_2] plants. These results are again consistent with the findings in a number of other studies (e.g. Eamus and Jarvis, 1989; Norby and O'Neill, 1991; Pettersson and McDonald, 1992; Townend, 1993).

In addition to the annual variation in our results, seasonal differences in biomass partitioning were observed. This was probably because of effects of elevated [CO_2] on the timing of bud phenology (Murray *et al.*, 1994). The late summer harvest of August 1991 showed that the plants in elevated [CO_2] were at that time larger than those in ambient [CO_2]; however by the winter (February 1992) this result had been reversed. Murray *et al.* (1994) showed that Sitka spruce raised in elevated [CO_2] set bud earlier in the autumn than Sitka spruce grown in ambient [CO_2]. Thus the shoot sink for fixed carbon is reduced earlier in the season in elevated [CO_2], allowing the still photosynthetically active plants to allocate more carbon to the roots. This alters the root/shoot ratio and changes the carbon budget of plants raised in elevated [CO_2] compared with plants growing in ambient [CO_2] conditions.

2.5 Conclusion

In conclusion, elevated [CO_2] may not necessarily enhance productivity. With a low nutrient supply, an increase in the root/shoot ratio may occur. If this is not accompanied by an overall increase in net carbon gain, above-ground harvestable timber production will be reduced. Despite such a possibility, valuable gains to the timber industry may be achieved on exposed nutrient poor sites, where increased root production may both enhance nutrient availability, and hence timber production, and increase wind stability. After 3 years of fumigation with nutritional conditions producing ~1.7% foliage nitrogen concentrations, we found no evidence of an increase in total plant biomass or shift in stem/branch ratio.

2.6 Summary conclusion

The results from this study concerning changes in the response to elevated [CO_2] over several growing seasons are of particular importance as are those attributed to growth in OTCs *per se*.

- After three growing seasons in elevated [CO_2] there was no significant increase in seedling biomass, but R/S was increased 35 and 10 % after one and three growing seasons, respectively. Although elevated [CO_2] had no effect on the allometric relationship between root and shoot dry mass. Throughout the experimental period there was no significant effect of elevated [CO_2] on needle parameters, although SLA, LMR and LAR were lower at the end than the start of the experiment. Annual relative growth rate was significantly higher in elevated [CO_2] at the end of the third growing season.

- Foliar nitrogen concentration was consistently lower in seedlings grown in elevated [CO_2] throughout the experiment, but there was no effect of [CO_2] on potassium or phosphorus concentration.
- Growth inside OTCs increased seedling biomass by 10.3 %. Weekly leader extension rates were higher for seedlings grown inside rather than outside OTCs, resulting in taller, heavier seedlings. Seedlings grown inside OTCs had a significantly lower R/S ratio than their counterparts grown outside.
- Growth inside OTCs had no significant effect on foliar nutrient concentrations, although total seedling nitrogen content was significantly smaller, as a result of less seedling biomass.

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CHAPTER 3

Assimilation and stomatal conductance responses of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) to elevated [CO_2] exposure.

Abstract

Sitka spruce (*Picea sitchensis* (Bong.) Carr.) seedlings were grown for two years in open-top chambers in either ambient (355 μmol mol^{-1}) or elevated (700 μmol mol^{-1}) atmospheric CO_2 concentrations or outside in an ambient [CO_2] control plot.

The photosynthetic responses of current year foliage (< 1 year old) to varying photosynthetic photon flux densities (0 to 1600 μmol m^{-2} s^{-1}) and CO_2 concentrations (40 to 850 μmol CO_2 mol^{-1}) were determined after 14 and 26 months exposure in their respective treatments. The elevated [CO_2] treatment 'directly' increased maximum photosynthetic rates and dark respiration rates but had no effect on stomatal conductance. There was no effect of long-term exposure to elevated [CO_2] on carboxylation efficiency but some evidence of enhanced electron transport rates. There was no 'indirect' evidence of 'down-regulation' of the photosynthetic response to elevated [CO_2] in either year.

There was no significant effect of open-top chamber on the PPFD response curve or maximum assimilation rate. However there was a highly significant effect of open-top chamber on the stomatal response curve to photon flux density, chamber grown plants had a higher g_s than those grown in the outside control plot.

Keywords: *elevated [CO_2], open-top chambers, photosynthesis, stomatal conductance, Dark respiration, Picea sitchensis*

3.1 Introduction

The present day global atmospheric CO_2 concentration is *ca.* 355 μmol mol^{-1}, and steadily increasing at an average annual rate of *ca* 1.6 μmol mol^{-1} (Keeling, 1993). The likely consequences for trees and forests of this increase in atmospheric CO_2 concentration fall into two categories, firstly the direct effects of [CO_2] *per se* on associated biological processes and hence plant productivity and secondly, the indirect effects of the increase in [CO_2] on regional climates. This study concentrates on the former, examining the impact of elevated [CO_2] on the photosynthetic mechanism of Sitka spruce during the second and third year of exposure to *ca.* 700 μmol mol^{-1}, an atmospheric CO_2 concentration that is likely to be achieved before the end of the next (21st) century.

An elevated atmospheric CO_2 concentration potentially allows plants to increase carbon fixation rate, reduce water loss by stomatal regulation, and possibly increase the efficiency of nitrogen use by reallocating nitrogen from ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco), all of which bode well for the future. However, despite such optimistic physiological predictions many studies examining the direct impact of elevated [CO_2] on plant productivity have reported less than expected stimulation in total plant biomass (Ceulemans and Mousseau, 1994; Murray *et al.*, 1996; Wullschleger *et al.*, 1995). Clearly further work is required in this field of research, as our current level of knowledge and understanding of how photosynthetic mechanisms in trees will respond to long term elevation of atmospheric CO_2 concentrations is, to say the least, scant and controversial. Even more uncertain is how CO_2-induced changes in photosynthetic capacity will affect growth and ultimately C accumulation.

A doubling of the present day atmospheric CO_2 concentration is likely to increase net photosynthetic rates (*A*) in the vast majority, if not all, C_3 tree species (Mousseau and Saugier, 1992; Luxmoore *et al.*, 1993; Ceulemans and Mousseau, 1994; Wullshleger *et al.*, 1995). However the extent to which *A* will be affected is likely to be both species and site specific. Recent studies on the influence of elevated CO_2 concentrations upon photosynthesis on the whole, report some degree of enhancement (see review by Luxmoore *et al.*, 1993). The exact amount of this enhancement is somewhat variable, with levels ranging from 3 % (Rouhier *et al.*, 1994) to 200 % in *Pinus eldarica* (Garcia *et al.*, 1994) stimulation in assimilation rate, for a doubling of CO_2 concentration.

Several studies have shown that after a period of growth (> 1-year) in elevated [CO_2] the initial increase observed in photosynthesis is lowered, i.e. down regulation of photosynthetic capacity occurs (e.g. Mousseau and Saugier, 1992; Gunderson and Wullschleger, 1994). This observation has been associated with a loss of activity and amount of Rubisco (Besford *et al.*, 1990; Wilkins *et al.*, 1994), mainly in studies where the experimental plant material was pot grown (Arp, 1991; Sage, 1994). In such conditions it is possible that the photosynthetic capacity is reduced as a result of end product inhibition; that is the enhanced supply of carbohydrates has exceeded the sink capacity of the plants, because the plants are pot bound or limited by some other environmental variable such as nutrient supply. When sink activity is reduced experimentally, by excision of all sinks from the source leaf, carbohydrates have been shown to accumulate rapidly in the source leaf, causing the abundance of *rbc*S transcripts, derived from the nuclear gene-family coding for the small subunit of Rubisco to decrease. Down regulation of Rubisco in this manner is accelerated in elevated [CO_2] (van Oosten and Besford, 1994).

Forests accumulate large amounts of carbon (100-500 t ha^{-1}), taking up twice that throughout the duration of their lifetime (Jarvis, 1989). They also represent 76 % of the worlds terrestrial biomass and 37 % of its bioproductivity (Ceulemans and Saugier, 1991), and thus constitute an important component in the global carbon budget. Therefore, it is essential to understand and quantify the long-term mechanisms controlling assimilation of CO_2 in trees in relation to the rising global atmospheric CO_2 concentration. This study, using open-top chambers, aims to estimate the impact which elevated [CO_2] will have on the photosynthetic process over the longer term (> two years) for an important C_3 tree 'crop' species, Sitka spruce (*Picea sitchensis* (Bong.) Carr.). The results will be separated into direct and indirect effects of aerial CO_2 concentration; direct effects are a result of the CO_2 concentration at the time of measurement, while indirect effects result from the CO_2 concentration the plants were grown in. In addition a comparison is made between the response in assimilation rates observed after 14 and 26 months exposure to elevated [CO_2].

3.2 Materials and Methods

3.2.1 Plant material

In June 1990, 2000 unflushed, 1+1 bare-rooted, Sitka spruce seedlings (Forestry Commission identity number 83(2015)S LOT2, provenance 20, origin Queen Charlotte Island) were taken from a cold store and potted into 2.0 dm^3 pots using a composite soil. The soil consisted of sphagnum peat, 5 mm quartz and sterilized loam in the ratio 13:4:3 by volume. A commercial fertiliser (Vitax Q4, N:P:K, 5.3:7.5:10, Vitax Ltd., Lancashire, UK) was added at 4 g dm^3 of compost to the soil and thoroughly mixed using a cement mixer. The plants were then randomised, 250 selected per chamber, and evenly distributed between 10 randomised blocks within each of eight open top chambers (2000 seedlings in total).

In March 1991, 30 from each of the eight chambers were repotted into 4.5 dm^3 pots using the above composite mix. An additional 30 plants were randomly selected from each of the CO_2 treatments, repotted and placed in two additional chambers, increasing the replicate number of chambers per treatment to five. In order to avoid end product inhibition of the photosynthetic process, in March 1992, 15 plants from each of the 10 chambers were repotted into 18.0 dm^3 pots using the same composite soil as before, top dressed with 22 g of fertiliser (Osmocote mini, N:P:K, 18:6:11, Osmocote Ltd, UK) and returned to their respective chambers. The plants were watered by capillary matting during 1990 and 1991, and because of increased pot size and hence soil volume, by trickle irrigation in 1992.

3.2.2 Open-top chambers and CO_2 treatments

Eight octagonal open-top chambers (OTCs) (Waytogrow Greenhouses Ltd, Essex, UK), were used in 1990. Four of the OTCs received ambient [CO_2] (*ca.* 355 μmol mol^{-1}) and four received elevated [CO_2] (*ca.* 700 μmol mol^{-1}). Each chamber was 2.7 m high with a floor area of 7.0 m^2, constructed from an octagonal aluminium frame with standard 3 mm horticultural glass side panels. For a more detailed description of the chambers and their properties see Fowler *et al.* (1989).

Ambient air was supplied to each chamber by individual fan units (EK31, Radial and Axial, Herts, UK). Prior to injection into all chambers, the ambient air was passed through a series of 10 impregnated, activated charcoal filters to remove ozone, sulphur dioxide and nitrogen dioxide (Emcel filters, Machine control, Sussex, UK). The ambient [CO_2] chambers then received this air directly via a polyethylene manifold (400 mm layflat tubing, McKinnon and Hay, Edinburgh, UK), 1.5 m above ground level. The elevated [CO_2] chambers received air which was supplemented with pure CO_2 to raise the ambient concentration by 355 μmol mol^{-1}, i.e. to approximately double the average present day concentration. The CO_2 concentration

inside the elevated chambers varied around 700 ± 80 μmol mol^{-1}, depending on the ambient concentration (which itself fluctuated diurnally around 355 ± 15 μmol $mol^{-1}CO_2$) and external windspeeds which affected ambient air incursion through the open top. For a full description of the CO_2 supply and monitoring system see Murray *et al.* (1994).

3.2.3 Laboratory gas exchange studies

During the summers of 1991 and 1992 rates of shoot photosynthesis (*A*), stomatal conductance (g_s), and transpiration (*E*), as a function of photosynthetic photon flux density (PPFD) and intercellular leaf CO_2 concentration (C_i) were measured using an open gas analysis system based on the one described by Jarvis and Ĉatský (1971), see below for full description.

Light response curves (*A/I*) were measured between 26 July and 24 August 1991 and 28 July to 7 September 1992 on a terminal shoot from one of the upper whorls of branches of each of five (1991) or six (1992) replicates from both the ambient and elevated CO_2, chamber grown Sitka spruce. In 1992 six replicates from the outside control plot were also measured. In 1991 photosynthesis was measured across a PPFD range of 0 and 800 μmol m^{-2} s^{-1}, and at treatment CO_2 concentrations only i.e., ambient [CO_2] plants were measured at 355 μmol mol^{-1} and elevated at 700 μmol mol^{-1}. In 1992 PPFD ranged between 0 and 1600 μmol m^{-2} s^{-1} and ambient [CO_2], elevated [CO_2] and outside treatments were measured at both CO_2 concentrations. All light response measurements started at a PPFD of 0 μmol m^{-2} s^{-1}.

Between 2 August and 8 September 1992 **CO_2 response curves** (A/C_i) were measured across a range of external CO_2 concentrations (C_a) between 40 and 850 μmol mol^{-1}, at PPFD saturation (1200 μmol m^{-2} s^{-1}). In order to avoid stomatal perturbations all CO_2 response measurements started at the growth CO_2

concentration, stepped down to the CO_2 compensation concentration (assumed to be *ca* 40 μmol mol^{-1}) and returned to their growth CO_2 concentration until assimilation equalled that at the start of the experiment, before rising in steps to 850 μmol mol^{-1}.

Throughout the sampling period three plants were brought into the laboratory on the evening prior to measurement and a terminal shoot from one of the upper whorl of branches was sealed in one of three purpose built hollow-floored, water-cooled leaf cuvettes. Dark respiration rates (R_m) were measured the following morning before switching on the lights. A/I and A/C_i responses were measured on the same shoot of each tree on consecutive days. A minimum of 40 minutes equilibration time was allowed at each PPFD or CO_2 concentration prior to recording the measurement.

The leaf cuvette dimensions were 180 mm x 80 mm x 95 mm. The side walls were constructed of glass and the top, bottom and both end walls constructed of metal. Each chamber was bi-laterally illuminated by mounting it directly between two metal halide lamps (Wotan power-star, HQI 250 W/NDL, Ian Fraser lighting, Edinburgh, U.K.). Neutral density filters (supplied by Strand Lighting Ltd., Isleworth, Middlesex, U.K.), were used to vary PPFD between 0 and 1600 μmol m^{-2} s^{-1}, without changing the spectral composition, which was measured using a standard hand held quantum sensor (SKP 215, Skye Instruments Ltd, Powys, U.K.) for each cuvette at each light level.

Leaf temperature in each of the three cuvettes was measured by Type K thermocouples adpressed to the underside of one needle in each cuvette. The thermocouple output was monitored by an electronic multichannel thermometer (Type 1624, Comark, UK). Chamber temperature was regulated by a water cooling system. A Pt-100 platinum resistance thermometer placed inside one of the cuvettes

to measure chamber air temperature was used to control the flow of cooling water through the cuvette floor, via a feedback temperature controller (CAL 6000 PID) coupled to an on/off solenoid water valve. The cooling water and cuvette air temperatures were thermostatically maintained at 11 °C and 19 °C ± 1 °C, respectively, and the reference dewpoint temperature was 10.8 °C.

The gas exchange system was an open path type as described by Jarvis and Čatský (1971). Air entering each cuvette was drawn from outside the building into a 10 dm^3 glass bottle, to buffer any sudden changes in ambient CO_2 concentration, and then passed through six gas washing bottles (two bottles per cuvette). In the case of the CO_2 response curves, a range of ambient CO_2 air concentrations (C_a), was obtained by a mass flow meter (FC260, Tylan General UK Ltd, Swindon, U.K.), which injected pure CO_2 into a stream of CO_2-free air prior to passing through the gas washing bottles. By altering the voltage across the mass flow meter a range of C_a concentrations was achieved. The bottles were held within a temperature controlled water bath, the first of the two bottles contained water to a depth one third of the bottles height and the second bottle was empty. The outside air was bubbled through sintered pipe ends in the first jar allowing equilibration of the inlet air to saturation at the bath water temperature before being fed into the cuvette. The second bottle acted as a safety trap to collect any potential excess moisture.

Air flow through each chamber was controlled via individual in-line rotameters (variable-area flow meter up to 5 $dm^3\ min^{-1}$, KDG Flowmeters, Sussex, England) and measured using a mass flow meter (FM360, Tylan General (UK) Ltd. Swindon, UK). The CO_2 concentration of cuvette inlet (reference) and outlet (analytical) air was measured using a bench-top infra-red gas analyser (IRGA, Type 225 Mk III, Analytical Development Co. Ltd., Hoddesdon, Herts) in differential mode. The dewpoint temperature of reference and analytical air streams was measured using

a cooled mirror dewpoint hygrometer (Series 3000, Mitchell, UK). Both reference and analytical air streams from all three cuvettes were continuously fed through the system and sampled for analysis through the IRGA and dew point meter manually via a gas switching unit.

The IRGA was fully calibrated at the start of each round of measurements using a cascade of three gas-mixing pumps (models 1 SA 18/3F, 1 SA 2 and 1 G 27/3F, Wosthoff, Bochum, Germany) and then daily during the experiment using two gas cylinders containing air of known CO_2 concentrations.

3.2.4 Calculation of parameters and statistical analysis

Each data set collected was analysed to yield assimilation rate, stomatal conductance and internal CO_2 concentration according to the model of von Caemmerer and Farquhar (1981).

3.2.4.1 CO_2 response curves

Each data set collected was analysed for subsequent modelling according to the equations based on the biochemical model of Farquhar *et al.* (1980). According to this model when Rubisco catalyses the oxygenation reaction of RuBP with one mol of O_2, 0.5 mol of CO_2 are released (Farquhar and von Caemmerer, 1982), and thus net assimilation of CO_2 (A) can be expressed by the equation:

$$A = V_c - 0.5\ V_o - R_d \qquad [3.1]$$

where V_c and V_o are the rates of carboxylation and oxygenation respectively, and R_d is CO_2 evolution from the mitochondria in the light (i.e. non-photorespiratory respiration) known as "day" respiration (Brooks and Farquhar, 1985).

Because the rate of *photosynthesis* is assumed to be limited by either the rate of electron transport (RuBP regeneration-limited net photosynthesis), A_{RuBP} [3.3], or the

amount, activity and kinetics of Rubisco (RuBP regeneration-saturated net photosynthesis), A_{carb} [3.4] the net rate of A can be expressed as [3.2]

$$A = \min \{A_{RuBP}, A_{carb}\} \quad [3.2]$$

$$A_{RuBP} = J_{max} \frac{C_i - \Gamma^*}{\alpha C_i + \beta\Gamma^*} - R_d \quad [3.3]$$

$$A_{carb} = V_{cmax} \frac{C_i - \Gamma^*}{C_i + K_c (1 + O/K_o)} - R_d \quad [3.4]$$

where J is the electron transport capacity, V_{cmax} is the maximum rate of carboxylation, C_i and O are internal leaf concentrations of CO_2 and O_2, respectively, Γ^* is the CO_2 compensation concentration in the absence of mitochondrial respiration, α and β are constants and K_c and K_o are Michaelis constants for carboxylation and oxygenation, respectively. The CO_2 response data were then fitted to this model using a reiterative optimisation program (Proc NLIN, SAS institute Inc., Cary, NC) and the parameters V_{cmax} and J_{max} derived.

3.2.4.2 Light response curves

Because the PPFD response data were collected at non-saturating CO_2 concentrations, C_a = 355 and 700 μmol mol^{-1}, it could not be assumed that the electron transport rate (J) solely limited photosynthesis, and therefore a less mechanistic model than the one proposed by Farquhar *et al.* (1980) was used to analyse these data. A non-rectangular hyperbolic function outlined in Jarvis *et al.* (1985) [3.5], and a reiterative optimisation program (Genstat 5) were used to

estimate the parameter values with the least mean square error. The measured input variables for the model are photon flux density (I), ambient CO_2 concentration (C_a), stomatal conductance (g_s) and assimilation rate (A). The model assumption is that photosynthesis is related to photosynthetic photon flux density (PPFD) by a non-rectangular hyperbola of the form:-

$$\theta(A + R)^2 - (\alpha I + A_{max} + R)(A + R) + \alpha I(A_{max} + R) = 0 \qquad [3.5]$$

Parameters of the model are α = initial slope of the A/I curve, θ = convexity coefficient (which defines the degree of curvature between the initial slope and the asymptotic value of A), A_{max} = the rate of PPFD saturated assimilation, g_m = mesophyll conductance, and R = rate of dark respiration.

This yielded fitted assimilation versus light flux density (A/I) curves for each CO_2 and chamber treatments in both years. The optimising routine of the Genstat package also estimated the following parameter values by the least mean square error method:- R, g_m, α and θ.

Analysis of variance was used to test the significance of difference between the measured maximum assimilation rates (A_{mmax}) and measured dark respiration rates (R_m) of chamber grown ambient and elevated CO_2 treatments and the outside control plot. These data were calculated from the A/I data sets where $I \geq 1200$ μmol m^{-2} s^{-1} (except for 1991 data, when $I = 800$ μmol m^{-2} s^{-1}) and $I = 0$ μmol m^{-2} s^{-1} for A_{mmax} and R_m, respectively.

3.2.4.3 Stomatal response curves

Standard nonlinear curves were fitted to each of the g_s/I response curves. A rectangular hyperbola of the form linear-divided by linear [3.6] best fitted the

measured data.

$$g_s = \alpha + \frac{\beta}{1 + \delta I} + \epsilon \qquad [3.6]$$

where α = the horizontal asymptote or maximum rate of g_s, β = vertical asymptote, δ = degree of curvature, and ε = error term.

The statistical differences between the [CO_2] and chamber treatments for A/I, A/C_i and g_s/I curves were determined by a combined curve analysis of variance (Ross, 1981). This technique tests the reduction in residual variance obtained by fitting a set of individual curves compared to the residual variance obtained from a common curve.

3.3 Results

3.3.1 Direct effect of CO_2 concentration on gas exchange

(i) PPFD response curves and dark respiration rates

Changes in photosynthetic rate as a function of photon flux density for Sitka spruce seedlings grown in open-top chambers, during their second and third year of growth in either ambient or elevated CO_2 are shown in Figure 3.1. The data presented are the net assimilation rates of trees measured at a range of PPFD in their respective growth CO_2 concentrations, i.e. trees grown in ambient [CO_2] were measured at 355 μmol mol^{-1} CO_2 and those grown in elevated [CO_2] were measured at 700 μmol mol^{1} CO_2. In both years the non-rectangular hyperbolic curve fitted both sets of [CO_2] treatment data well, with 86.2 % and 89.5 % of the variance accounted for (in the ambient and elevated [CO_2] treatments, respectively) after 14 months and 92.7 % and 97 % accounted for (in the ambient and elevated [CO_2] treatments, respectively) after 26 months. Pair-wise comparisons between response curves fitted to each [CO_2]

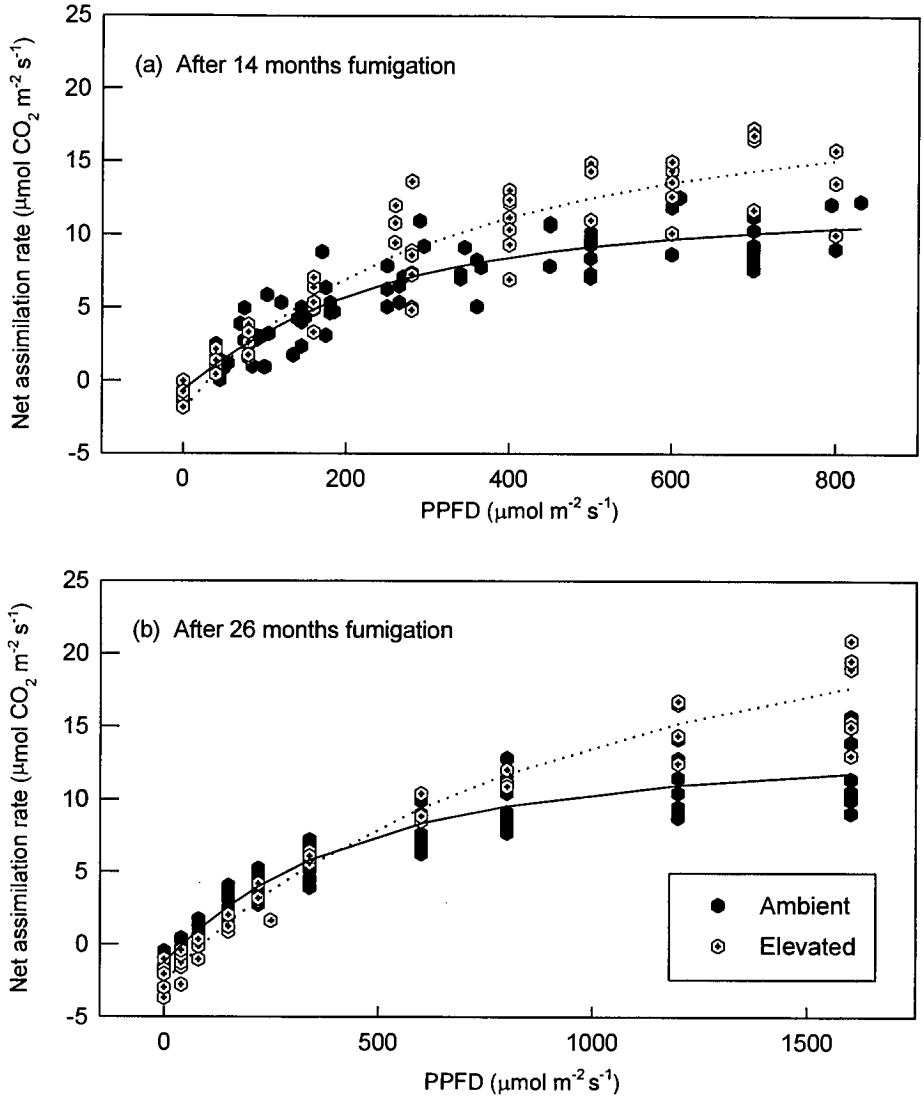

Figure 3.1: Changes in assimilation rate of Sitka spruce seedlings as a function of photosynthetic photon flux density after (a) 14 months and (b) 26 months growth in elevated or ambient CO_2. The curves are the best fits to the non-rectangular hyperbolic equation from the model of Jarvis *et al.* (1985) between treatments (dotted lines = elevated fitted curves and solid lines = ambient fitted curves).

treatment were significantly different after both treatment periods (Table 3.1, $p < 0.001$). This demonstrates a highly significant "direct" effect of CO_2 concentration on the photosynthetic response curve to PPFD for Sitka spruce.

Table 3.1: Combined curve analysis of variance tables (Ross 1981) for curves fitted to the CO_2 exchange rates of Sitka spruce shoots after 14 and 26 months, using the theoretical model of Jarvis *et al.* (1985). Statistical analysis is given for plants grown and measured in ambient [CO_2] compared with those grown and measured in elevated [CO_2].

Curve comparisons	df	ss	mean ss	F ratio	*p* value
After 14 months fumigation					
Ambient *vs* Elevated [CO_2]	4	195.6	48.9	17.85	**<0.01**
Residuals	137	375.4	2.74		
After 26 months fumigation					
Ambient *vs* Elevated [CO_2]	4	199.36	49.84	31.19	**<0.001**
Residuals	116	185.33	1.598		

After 14 months plants raised and measured in elevated [CO_2] had a 51 % higher maximum assimilation (A_{mmax}) and a 76 % higher dark respiration (R_m) rate compared with those raised and measured in ambient [CO_2], Table 3.2.

Table 3.2: Mean maximum assimilation rates (A_{mmax}, μmol m^{-2} s^{-1}) and mean dark respiration rates (R_m, μmol m^{-2} s^{-1}) for chamber grown Sitka spruce seedlings after 14 months in ambient and elevated [CO_2]. Measurements were made at treatment CO_2 concentrations, 20 °C and a photon flux density of 800 and 0 μmol m^{-2} s^{-1}, respectively. Values are means ± 1 SEM.

Growth CO_2 treatment	Measurement CO_2 concentration	R_m	A_{mmax}
Ambient	*ca.*355 µmol mol^{-1}	-0.66 ± 0.12	9.85 ± 0.57
Elevated	*ca.*700 µmol mol^{-1}	-1.16 ± 0.11	14.86 ± 0.99

After 26 months although the absolute values of both (A_{mmax}) and (R_m) were higher than in the previous year, the trends were the same. A_{mmax} and R_m of the elevated [CO_2] seedlings were directly stimulated by 58 % and 88 %, respectively. An increase in air temperature of 5 °C increased R_m of ambient [CO_2] grown and measured seedlings by 36 % and elevated [CO_2] grown and measured seedlings by 71 % (Table 3.3). Because temperature stimulation of R_m rates was not uniform

across both [CO_2] treatments, the net increase in R_m rates of elevated treated and measured plants compared with ambient treated and measured plants at 20 °C was 138 %.

Table 3.3: Maximum assimilation rates of Sitka spruce seedlings at 20 °C (A_{mmax}, μmol m^{-2} s^{-1}), and mean dark respiration rates at 15 and 20 °C (R_m, μmol m^{-2} s^{-1}), after 26 months growth in OTCs supplied with ambient or elevated [CO_2] and outside in a control plot. Seedlings were measured at 355 µmol mol^{-1} CO_2 and 700 µmol mol^{-1} CO_2. Values are treatments means ± 1 SEM.

Treatment	Measurement CO_2 concentration	R_m at 15 °C	R_m at 20 °C	A_{mmax}
Ambient	*ca.*355	-1.04 ±0.19	-1.41 ± 0.17	11.32 ± 0.73
Elevated	*ca.*355	-1.24 ± 0.24	-1.99 ± 0.19	10.00 ± 0.84
Outside	*ca.*355	-1.37 ± 0.11	-1.89 ± 0.13	10.71 ± 0.38
Ambient	*ca.* 700	-1.58 ± 0.19	-3.04 ± 0.12	15.49 ± 1.31
Elevated	*ca.* 700	-1.95 ± 0.12	-3.35 ± 0.35	17.92 ± 1.17
Outside	*ca.* 700	-2.19 ± 0.01	-2.64 ± 0.01	15.00 ± 1.15

Table 3.4 gives the estimated parameter values from the model (Jarvis *et. al.*, 1985) fitted to the data collected after 14 months fumigation. It shows that elevated [CO_2] grown seedlings had a higher initial slope (α), R and convexity coefficient (θ) but a lower mesophyll conductance (g_m) compared with ambient [CO_2] grown seedlings when measured at their respective CO_2 treatment concentrations. The similarity between the estimated R (Table 3.4) and measured (Table 3.2) R_m value serve to verify the goodness of fit of the model for these data.

Results from the estimated model parameters obtained from data collected on seedlings grown in ambient or elevated [CO_2] for 26 months are presented in Table 3.5. When seedlings were grown and measured at the same CO_2 concentration R, and g_m were still lower in ambient than elevated [CO_2] treatments. However, the

absolute values of both R, and g_m differed between years, after 26 months treatment values of R were higher and g_m lower than after 14 months (Table 3.5). In addition after 26 months growth in their respective [CO_2] treatments, there was no longer a difference between the initial slopes and θ could only be fitted to seedlings from the ambient [CO_2] treatment.

Table 3.4: Estimated parameter values from the theoretical model (Jarvis *et al.*, 1985) for Sitka spruce seedlings grown in OTCs for 14 months in either ambient or elevated [CO_2] and measured at treatment CO_2 concentration (α = initial slope of A/I curve, g_m = mesophyll conductance (mmol m^{-2} s^{-1}), R = dark respiration (μmol m^{-2} s^{-1}), and θ = convexity coefficient).

Parameter values	Ambient [CO_2]	Elevated [CO_2]
α	0.046	0.050
g_m	42.27	30.26
R	0.628	1.020
θ	0.513	0.557

(ii) Stomatal conductance

Figure 3.2 shows the direct response (plants grown and measured at the same CO_2 concentration) of stomatal conductance (g_s) to photon flux density (PPFD) of seedlings grown in either ambient or elevated CO_2 for 26 months. In both [CO_2] treatments g_s increased sharply up to 500 μmol m^{-2} s^{-1} reaching a plateau at approximately 800 μmol m^{-2} s^{-1}. Rectangular hyperbolas of the form linear-divided by linear fitted all data well with 78% and 77% of the variation accounted for in the ambient and elevated treatments respectively. Combined curve analysis of the fitted rectangular hyperbolas showed no significant difference between the functional response of g_s to PPFD for ambient [CO_2] treated and measured plants compared with elevated [CO_2] treated and measured plants.

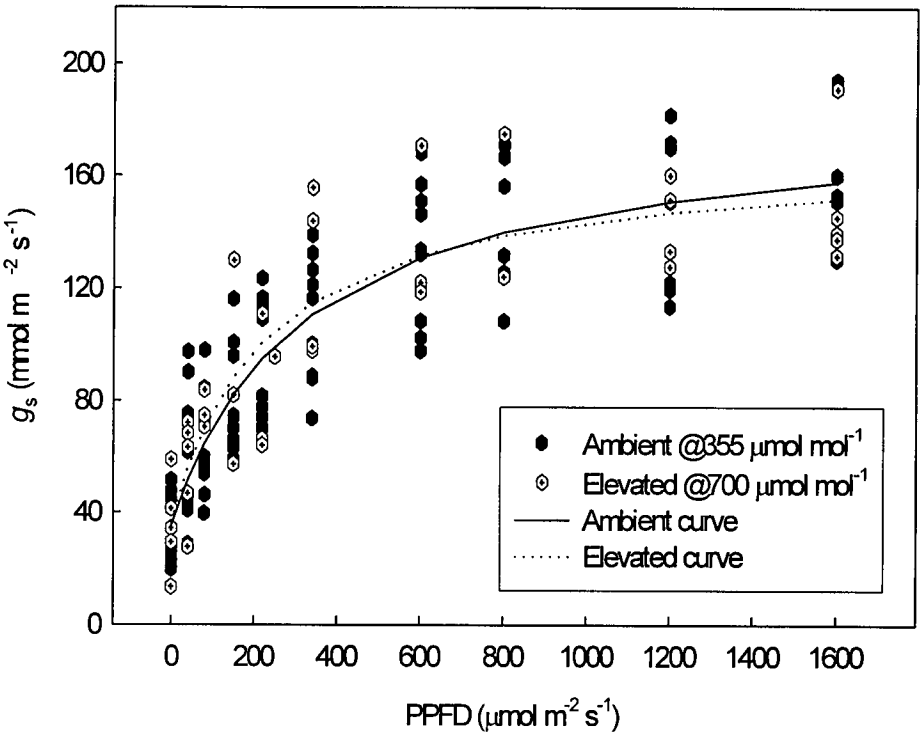

Figure 3.2: Changes in stomatal conductance of Sitka spruce seedlings as a function of photosynthetic photon flux density after 26 months growth in ambient and elevated [CO_2]. Measurements were made on seedlings grown and measured at the same CO_2 concentrations. The curves are best fits to the rectangular hyperbola of the form linear-divided by linear.

3.3.2 Indirect affects of CO_2 concentration on gas exchange

(i) Light response curves and dark respiration rates

A comparison between ambient and elevated CO_2 treated plants measured at both treatment CO_2 concentrations after 26 months fumigation showed no significant indirect effect of CO_2 fumigation on A_{mmax} (Table 3.5). Figure 3.3 shows measured net assimilation rate and fitted response curves as a function of PPFD for all treatments at both 355 and 700 µmol CO_2 mol^{-1}. Again the non-rectangular

hyperbolic curve fitted all data combinations well (between 90-97% of variance accounted for). A pair-wise comparison between the response curves fitted to each treatment at 355 μmol CO_2 mol^{-1} showed no significant difference (Figure 3.3(a)). However, the elevated [CO_2] treatment had a significantly different light response curve compared to the ambient and outside treatments when measured at 700 μmol CO_2 mol^{-1} (Figure 3.3(b), $p = 0.05$).

Table 3.5: Estimated parameter values from the model (Jarvis *et al.*, 1985) for Sitka spruce seedlings grown in OTCs in either ambient or elevated [CO_2] and grown outside in a control plot. All treatments were measured at 355 μmol mol^{-1} CO_2 and 700 μmol mol^{-1} CO_2 after 26 months of treatment (α = initial slope of *A/I* curve, g_m = mesophyll conductance (mmol m^{-2} s^{-1}), R = dark respiration (μmol m^{-2} s^{-1}), and θ = convexity coefficient).

Treatment	α	g_m	R	θ
Measured at 355 μmol mol^{-1}				
Ambient	0.030	50.27	1.28	0.41
Elevated	0.033	51.11	1.58	0.00
Outside	0.031	54.92	1.63	0.00
Measured at 700 μmol mol^{-1}				
Ambient	0.030	30.47	2.04	0.59
Elevated	0.031	49.17	2.60	0.00
Outside	0.030	40.44	2.42	0.00

Mean measured dark respiration rates of elevated [CO_2] treated plants measured at 355 μmol CO_2 mol^{-1} were significantly higher at both 15 and 20 °C compared with those of ambient [CO_2] treated plants (Table 3.3). Elevated [CO_2] treated plants had a higher R_m than ambient [CO_2] treated plants *per se*, though the degree of stimulation varied between 10 and 41 % depending on both CO_2 concentration and air temperature. These results are comparable with the estimated parameter R values from the model (Jarvis *et al.*, 1985) where modelled R values for elevated [CO_2] treated plants were higher than those of the ambient [CO_2] treated plants (Table 3.5).

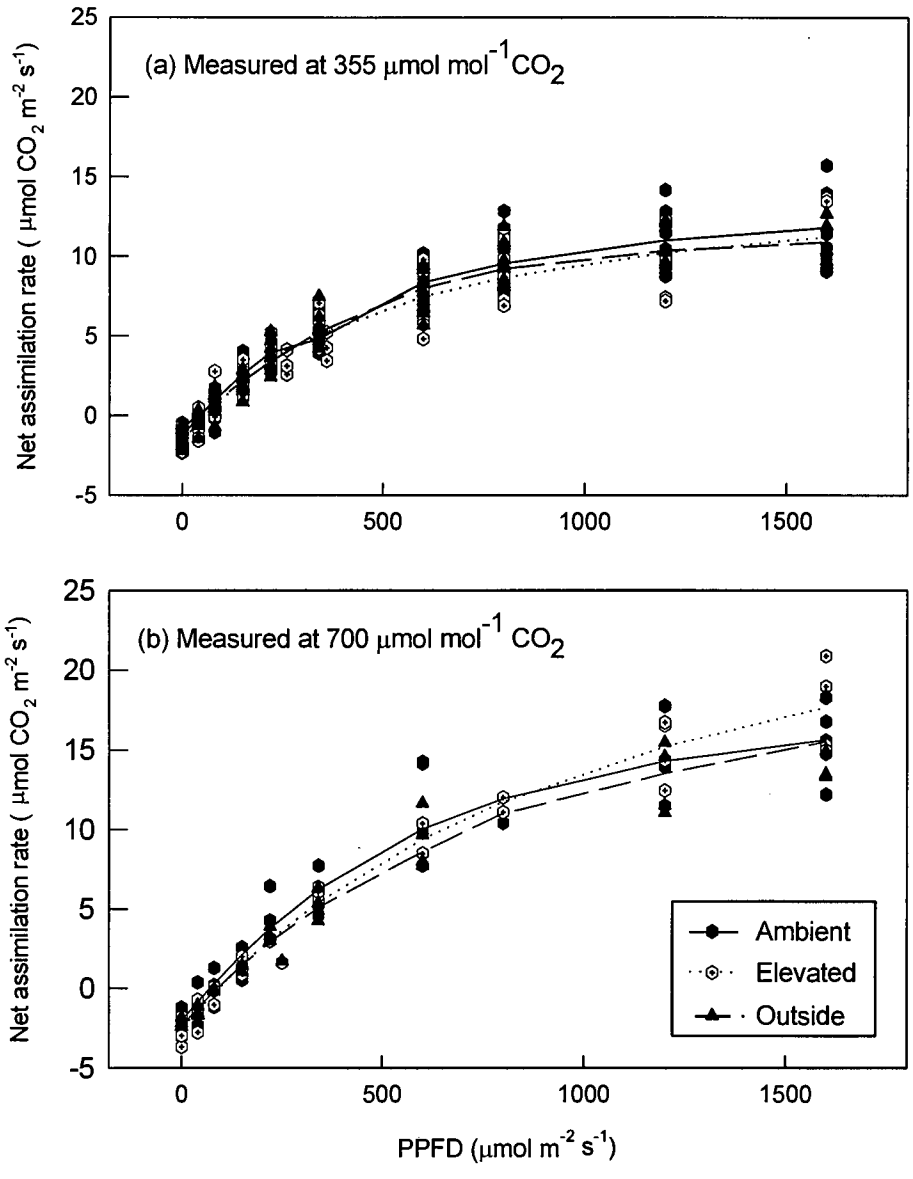

Figure 3.3: Changes in assimilation rate of Sitka spruce seedlings as a function of photosynthetic photon flux density measured at (a) 355 μmol mol^{-1} CO_2 and (b) 700 μmol mol^{-1} CO_2), for ambient (plants grown in open-top chambers in 355 μmol mol^{-1} CO_2), elevated (plants grown in open-top chambers in 700 μmol mol^{-1} CO_2) and outside (plants grown outside in a control plot under ambient CO_2 concentrations). The curves are the best fits to the non-rectangular hyperbolic equation from the model of Jarvis *et al.* (1985) between treatments.

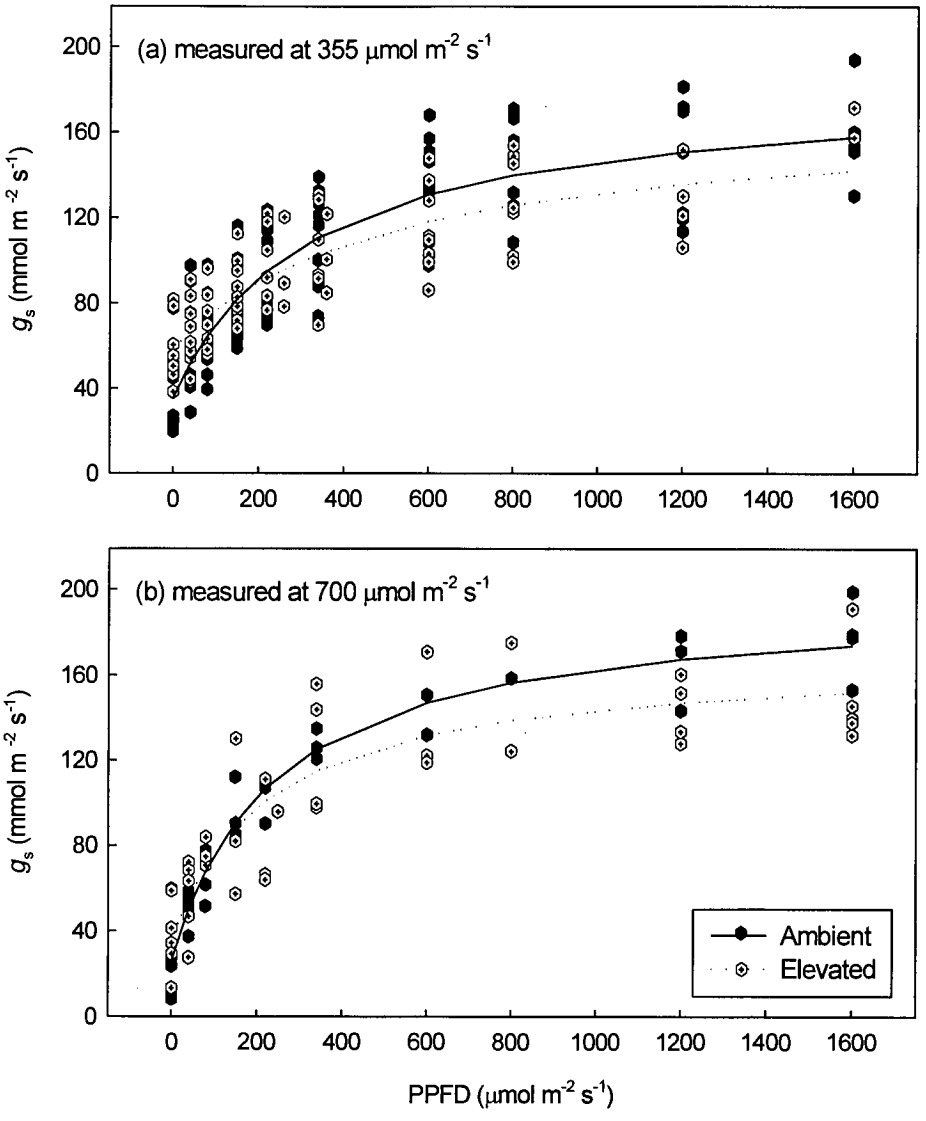

Figure 3.4: Changes in stomatal conductance of Sitka spruce seedlings as a function of photosynthetic photon flux density after 26 months exposure to ambient or elevated [CO_2] and measuring at (a) 355 and (b) 700 μmol mol^{-1} CO_2. The curves are best fits to the rectangular hyperbola of the form linear-divided by linear.

(ii) Stomatal conductance

Stomatal response (g_s) to photon flux density for ambient and elevated [CO_2] grown seedlings at both CO_2 concentrations are presented in Figure 3.4. Figure 3.4 also includes fitted curves of the form linear-divided by linear for each data set at both CO_2 concentrations. Rectangular hyperbolas fitted all sets of data well with 78 and 94 % of the variance accounted for in the ambient [CO_2] grown seedlings and 67 and 77 % of the variance accounted for in the elevated [CO_2] grown seedlings, at 355 and 700 μmol mol^{-1} CO_2, respectively. Combined curve analysis conducted at both CO_2 concentrations showed significant differences between the chamber grown ambient and elevated [CO_2] seedlings when analysed at the same CO_2 measurement concentration ($p > 0.05$), i.e. ambient and elevated [CO_2] seedlings measured at 355 μmol mol^{-1} were significantly different. However, there was no significant effect of measurement CO_2 concentration on the g_s response of seedlings to PPFD within each [CO_2] treatment.

3.3.3 CO_2 response curves

Figure 3.5 summarises the CO_2-response of net photosynthesis for Sitka spruce after 26 months growth in either ambient or elevated [CO_2]. To remove additional effects due to stomatal conductance, the rate of photosynthesis is plotted against intercellular (C_i) rather than ambient (C_a) CO_2 concentrations. The mechanistic model of Farquhar *et al.* (1980), represented in Figure 3.5 by the lines, fitted all data sets well with 84 and 79 % of the variance accounted for in seedlings grown in ambient and elevated [CO_2], respectively. In this study, pair-wise comparisons between the curves fitted to Sitka spruce plants grown at elevated CO_2 concentrations and those grown at ambient CO_2 concentrations showed that they were highly significantly different ($p < 0.01$). Table 3.6 lists the parameter values obtained from fitting individual A/C_i response curves to each of the ambient, elevated and outside data sets.

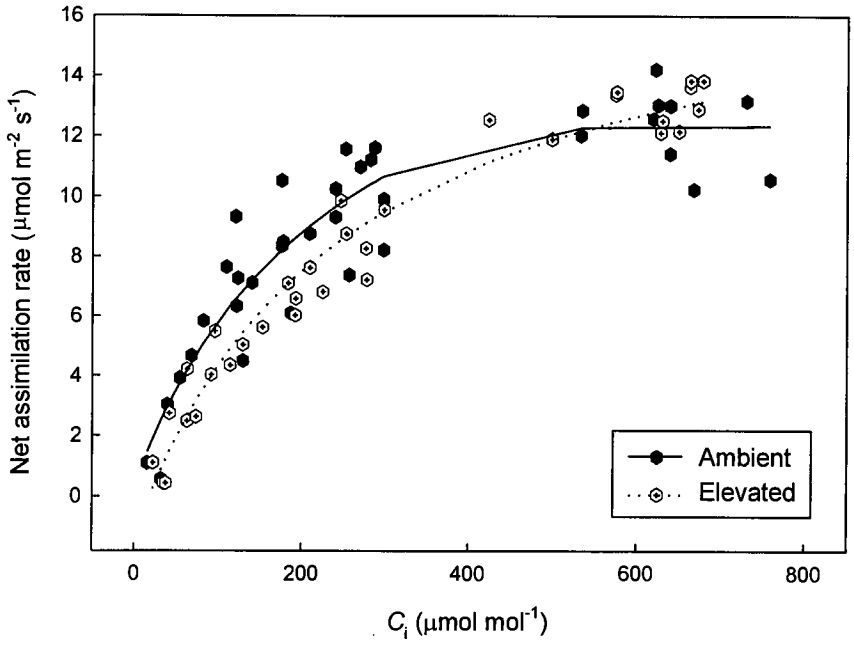

Figure 3.5: Changes in assimilation rate of Sitka spruce seedlings as a function of intercellular leaf CO_2 concentration (C_i) for ambient (plants grown in open-top chambers in 355 μmol mol^{-1} CO_2) and elevated (plants grown in open-top chambers in 700 μmol mol^{-1} CO_2). The curves are the best fits to the mechanistic model of Farquhar *et al.* (1980) between treatments.

V_{cmax} of seedlings grown in elevated [CO_2] was only slightly increased by 3.6 % compared with those grown in ambient [CO_2]. However there was a much bigger effect on J_{max} which was stimulated by 21 % in elevated [CO_2].

Figure 3.6 shows the linear relationship between ambient CO_2 concentrations and internal CO_2 concentrations for all treatments r^2 = 0.98. Overall C was approximately 75 % of C_a and [CO_2] treatment had no significant effect on the relationship between external (C_a) and internal (C_i) CO_2 concentration.

Table 3.6: Estimated parameter values, V_{cmax} and J_{max} obtained from the mechanistic Farquhar *et al.* (1980) model for Sitka spruce seedlings grown in OTCs under either ambient or elevated [CO_2] and grown outside in a control plot. Measurements were made during August and September 1992.

	CO_2 treatment		
Parameters	Ambient	Elevated	Outside
V_{cmax} (μmol mol^{-1})	19.3 ± 2.0	20.0 ± 0.5	18.0 ± 0.3
J_{max} (μmol m^{-2} s^{-1})	49.0 ± 03.7	59.2 ± 1.5	52.6 ± 5.6

3.3.4 Open top chamber effect

(i) Light response curves and dark respiration rates

Figure 3.7 shows assimilation rate as a function of PPFD from 0 to 1600 μmol m^{-2} s^{-1} for seedlings grown inside open top chambers (ambient) and outside in a control plot (outside) measured at both 355 and 700 μmol CO_2 mol^{-1}. Non rectangular hyperbola curves fitted all data sets well with 92.7 and 93.9 % accounted for in ambient treated plants at 355 and 700 μmol CO_2 mol^{-1} respectively, and 91.9 and 96.1 % accounted for in outside treated plants at 355 and 700 μmol CO_2 mol^{-1} respectively. Combined curve analysis showed that there was a significant effect of open top chamber on the net photosynthetic response curve to light intensity, PPFD at 350 μmol CO_2 mol^{-1} ($p = 0.05$) but not at 700 μmol CO_2 mol^{-1}.

Mean A_{max} values were always lower for the outside treated plants compared to ambient [CO_2] treated plants though not significantly so (Table 3.3). There was no consistent trend between R_m and R values of ambient and outside treated plants, though generally those from the outside treatment had higher R_m and R values than those from the ambient treatment (see Tables 3.3 and 3.5).

(ii) Stomatal conductance

Figure 3.8 shows the comparison between the response of stomatal conductance (g_s)

to PPFD for ambient and outside treated Sitka spruce plants measured at 355 μmol CO_2 mol^{-1} (Figure 3.8(a)) and 700 μmol CO_2 mol^{-1} (Figure 3.8(b)). A rectangular

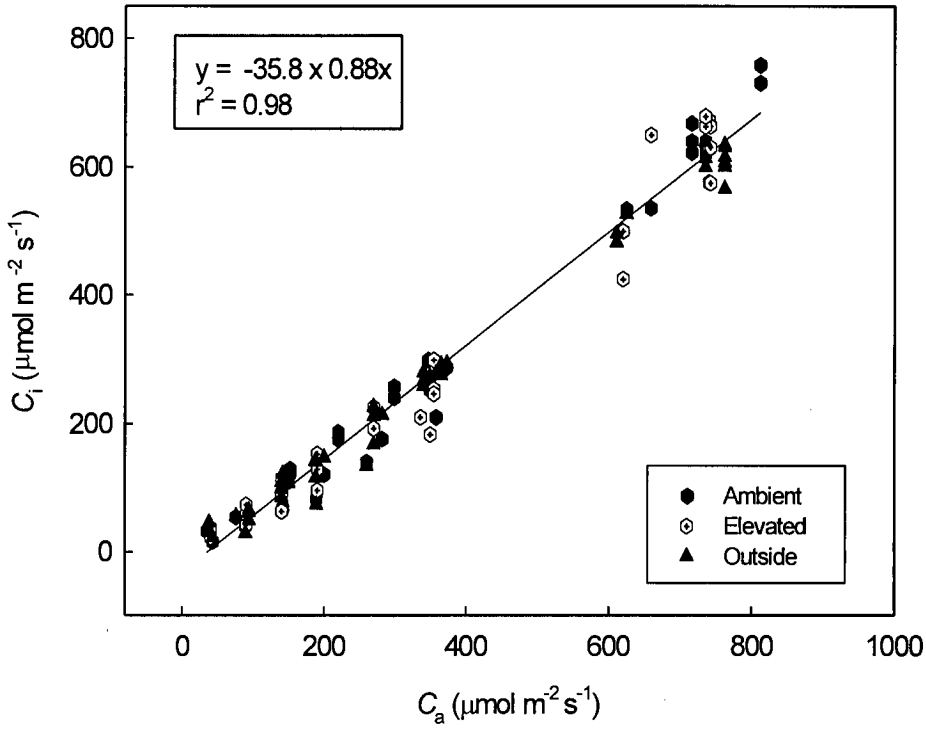

Figure 3.6: The relationship between ambient air CO_2 concentration (C_a) and internal foliar CO_2 concentration (C_i) for ambient (plants grown in open-top chambers in 355 μmol mol^{-1} CO_2), elevated (plants grown in open-top chambers in 700 μmol mol^{-1} CO_2) and outside (plants grown outside in a control plot under ambient CO_2 concentrations) Sitka spruce seedlings. Solid line indicate linear relationship $r^2 = 0.98$.

hyperbola of the form linear-divided by linear fitted the data well with 78 and 94 % of the ambient treated plants variation accounted for at 355 and 700 μmol CO_2 mol^{-1} respectively and 77.5 and 82.4 % accounted for in the outside treated plants at 355 and 700 μmol CO_2 mol^{-1}, respectively. Combined curve analysis showed a highly

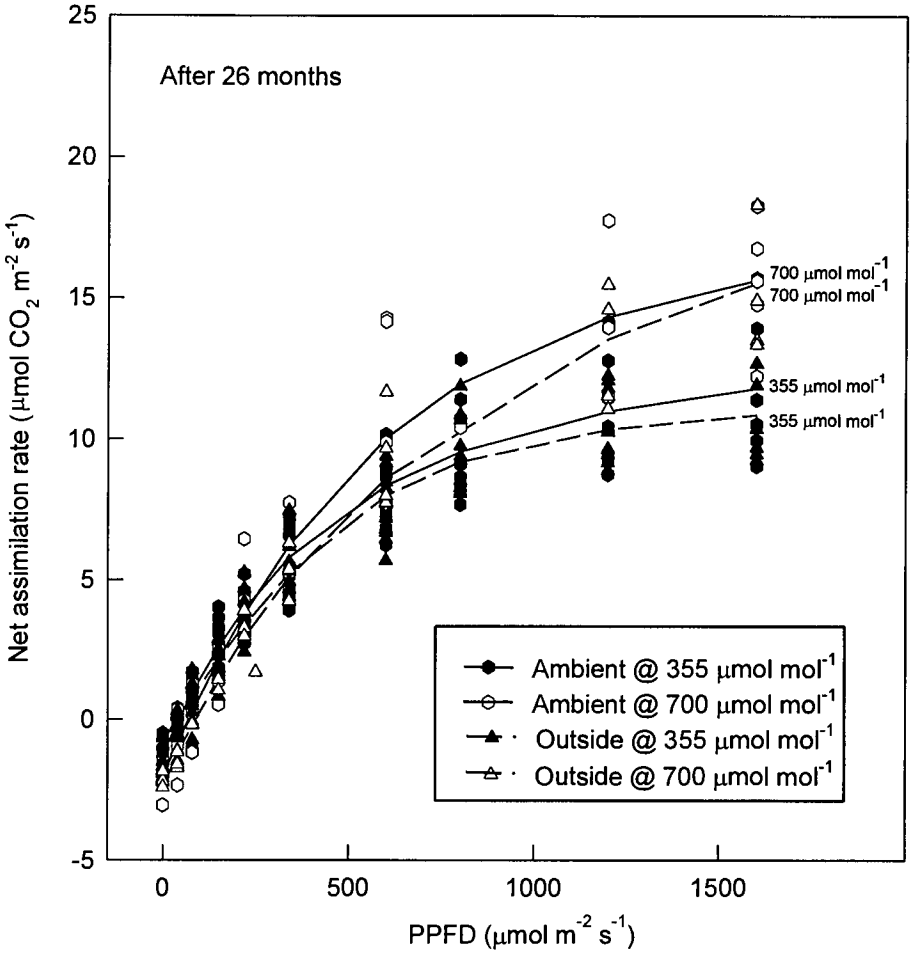

Figure 3.7: Changes in assimilation rate of Sitka spruce seedlings as a function of photosynthetic photon flux density measured at 355 μmol mol^{-1} CO_2 and 700 μmol mol^{-1} CO_2), for ambient (plants grown in open-top chambers in 355 μmol mol^{-1} CO_2), and outside (plants grown outside in a control plot under ambient CO_2 concentrations). The curves are the best fits to the non-rectangular hyperbolic equation from the model of Jarvis *et al.* (1985) between treatments.

significant effect of open to chamber on the stomatal response curve to PPFD (p = 0.001 for both CO_2 concentrations). Plants grown inside open top chambers (i.e. ambient treatment) had a consistently higher stomatal conductance compared with those grown outside in the control plot at all light levels and both CO_2 measurement concentrations (Figure 3.8).

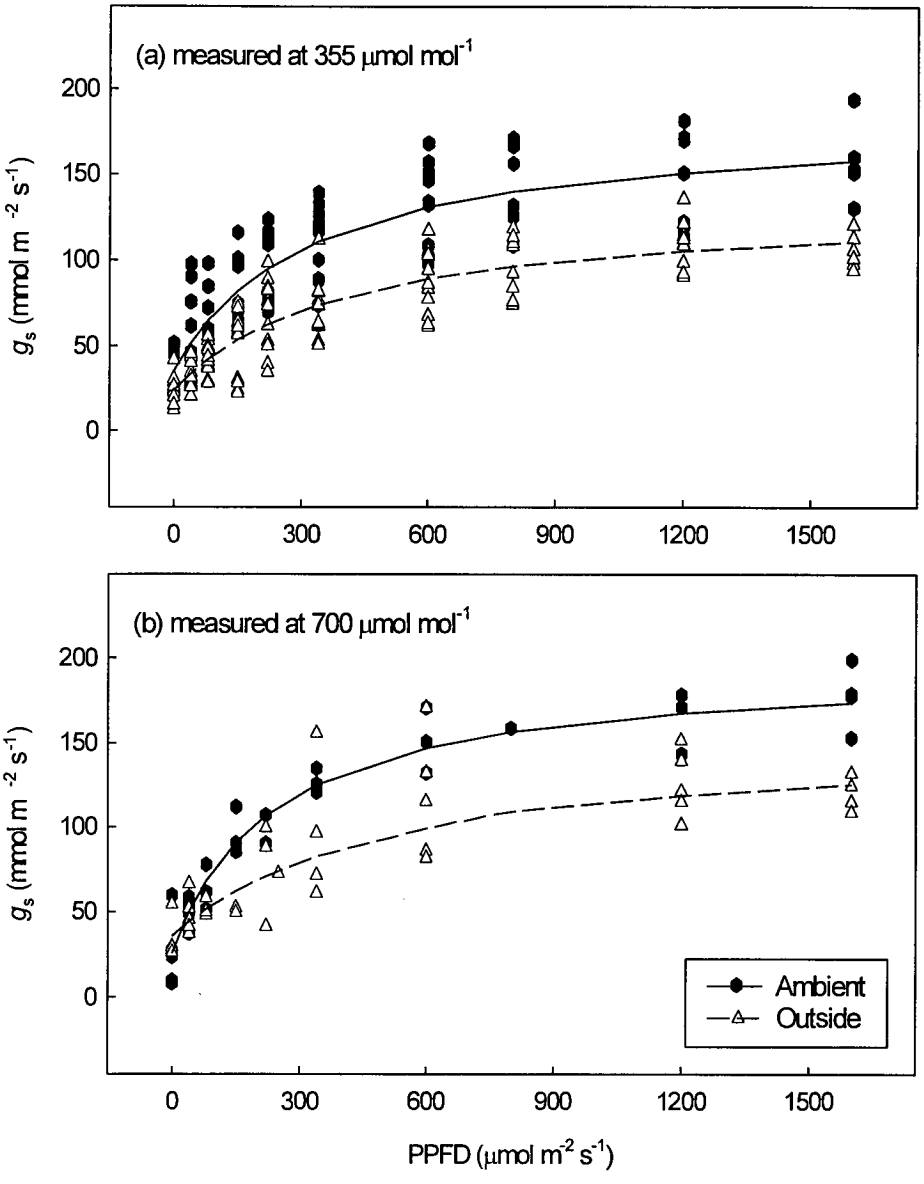

Figure 3.8: Changes in stomatal conductance of Sitka spruce seedlings as a function of photosynthetic photon flux density after 26 months growth inside an OTC in ambient [CO_2] and outside in a control plot. Seedlings were measured at (a) 355 μmol mol^{-1} CO_2 and(b) 700 μmol mol^{-1} CO_2. The curves are best fits to the rectangular hyperbola of the form linear-divided by linear.

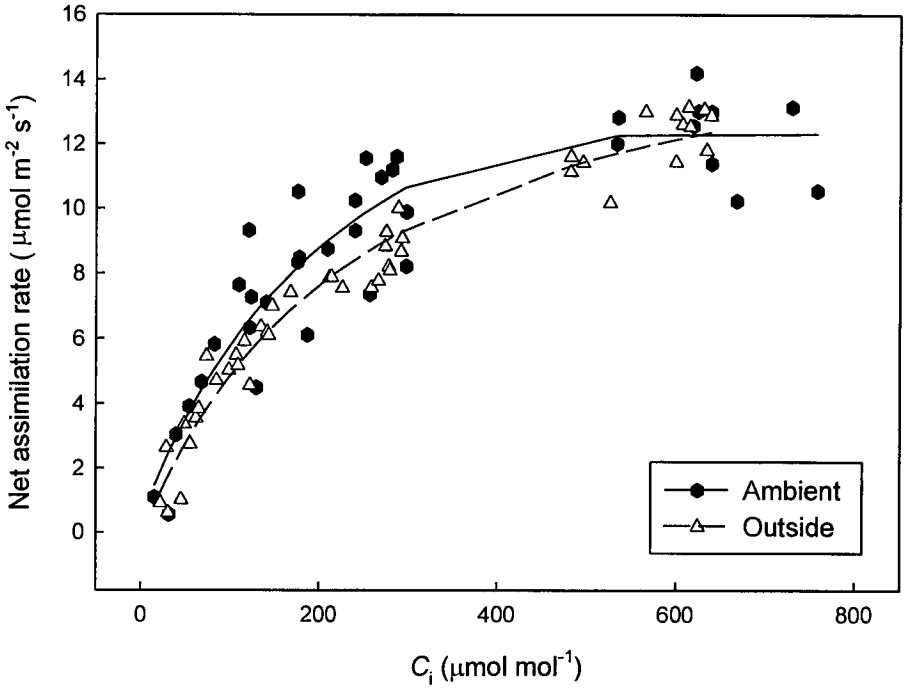

Figure 3.9: Changes in assimilation rate of Sitka spruce seedlings as a function of intercellular leaf CO_2 concentration (C_i) for ambient (plants grown in open-top chambers in 355 μmol mol^{-1} CO_2) and outside (plants grown outside OTCs in a control plot under ambient [CO_2]. The curves are the best fits to the mechanistic model of Farquhar *et al.* (1980) between treatments.

(iii) CO_2-response curves

Figure 3.9 summarises the CO_2-response of net photosynthesis for Sitka spruce after 26 months growth either inside an OTC or outside in a control plot, both under ambient CO_2 concentrations. Pair-wise comparisons between the ambient and outside A/C_i response curves (represented in Figure 3.9 by the lines) fitted using the Farquhar *et al.* (1980) model, showed that there was a significant effect of OTC ($p < 0.001$).

The Farquhar *et al.* (1980) model fitted the outside data set well, estimated parameter values obtained from the model are presented in Table 3.6. Despite the significant difference between the fitted curves there was little effect of OTC on either V_{cmax} or J_{max}. The V_{cmax} and J_{max} of seedlings grown inside OTCs were 7 % higher and 7 % lower, respectively compared with those grown outside in the control plot (Table 3.6).

3.4 Discussion

3.4.1 Photosynthetic response

One of the primary direct effects of elevated atmospheric CO_2 concentration on plants with the C_3 carbon fixation pathway is almost always an overall stimulation in net photosynthesis (Bowes, 1991; Idso and Kimball, 1992; Wullschleger *et al.*, 1995). These results are well accounted for by mechanistic equations of photosynthesis where A can be considered as limited by either the capacity of ribulose 1-5 bisphosphate carboxylase/oxygenase (Rubisco) or limited by the rate of ribulose 1,5-bisphosphate (RuBP) regeneration through the C_3 photosynthetic carbon reduction cycle (PCR) (von Caemmerer and Farquhar, 1981; Farquhar *et al.,* 1980). Laboratory studies have shown that across the range of C_a which have occurred over geological time scales (150-600 μmol mol^{-1}), an increase in the rate of foliar photosynthetic CO_2 assimilation rate (A) with increasing C_a is usually observed (Lloyd and Farquhar, 1996). However, a disparity exists as to the extent of the photosynthetic stimulation under enhanced atmospheric CO_2 concentrations, values range from as little as 3 % in *Castanea sativa* (Rouhier *et al.*, 1994) to as much as 200 % in *Pinus eldarica* (Garcia *et al.*, 1994). Results presented here confirm the above findings with maximum net assimilation rates being stimulated by 51 and 58 % after growing seedlings in double present day atmospheric [CO_2] for 14 and 26 months, respectively. The magnitude of this result is consistent with many studies

conducted in either the field or in large pots (Sage, 1994; Liu and Teskey, 1995; Rey, 1997).

In many instances studies which have initially reported a stimulation in net photosynthetic rates under elevated [CO_2] concentrations, subsequently report a decline in this stimulation after a longer period of fumigation (Tissue & Oechel, 1987; Ceulemans and Mousseau, 1994; Sage, 1994). This type of acclimation to elevated atmospheric [CO_2], known as 'down-regulation', is often attributed to an end-product feedback inhibition of photosynthesis. Feedback inhibition results from an imbalance between the supply 'source' and demand 'sink' of photo-assimilates. In this study, 'down-regulation' did not occur over the 26 month period of the experiment with photosynthetic stimulation of A_{mmax} remaining above 50 %. In fact, the instantaneous stimulation in A_{mmax} of seedlings grown at ambient [CO_2] but measured at 700 μmol mol^{-1} was only 37%.

Because assimilation is controlled by either the slowest or rate-limited step in either the light reactions, electron transport system or the photosynthetic carbon reduction (PCR) cycle, it is not surprising that changes in atmospheric CO_2 concentrations have a major impact. Results presented here show a highly significant effect of CO_2 concentration on the photosynthetic response curves to PPFD. Both the initial slopes (RuBP-regeneration limited, i.e. supply of ATP and NADPH is restricted because of inadequate PPFD) and PPFD-saturated levels (Rubisco limited) were higher in the elevated compared to the ambient treated plants when measured directly, i.e. grown and measured in the same atmospheric CO_2 concentration. If the PPFD response curves of seedlings from the elevated [CO_2] treatment are examined indirectly (i.e. elevated [CO_2] treated plants measured at 355 μmol CO_2 mol^{-1}) and compared with those from the ambient [CO_2] grown and measured seedlings there was no significant

difference between response functions, again suggesting that there was no downward photosynthetic acclimation of either the electron transport mechanism or PCR cycle. There are two possible and indeed likely scenarios which would explain our apparent lack of 'down-regulation': (i) 'down-regulation' is not only a function of end product synthesis limitation but is a species mediated response which Sitka spruce does not exhibit, and/or (ii) our experimental protocol did not lead to plants becoming pot bound or nutrient deficient (Murray *et al.*, 1996) and hence sink limited. Both scenarios are supported by results from a comparative study on seedling Sitka spruce (Barton, 1997). Barton (1997) reported that the weak down-regulation of photosynthesis in response to growth in elevated [CO_2] and low nutrient supply rate could be explained entirely by a reduction in leaf nitrogen concentration. Therefore, under nutrient deficient conditions down-regulation was a function of shifts in internal N partitioning rather than elevated [CO_2] *per se*.

It has been widely postulated that because C_3 species have the ability to optimise resource allocation, primarily nitrogen, between, Rubisco, chlorophyll and thylakoid proteins, i.e. between carboxylation, light harvesting processes and electron transport, elevated CO_2 will also cause functional changes in the photosynthetic mechanism (Sage, 1994; Wullschleger, 1993; Long *et al.*, 1994). Such a response to elevated [CO_2] may be attributed to the change in CO_2 concentrations *per se* or as a result of a potential indirect effect on plant nutrient status and hence nutrient use efficiency (see chapters 4 and 5).

Results from A/C_i analysis give us some insight into the underlying biochemical changes involved behind the photosynthetic responses often observed in plants growing in elevated atmospheric CO_2 concentrations (Gunderson and Wullschleger, 1994; Farquhar *et al.*, 1980). Despite the widely held concept that as CO_2

concentration increases the efficiency of Rubisco increases, leading to a reduction in its content and hence the occurrence of 'down-regulation' when plants are measured indirectly (Drake *et al.*, 1997), this study and many others have found no such response (Gunderson *et al.*, 1993; Arp and Drake, 1991; Barton, 1997). V_{cmax} (or carboxylation rates) observed in this study although lower than those presented for *Picea sitchensis* by Barton (1997) are similar to the late summer values found in field grown *Betula pendula* (Rey and Jarvis, 1997). Rey (1997) showed that V_{cmax} values decrease over the growing season, irrespective of experimental treatment. Therefore, seasonal downregulation of photosynthesis should be taken into account when comparing photosynthetic results obtained across a number of species and studies.

J_{max} values in this study were increased by elevated [CO_2] indicating an increase in electron transport capacity. However, since V_{cmax} did not decline in elevated [CO_2] it is unlikely that nitrogen was redistributed from Rubisco to proteins involved in electron transport. However, it is possible that as a result of the effect of elevated [CO_2] on TLA and SLA (see chapter 2, Murray *et al.*, 1996), needle structure was altered in elevated [CO_2] grown seedlings. Because the PPFD actually intercepted by the shoot depends on needle shape, internal structure, orientation and proximity to other needles on the shoot (Ludlow and Jarvis, 1971), any [CO_2] induced change in needle structure and arrangement may have resulted in more efficient light harvesting structure and consequently 'apparent' enhanced J_{max} rates.

3.4.2 Respiration rates

Whole plant respiration is a major component in the total carbon budget of higher plants with estimates indicating about half the net carbon fixed in photosynthesis being lost via respiration (Farrar, 1985). Therefore, it is important to understand potential impacts of elevated CO_2 concentrations on respiration. Plants from an

elevated [CO_2] environment are often reported to have decreased rates of respiration (Bunce, 1990; Idso and Kimball, 1993; Wang *et al.*, 1995; Drake *et al.*, 1997) or show no apparent change in the rate of respiration (Poorter *et al.*, 1992; Liu and Teskey, 1995) when measured at any given CO_2 concentration (see Amthor (1991) for a review of respiratory responses to CO_2 concentration). However, this is far from a universal response to elevated [CO_2] as increases in dark respiration have also been reported (Hrubec *et al.*, 1985; Townend, 1993). In this study, the measured dark or 'night-time' respiration rates (R_m) of Sitka spruce shoots (branch and needles) were up to 75% higher when grown and measured in elevated [CO_2] compared with ambient [CO_2].

Because respiration rates increase when respiratory products (i.e. ATP, NAD(P)H and C-skeleton intermediates) are consumed at increased rates (Amthor, 1995), changes in respiration may be a direct result of changes in C-accumulation and/or C-partitioning. In the longer term an increase in the demand for end products as a result of growth stimulation, will lead to increased respiration rates not only in the sinks but also in the source leaves supplying those sinks (Amthor, 1993). R_m increased by 73 % and 188 % over the course of one year for the ambient and elevated [CO_2] grown seedlings, respectively. This result supports the concept that bigger plants have a greater metabolic cost of supply and maintenance and hence a higher rate of respiration.

At the end of this study, despite a 50 % stimulation in A_{mmax}, seedlings grown in elevated [CO_2], though slightly taller, did not have a significantly larger dry mass than those grown in ambient [CO_2] (Chapter 2; Murray *et al.*, 1996). There was however an increase in the allocation of dry mass to roots in elevated [CO_2]. Since respiration of roots is know to be significantly higher (g for g) than aboveground

biomass, both the demand for and loss of carbon from this sink will be increased in elevated [CO_2]. Therefore, much of the additional carbon fixed under elevated CO_2 may be lost via enhanced respiratory costs in maintaining a larger root biomass and increased source leaf respiration *per se*. In addition, much of the additional carbon fixed may be lost via respiratory processes as a result of carbohydrate accumulation in the source leaves (Farrar and Williams, 1991) during periods of slow root growth.

3.4.3 Stomatal conductance

Stomatal conductance has often been shown to decrease in elevated [CO_2] (Eamus *et al.*, 1993; Overdieck and Forstreuter, 1994) . This has led to the speculation that in elevated atmospheric CO_2 concentrations water use efficiency (WUE) will increase, via the regulatory control of stomata on transpiration rates. However more recently an increasing number of studies have shown a minimal or none existent response of stomatal conductance to elevated [CO_2] (Bunce, 1992; Ellsworth *et al.*, 1995; Gunderson *et al.*, 1993; Liu and Teskey, 1995). Results presented here also indicate no significant effect of [CO_2] on the functional relationship between stomatal conductance and PPFD. It has been suggested that such findings result from more favourable experimental protocols, where plants are grown directly in the soil or in large pots with adequate nutrients and water supplies (Eamus, 1996). In studies where root restriction is not a problem, i.e. the plants are not pot bound, shifts in the functional relationship between roots and shoots in favour of roots can freely be made. Thus enabling the plant to sequester more water without having to alter its stomatal conductance (Eamus, 1996). The consequences of such an hypothesis under increased CO_2 concentrations is an ultimate increase in water use per tree, not an increase in water use efficiency.

3.4.4 OTC effect

In this study, OTCs had little or no effect on photosynthetic processes, although in general assimilation rates were higher and respiration rates lower for chamber grown seedlings. However, in contrast to the effect of CO_2 concentration on stomatal conductance, OTCs significantly ($p = 0.001$) increased stomatal conductance across a range of PPFD and at both treatment CO_2 concentrations. This result is likely to be an artifact of the effect of open top chambers on leaf morphology rather than a result of internal feedback mechanism. Cape and Percy (1993) studied the effect of growth environment on needle epicuticular wax production and morphology of three spruce species and showed that growth inside OTCs affected needle epicuticular wax characteristics and wettability. Therefore, because stomata respond independently to water-vapour saturation deficit and the waxy cuticle is a very effective barrier to water loss, changes in its composition will affect the apparent stomatal conductance.

Although stomatal conductance was higher in the OTCs than outside, the C_i/C_a remained constant (*ca.* 0.75), an assumption frequently adopted by modellers who assume a typical ratio of about 0.7 for C_3 species (McMurtrie and Wang, 1993).

3.5 Conclusions

The generally held view that elevated atmospheric CO_2 concentrations will lead to an increase in resource use efficiency, as a result of higher photosynthetic rates, enhanced WUE and NUE, in turn leading to an stimulation in biomass productivity (Drake *et al.*, 1997), may not be true in all cases. In reality, species responses to elevated [CO_2] are likely to be diverse; the amount of contradictory literature currently published is testament to this. In addition, it would be naive to assume no interaction between plant physiological responses to elevated [CO_2] and other environmental variables (see Chapter 5 for interactions with nutrition).

Increasingly, longer term studies report little or no stimulation in plant biomass accumulation and stomatal conductance (see review by Eamus, 1996). In the present study, elevated [CO_2] significantly enhanced photosynthetic and dark respiration rates of Sitka spruce shoots and did not significantly decrease stomatal conductance. An enhanced dark respiration rate under elevated CO_2 is likely to result in some of the additional CO_2 fixed during photosynthesis being respired back into the atmosphere, thus reducing the potential for carbon sequestration of this species.

This study, also highlights the need for great care to be taken when extrapolating experimental findings beyond the bounds of experimental trees in controlled environments. Results from this study show a particular effect of OTC on stomatal conductance. Chamber grown seedlings had a higher g_s than those grown outside, a result which affects any implications concerning the absolute effect of elevated CO_2 on WUE of Sitka spruce.

3.6 Summary conclusions

In this study, photosynthetic responses to elevated [CO_2] were basically similar after 14 and 26 months of continuous exposure.

- Shoot photosynthesis was directly enhanced by elevated [CO_2] after 26 months exposure inside OTCs. PPFD saturated photosynthetic rates of seedlings grown in elevated [CO_2] were on average 55 % higher than in ambient [CO_2] when measured at growth [CO_2].
- There was no significant effect of exposure duration on the photosynthetic capacity. The instantaneous stimulation of 700 μmol mol^{-1} CO_2 on photosynthesis of ambient [CO_2] treated seedlings was around 40 %.

- There was no evidence of down-regulation after exposure to elevated [CO_2] of 14 or 26 months, both V_{cmax} and J_{max} were higher in elevated [CO_2] grown seedlings, probably as a result of seedlings having adequate sinks and nutrient supply rates.
- Elevated [CO_2] increased dark respiration rates across both exposure periods. This was probably as a result of enhanced sink demands being placed on source shoots.
- There was no evidence of a reduction in stomatal conductance after either exposure period to elevated [CO_2]. Therefore, elevated [CO_2] is unlikely to enhance WUE of Sitka spruce.
- There was no significant effect of OTCs on photosynthetic processes. There was a highly significant effect of OTC on stomatal conductance probably as a result of changes in needle and shoot morphology.

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CHAPTER 4

Effect of elevated [CO_2] and varying nutrient supply rate on the growth, biomass partitioning and foliar mineral nutrition of Sitka spruce (*Picea sitchensis* (Bong.) Carr.)

Abstract

Sitka spruce (*Picea sitchensis* (Bong.) Carr.) seedlings were supplied with either 0.1(low-N), 0.5 (medium-N) or 2.0 (high-N) x optimum nutrient solutions and grown outside in a control plot or inside open-top chambers under ambient, 355 or elevated, 700 μmol mol^{-1} CO_2. After 24 months [CO_2] treatment and 12 months nutrient treatment there were significant [CO_2], nutrient and CO_2 x nutrient differences in total dry mass, total plant height, root collar diameter and biomass allocation. The effect of elevated CO_2 on biomass accumulation was largest in the high nutrient treatment; 37% stimulation compared with 15.9 % in the low nutrient treatment. Chamber grown plants supplied with the high nutrient treatment were 179 and 286% bigger than those receiving the low nutrient treatment, for ambient and elevated [CO_2] treatments respectively. Increasing nutrient supply rate significantly increased carbon allocation to all section of the plant irrespective of CO_2 concentration. Total root biomass was increased by both elevated CO_2 and decreasing nutrient supply rate. Elevated [CO_2] significantly reduced the amount of biomass allocated to stems in plants receiving the low nutrient treatment but had no effect at the high nutrient level. Increasing nutrient supply rate from the low to high treatment decreased R/S by 188, 124 and 186 % in the elevated, ambient and outside control treatments.

Total leaf area (TLA) and total leaf dry mass (TLM) were both significantly increased by increasing CO_2 concentration and nutrient supply rate. There was no significant effect of elevated CO_2 on SLA, LAR or LMR, although there was a highly significant effect of nutrient treatment. There was a highly significant effect of nutrient treatment on foliar N, P and K concentrations. Elevated [CO_2] significantly reduced foliar N concentrations by 44, 11 and 8 % in the low, medium and high nutrient treatments, respectively. There was no significant [CO_2] effect on P or K concentrations though they were consistently lower in the elevated [CO_2] treatment. Total carbon content was approximately 51% across all treatments. Both elevated CO_2 and decreasing nutrient supply rates significantly increased C/N ratios.

OTCs had no significant effect on total biomass or specific leaf parameters. Plants grown inside OTCs were 25% taller with a bigger stem mass than those grown outside. OTCs reduced the allocation of carbon to roots, with the biggest effect seen in the low nutrient treatment. OTCs *per se* changed the carbon allocation pattern of Sitka spruce.

Key words: Picea sitchensis, elevated CO_2, open-top chambers, nutrients, growth, biomass.

4.1 Introduction

Growth rate, and hence ultimate biomass accumulation, of woody plants has been shown to increase in response to increasing atmospheric carbon dioxide concentration [CO_2] across a range of crop species (see review by Cure and Acock, 1986) and coniferous and broadleaved tree species (see reviews by Eamus and Jarvis, 1989; Ceulemans and Mousseau, 1994; Idso and Idso, 1994). This is generally an indirect result of the direct effect of elevated atmospheric CO_2 concentration on the plants physiological processes, namely photosynthesis, photorespiration, respiration and transpiration (Jackson *et al.*, 1994). Because current-day concentrations of atmospheric CO_2 are limiting to photosynthesis any increase in atmospheric CO_2 will enhance net photosynthetic rates and hence biomass accumulation. However, if Liebig's law of the minimum, which states that 'the environmental resource present in the least amount will determine plant growth' applies, then increased growth under future elevated CO_2 concentrations may not necessarily occur to the extent predicted by many researchers (Kirschbaum, *et al.*, 1994). Current estimates given in a recent review by Poorter (1993) of the amount of growth stimulation to be expected in elevated [CO_2] range from around 41% for C_3 species to 15 % for CAM species, with C_4 plants falling in the middle at 22 %. However, such estimates of the degree of growth stimulation under elevated [CO_2] have been shown to be reduced under nutrient limiting conditions (Brown and Higginbotham, 1986; Conroy *et al.*, 1992; Bazzaz and Fajer, 1992; Wong, *et al.*, 1992; Ceulemans and Mousseau, 1994).

There are several possible mechanisms whereby nutrient availability and uptake rates may interact with enhanced atmospheric CO_2 concentrations. Firstly, adjustments in the distribution of the nutrient pool within the plant or in metabolic requirements could lower nutrient demand and increase nutrient use efficiency (NUE). For example, if the efficiency of ribulose 1, 5-bisphosphate carboxylase (Rubisco) is

higher in elevated [CO_2], less N would be needed per unit dry matter increment. Indeed it is now generally accepted that the nitrogen (N) concentration in leaves and other organs of plants grown in elevated [CO_2] is lower than in plants cultivated in ambient [CO_2] the so called 'dilution effect'- (Overdieck, 1990). This has been found to occur across a range of nutrient concentrations and soil types and is therefore, thought to occur irrespective of nitrogen availability in the soil (Conroy *et al.*, 1992; El Kohen *et al.*, 1992). It has been suggested that plants maximise their resource-use efficiency by allocating N to maintain a balance between photosynthetic and non-photosynthetic processes (Field and Mooney, 1986).

Secondly, nutrient acquisition rates could increase if fine root growth is stimulated. It is well known that nutrient deficiency enhances biomass allocation to roots. Therefore it is not surprising that given the amount of studies reporting reduced tissue nitrogen concentrations in elevated [CO_2], a number of them have reported a proportionally larger stimulation of root biomass (Bazzaz, 1990; Rogers, *et al.*, 1993; Murray *et al.*, 1996). However, it has been shown that the response of the root fraction (the ratio of root to total plant dry mass) to elevated [CO_2] is not consistent among species and varies with growth conditions (Farrar and Williams, 1991; Stitt, 1991; Eamus and Jarvis, 1989). Nevertheless, even in the case of free nutrient supply, when an increase in CO_2 concentration generally results in a relative enhancement of the above ground plant parts, total root biomass is also increased compared with plants in ambient [CO_2] (Eamus and Jarvis, 1989).

Thirdly, nutrient supply may increase through stimulation of biological activity in the soil and rhizosphere, as a result of soil mineralisation through the exudation of C (van Veen *et al.*, 1989). Zak *et al.* (1993) suggests that elevated [CO_2] will lead to increased mineralisation rates as a direct result of increased root activity.

In addition, internal nutrient imbalances are as important to a plants well being and ability to respond to elevated [CO_2] as nutrient availability *per se* (Linder, 1995). The interactions between [CO_2], biomass production and nutrient concentration in leaves are quite different for nitrogen and phosphorus (Conroy, 1992). Shifts in the relative rates of carbon fixation and photorespiration by changing [CO_2] will alter the demand for phosphorus in leaves, because carbon fixation requires phosphate, whereas photorespiration releases bound phosphorus. Therefore, in contrast to nitrogen, a higher phosphorus concentration in the leaf may be needed in elevated [CO_2] to maximise production (Conroy, 1992).

If increasing atmospheric [CO_2] *per se* not only affects biomass accumulation but also impacts on the allocation of biomass within the tree itself, it is important to establish how such changes in allocation patterns interact with other environmental variables. Because of the strong influence nutrient availability *per se* has on biomass allocation and tree productivity, the interaction between it and [CO_2] is of particular interest. This is especially true for a forest crop species such as Sitka spruce (*Picea sitchensis* (Bong.) Carr.), which is of primary importance as a timber crop for the forestry industry and is frequently grown in infertile habitats where it is limited by nutrient deficiencies.

The present study was set up to explore the interactive effect of atmospheric CO_2 concentration [CO_2] and nutrient availability on growth, biomass accumulation and allocation, and foliar nutrient status of Sitka spruce seedlings.

In order to be able to supply nutrients 'according to demand' or to control growth rate by regulating the addition rate of nutrients, one needs to know that the nutrients available for uptake will equal the rate of supply of nutrients. This is seldom the case in a 'normal' or in this study a 'composite' soil, where both biological and chemical

processes can affect the availability of individual nutrient elements. One approach to this problem, currently being practised in forest experiments, is to set target values of plant nutrient concentrations in the trees being studied and, on the basis of foliar analysis and predicted growth responses, it is then possible to calculate the appropriate supply rate of mineral fertiliser appropriate to achieve the set target figure (Linder, 1995). In this study, we set a target value of 2.0 % nitrogen by mass in the foliage and then supplied 2.0, 0.5 and 0.1 times the estimated amount of nutrients required to maintain this percentage, thus creating a range of nutrient regimes. In longer term studies, it is then possible subsequently to analyse foliar and soil water nutrients to show the extent to which the target has been met and to use this information to alter the proportions and amounts of nutrient to be added on the next (e.g. weekly, monthly or annual) occasion of fertiliser application.

4.2 Methods and materials

4.2.1 Plant material

In March 1992, 330 one-year-old Sitka spruce seedlings, identity number 83(1012)LOT3, provenance 10, Queen Charlotte Islands, which had been raised in ambient or elevated CO_2 concentrations for 12 months in glasshouse CO_2 exposure tunnels, were potted into 2 dm^3 pots containing unfertilised composite soil. The soil consisted of sphagnum peat, 5 mm quartz and sterilised loam in the ratio 13:4:3 by volume. Thirty plants were placed randomly in each of 10 open-top chambers (OTCs) and in an outside control plot, giving 30 plants per chamber and 30 plants outside, a total of 330 plants. Within the outside plot, six plants were randomly placed in each of five blocks

4.2.2 Growth conditions

OTCs were used to expose the plants to two different atmospheric CO_2

concentrations, ~355 μmol CO_2 mol^{-1} (ambient CO_2 treatment) and ambient + 350 μmol CO_2 mol^{-1} (elevated CO_2 treatment). Five OTCs were used per CO_2 treatment. The chambers had a floor area of 7.0 m^2 and height of 2.3 m. Ambient air or ambient air supplemented with additional CO_2 was passed through a series of charcoal filters and pumped directly into each OTC via a polythene manifold 1.5 m above ground level. For a full description of open-top chamber properties and the CO_2 fumigation facility see Chapter 2, Fowler *et al.* (1989) and Murray *et al.* (1996). CO_2 concentrations were monitored in each of the OTCs. OTCs receiving the ambient treatment had an average CO_2 concentration of 355 ± 15 µmol mol^{-1}, as a result of diurnal fluctuations and those receiving the elevated treatment had an average CO_2 concentration of 700 ± 80 µmol mol^{-1}, as a result of ambient fluctuations and changes in external windspeed. In addition to the [CO_2] treatments, an outside control plot (outside treatment) was set up to examine the effect of OTCs *per se*. Throughout the year, all seedlings were watered to field capacity by trickle irrigation as required. On days when nutrients were applied seedlings were watered first thing in the morning, one hour prior to nutrient application.

4.2.3 Nutrient treatments

4.2.3.1 Application techniques

Throughout the 1992 growing season, 10 dm^3 of one of three balanced nutrient solutions was applied weekly to each of the plants using a hand held pipette (Varipette 4720, Eppendorf, Hamburg). Nutrient supply rates were based on the Ingestad technique (Ingestad and Lund, 1986), which matches the addition rate of nutrients to plant growth rates. The nutrient addition rate was calculated based on previous growth measurements of Sitka spruce seedlings (see section *4.2.3.2 Growth analysis for nutrient application rate*) and weekly leader extension rates. Relative nutrient proportions required for optimum growth were assumed to be similar to

those described for *Picea abies* by Ingestad (1979), and are given in Table 4.1. A nitrogen concentration of 2 % by mass in current-year foliage was assumed to be optimum for Sitka spruce seedlings. Three rates of nutrient supply were selected to give 2 x optimum

Table 4.1: Chemical composition of nutrient solution applied to *Picea sitchensis* seedlings throughout the 1992 growing season. Solutions *B* and *C* were mixed in the ratio 1.7 : 1.0, respectively just prior to application, giving an equivalent of 37 g of nitrogen dm^{-3}.

Solution B		g l^{-1}
Ammonium nitrate	NH_4NO_3	140.2
Potassium nitrate	KNO_3	37.2
Potassium dihydrogen phosphate	KH_2PO_4	41.3
Potassium sulphate	K_2SO_4	14.0
Solution C		
Nitric acid	HNO_3	1.6 (cm^3)
Calcium nitrate	$Ca(NO_3)_2$	14.3
Magnesium nitrate	$Mg(NO_3)_2$	26.0
Manganese sulphate	$MnSO_4$	0.55
Boric acid	H_3BO_3	0.57
Cuprous chloride	$CuCl_2$	0.032
Zinc sulphate	$ZnSO_4$	0.036
Sodium molybdate	Na_2MoO_4	0.007
Ferric sulphate	$Fe_2(SO_4)_3$	2.5

(high-N), 0.5 x optimum (medium-N) and 0.1 x optimum (low-N) foliar nitrogen contents. Each nutrient treatment was applied to 10 of the 30 plants in each of the ten chambers and to two of the six plants in each of the five outside control blocks. Thus there were 10 plants per nutrient treatment per chamber and two plants per nutrient treatment per outside block.

4.2.3.2 Growth analysis for nutrient application rate

Total plant biomass was predicted throughout the experiment using a linear regression of the form:

$$M = aD^2H$$

where M = total plant dry mass(g), H = total plant height (mm), D = root collar diameter (mm) at ground level and a = a constant of proportionality. Linear regressions were fitted for Sitka spruce using data obtained from an initial harvest of 20 plants from each of the elevated and ambient CO_2 treatments prior to bud burst, 18 March 1992 (Genstat 5). The relationship between root collar diameter, plant height and total biomass is shown in Figure 4.1, $r^2 = 0.75$.

This relationship was then used to calculate the subsequent weekly nutrient supply required to maintain the nitrogen concentration of 2.0 % dry mass in the needles, which was then multiplied by 2.0, 0.5 and 0.1 for the high-N, medium-N and low-N treatments respectively.

4.2.4 Growth analysis

4.2.4.1 Biomass harvests

At the start of the experiment (18 March 1992), 20 plants from each of the elevated and ambient CO_2 glasshouse tunnels were randomly selected and destructively harvested, giving 40 plants in total. The harvested seedlings were separated into roots, needles and shoots and dried to a constant mass, this took three days at 80 °C in a forced draught oven (Apex drier 14:E, Apex construction Ltd, Kent, England). The dried plant material was then weighed on a calibrated balance (Sartorius, Northern balance consultancy, Tyneside, England). At the subsequent final harvest in February 1993, the plant material was further subdivided into current and previous years' needles, stem and branch wood. This harvest included a random sample of five plants from each of the nutrient treatments within each of the 10 open-top chambers and one plant from each of the outside control blocks, giving

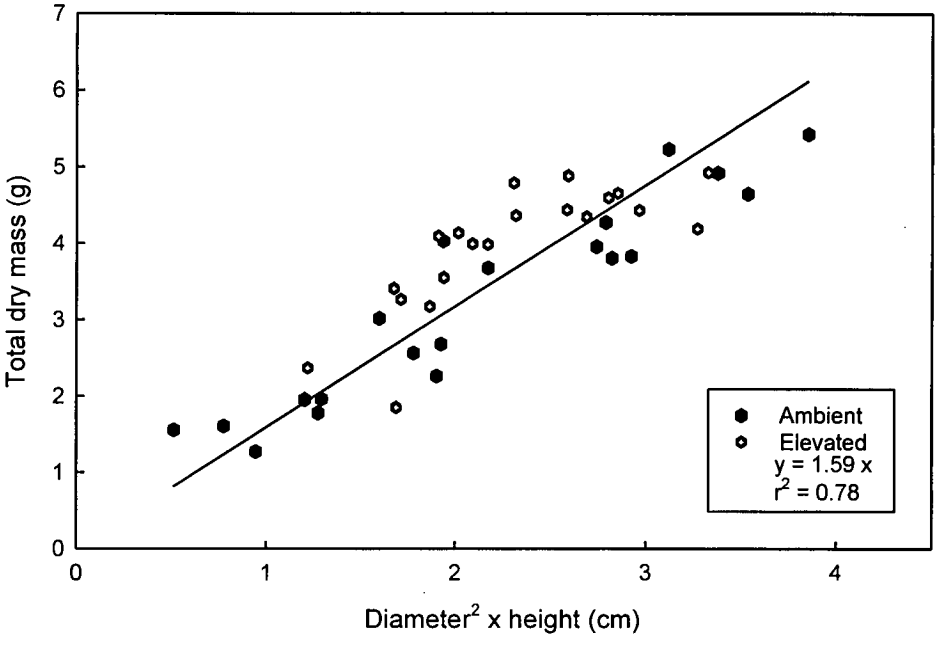

Figure 4.1: The regression between the measured root collar diameter and plant height versus total plant biomass on 18 March 1992 (initial harvest) for Sitka spruce seedlings raised in either ambient or elevated [CO_2] exposure tunnels for 12 months (n=40).

25 plants per nutrient treatment in each of the ambient and elevated CO_2 chamber treatments and five plants per nutrient treatment in the outside control treatment, totalling 165 plants. At each harvest plant height, root collar diameter and leaf area were also measured on each of the harvested seedlings. Projected needle area was determined using an image analysis system (microscale IIR, Digithurst, Royston, England). Needles were placed on a light box to increase edge definition, and black and white video images were digitised at 512 x 512 pixel resolution. Threshold settings for binary imaging were determined prior to measurements, using calibration

standards. Total seedling leaf area (TLA, cm^2), total seedling leaf dry mass (TLM, g), leaf area ratio (LAR, $cm^2\ g^{-1}$), specific leaf area (SLA, $cm^2\ g^{-1}$) and leaf mass ratio (LMR, $g\ g^{-1}$) were calculated for the February 1993 harvest using the following equations:

$$Leaf\ area\ ratio = L_a / M \qquad [4.1]$$

$$Specific\ leaf\ area = L_a / L_m \qquad [4.2]$$

$$Leaf\ mass\ ratio = L_m / M \qquad [4.3]$$

where L_m= total seedling needle dry mass, L_a= total seedling projected needle area and M= total seedling dry mass.

4.2.4.2 Weekly leader extension rates

Throughout the 1992 growing season weekly leader extension measurements were made on all chamber grown and outside control plot plants. Weekly relative growth rates (R_w) were computed using the differential equation (Hunt, 1978):

$$R_w = [\ln H_2 - \ln H_1] / (T_2 - T_1) \qquad [4.4]$$

where H_1 and H_2 are leader height at times T_1 and T_2, previous weeks and current weeks measurement, respectively. Since this form of sampling was not destructive, consecutive measurements were made on the same plants and pairing was not necessary.

Relative growth rates (RGR) of biomass accumulation were obtained from estimates of initial dry mass (M_1) of the plants made using the linear regression of total dry mass on root collar diameter and total plant height (Figure 4.1); obtained from the initial (March 1992) harvest data and final dry mass (M_2) from the measured values of the February 1993 harvest. This was then divided by the individual treatment growing seasons (G_{season}) for each of the respective CO_2 x nutrient treatments, i.e. the number of days between the date of budburst and budset to give a mean daily RGR for the growing season (R_{sm}) and by 365 to give a mean daily RGR for the entire year R_{dm}.

$$R_{sm} = [\ln M_2 - \ln M_1] / (G_{season}) \quad [4.5]$$

$$R_{dm} = [\ln M_2 - \ln M_1] / 365 \quad [4.6]$$

4.2.5 Nutrient analysis

In February 1993, nitrogen (N), phosphorous (P), potassium (K) and total structural and non-structural carbon (C) were measured in both current (< 1- year old) and previous (> 1- year old) years needles removed from the seedling branches. Each of the needle age classes were bulked by chamber or outside control plot for each of the three nutrient treatments, sub-sampled then ground using a Wiley mill (type DCFH48, Glen Creston, Stanmore, England) to < 0.8 mm, in preparation for the above analyses. This gave 66 samples in all, 5 per needle age class per nutrient treatment for each of the chamber treatments and one per needle age class per nutrient treatment in the outside control plot. An aliquot of the ground material was re-dried in an air-circulated oven at 105 °C for 3 h, and 350 mg of the oven-dry sample was digested by a modified Kjeldahl procedure in the presence of H_2O_2 and using Li_2SO_4 to increase boiling point and Se as catalyst (Parkinson and Allen 1975). Concentrations of N and P were measured by continuous flow colorimetry (Skalar

analytical) via indolephenol blue and molybdenum blue respectively and K was measured by flame emission spectrometry (Corning Flame photometer 430). Samples were prepared for C analysis by initially grinding using a Wiley mill (type DCFH48, Glen Creston, Stanmore, England) to < 0.8 mm then ball milling to a fine particle size. C was then measured using elemental analysis (Carlo Erba Strumentazione, Mod 1106, Fison Instruments, Sussex, England).

The total nutrient content of both current (< 1 -year old) and previous (> 1 -year old) years foliage was calculated from measured nutrient concentrations and their corresponding tissue dry mass for each plant component.

4.2.7 Statistical analyses

Differences in measured variables were tested by analysis of variance using Genstat 5 software (Payne *et al.* 1987). A randomised split plot design was used with chamber as the main plot and plants the subplots. Seedling dry mass was calculated from stem height and basal diameter over the growing season using regression analysis (SigmaPlot, Jandel Scientific, Germany).

4.3 Results

4.3.1 Effects of elevated CO_2 and nutrition on growth and biomass production

Results from the initial (18 March 1992) harvest of one-year-old Sitka spruce seedlings after exposure for 12 months to ambient or elevated [CO_2] are presented in Table 4.2. At the start of the experiment, seedlings from the elevated [CO_2] tunnels were 26 % larger than those from the ambient [CO_2] tunnels ($p = 0.023$). There was no significant difference in root/shoot allometry between seedlings grown in the CO_2 treatments, as the dry mass of both roots and needles was stimulated evenly by elevated [CO_2].

Table 4.2: Initial biomass parameters from 18 March 1992 harvest of 40, one-year-old Sitka spruce [*Picea sitchensis* (Bong.) Carr.] after germination and growth for 12 months in either ambient (355 μmol mol^{-1}) or elevated (700 μmol mol^{-1}) CO_2. Values presented are means and significance obtained from t-test (n= 20).

Parameter	ambient [CO_2]	elevated [CO_2]	*p* value [CO_2] treatment
Height (mm)	213.7	229.8	0.108
Root collar diameter(mm)	3.049	3.197	0.379
Stem (Needles + wood)(g)	1.276	1.49	0.070
Branches (Needles+wood) (g)	1.018	1.322	0.083
Wood (g)	0.89	0.968	0.493
Needles (g)	1.404	1.844	**0.011**
Roots (g)	0.853	1.16	**0.004**
Total dry mass (g)	3.15	3.97	**0.023**
Root / shoot ratio	0.38	0.422	0.089

The final (February 1993) growth response values of seedlings grown in either ambient or elevated [CO_2] and treated with one of three nutrient regimes are presented in Table 4.3, along with results obtained from the outside control treatment. After two years of exposure to elevated [CO_2] and 12 months nutrient treatment, there were significant CO_2, nutrient and CO_2 x nutrient treatment differences in total dry mass, total plant height and root collar diameter (Table 4.3) between ambient [CO_2] and elevated [CO_2] treated seedlings. Elevated [CO_2] increased total dry mass by 37 % and 15.9 % under the high-N and medium-N treatments, respectively but had no effect in the low-N treatment. The stimulation in dry mass production between the low-N and high-N treatments was 179 % and 286 % for ambient and elevated CO_2 treatments, respectively. Seedlings were taller with larger root collar diameters when grown in elevated CO_2 compared with ambient CO_2 in all but the low-N treatment. Plate 4.1 shows a visual example of the

Plate 4.1: Visible effect from left to right, of high-N, medium-N and low-N supply rates on Sitka spruce seedlings growing inside OTCs after 5 months of nutrient treatment.

difference produced by the three nutrient treatments toward the end of the growing season (18 August).

Table 4.3: Effects of CO_2 concentration, nutrient supply rate and open top chamber on growth parameters of 2-year-old Sitka spruce after 23 months CO_2 and 12 months nutrient treatment. Values presented are the treatment means and significance level (n = 5, ANOVA). Significant differences within both the CO_2 and nutrient treatments, for each of the measured parameter are indicated by a different letter (LSD). Letters presented across rows are CO_2 treatment differences and within columns are nutrient treatment differences.

Parameter	x optimum nutrient treatment	OTC and ambient CO_2	OTC and elevated CO_2	Outside and ambient CO_2	*p* value		
					CO_2	nutrient	CO_2 x nutrient
Total dry mass (g)	0.1 a	27.42	27.2	25.39	<0.001	<0.001	<0.001
	0.5 b	41.55	48.14	42.99			
	2.0 c	76.49a	104.91b	77.27a			
Height (mm)	0.1 a	463.4	379.8	370.4	0.013	<0.001	<0.001
	0.5 b	519.1	575.9	540.0			
	2.0 c	707.6a	746.9a	568.2b			
Root collar diameter (mm)	0.1 a	9.02	8.98	8.01	0.005	<0.001	0.017
	0.5 b	10.25	11.38	9.79			
	2.0 c	13.11a	15.23b	13.90a			

4.3.2 Leader extension rates

Differences in weekly relative extension rates (R_w) are presented in Figure 4.2. The R_w growth pattern was similar for seedlings grown in both [CO_2] treatments, across all three nutrient regimes, peaking around 8 June (Julian day 160) and falling at varying rates, depending on [CO_2] and nutrient treatment, to zero on or before 7 October (Julian day 280). Elevated [CO_2] and high-N supply rates produced seedlings with the highest summer R_w, 0.009 day^{-1} compared with 0.0075 day^{-1} for comparative seedlings growing in ambient [CO_2]. The mean annual relative extension rate (R_{aw}) was 10 % higher in the elevated compared to the ambient [CO_2] treatment for seedlings with high-N supply rate (Figure 4.3(a), $p = 0.05$). However, there was no significant effect of CO_2 concentration on R_{aw} with the low-N supply

rate. There was a bigger effect of nutrient treatment on R_{aw} under elevated [CO_2] compared with ambient [CO_2]. R_{aw} was stimulated by 96 % and 57 % when comparing seedlings supplied with the low-N and high-N rates for the ambient and elevated [CO_2] treatments, respectively. Seedlings supplied with the low-N rate had a significantly lower R_{aw} when grown in elevated [CO_2] compared to ambient [CO_2].

4.3.3 Relative growth rates

The mean RGRs expressed per growing season day (R_{sm}) and per day of the year (R_{dm}) are presented in Figure 4.3 (b) and (c) for each of the [CO_2] and nutrient treatments. Both [CO_2] and nutrient treatment had a significant effect on R_{sm} and R_{dm} ($p = 0.039$ for [CO_2] treatment on R_{dm} and $p < 0.001$ for [CO_2] treatment on R_{sm} and nutrient treatment on R_{sm} and R_{dm}). Nutrient supply rate had a bigger effect on both R_{dm} and R_{sm} than elevated [CO_2]. Increasing the nutrient supply rate from low-N to high-N stimulated R_{dm} on average by 52 % and R_{sm} by 26 %, while growth in elevated [CO_2] increased R_{dm} by 15.2 % and R_{sm} by 16.8 %.

4.3.4 Biomass allocation

Allocation of biomass between roots, stem, branches and needles for each of the CO_2 and nutrient treatments obtained from the February 1993 harvest are presented in Figure 4.4 (a) and (b). Both [CO_2] and nutrient treatment had a highly significant effect on biomass allocation to all components of the seedling ($p < 0.001$ except for the branches where $p = 0.048$). Increasing nutrient supply rate increased the biomass of all seedling components; roots, stems, branches and needles, in both of the OTC [CO_2] treatments and the outside control treatment (Figure 4.4(a)). Elevated [CO_2] increased root biomass irrespective of nutrient treatment compared to both the ambient [CO_2] and outside control treatments, although the biggest effect was in combination with the low-N supply rate (Figure 4.4(b)). Elevated [CO_2] increased

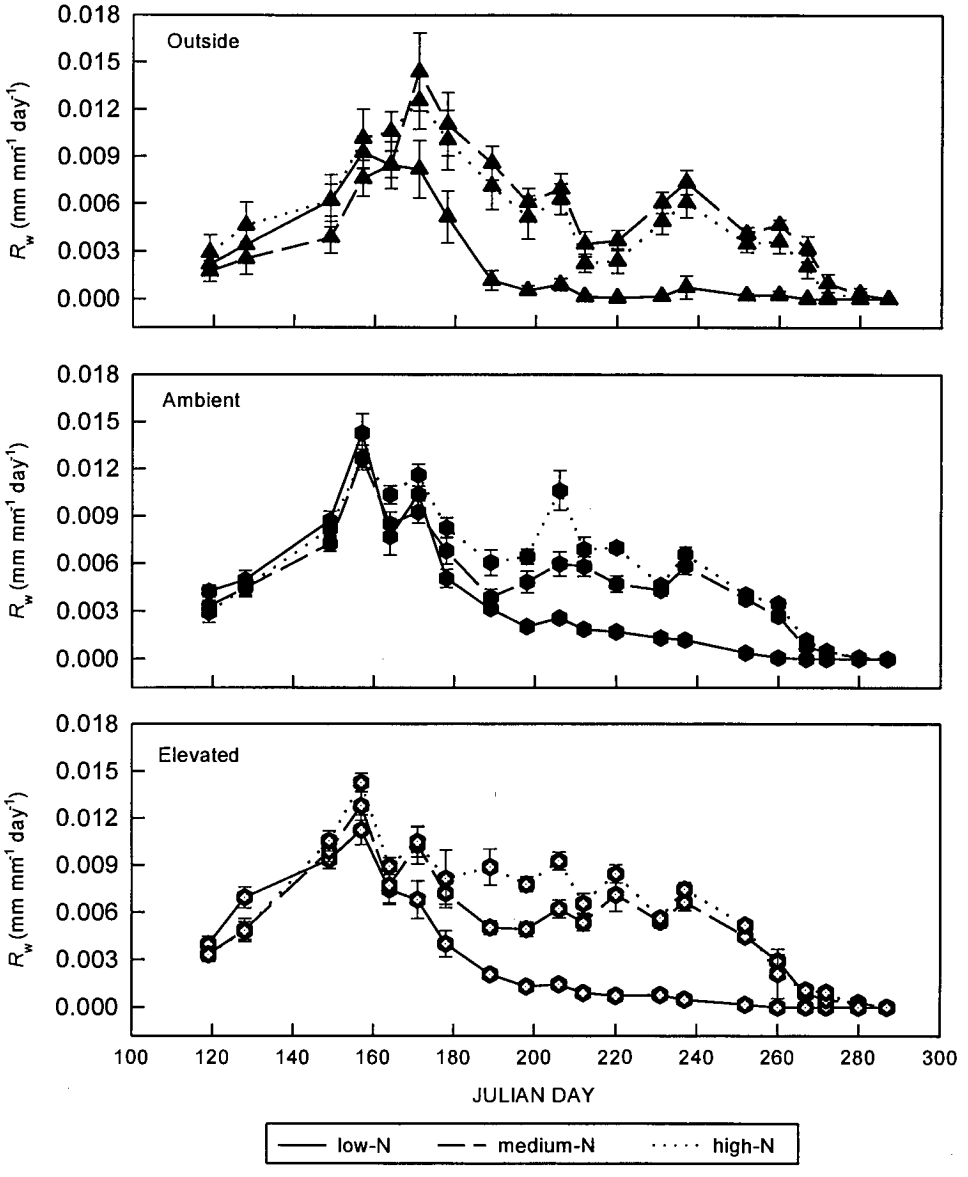

Figure 4.2: Effects of OTC, CO_2 concentration and nutrient application rate on relative weekly leader extension rates. For this and subsequent figures Outside = seedlings grown in an outside control plot under ambient CO_2 concentrations (*ca* 355 µmol mol^{-1}), Ambient = chamber grown, ambient CO_2 (*ca* 355 µmol mol^{-1}) treated seedlings, Elevated = chamber grown, elevated [CO_2] (*ca* 700 mol.. mol^{-1}), and low-N = 0.1, medium-N = 0.5 and high-N = 2.0 x optimum nutrient supply rates. Each point is the mean ± 1 SEM (n = 50 except for Outside treatment where n = 10)

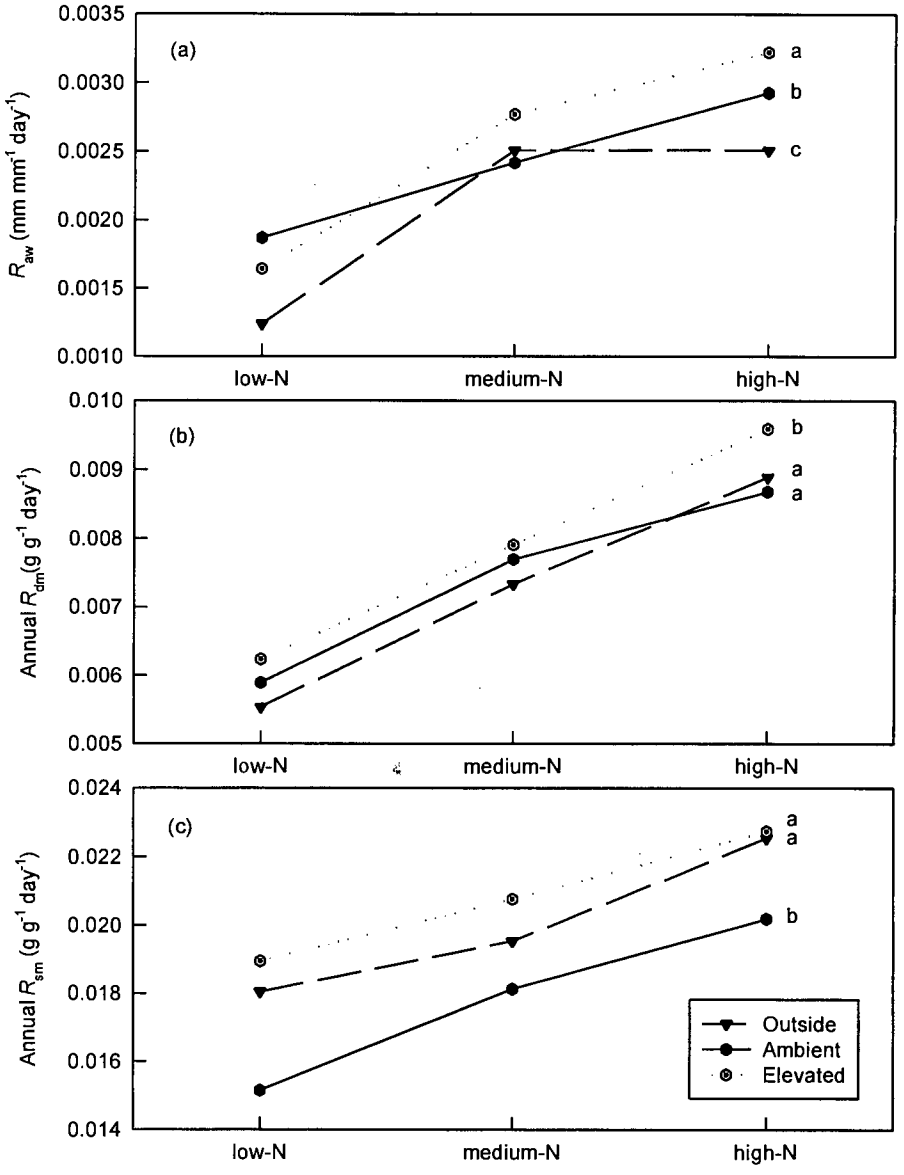

Figure 4.3: Effects of OTC, CO_2 concentration and nutrient application rate on (a) mean annual leader extension rates (R_{ah}), (b) annual total mass growth rates (R_{dm}) and (c) seasonal total mass growth rates (R_{sm}). Values are treatment means and lines followed by different letters indicate significant chamber and CO_2 treatments differences (ANOVA, LSD).

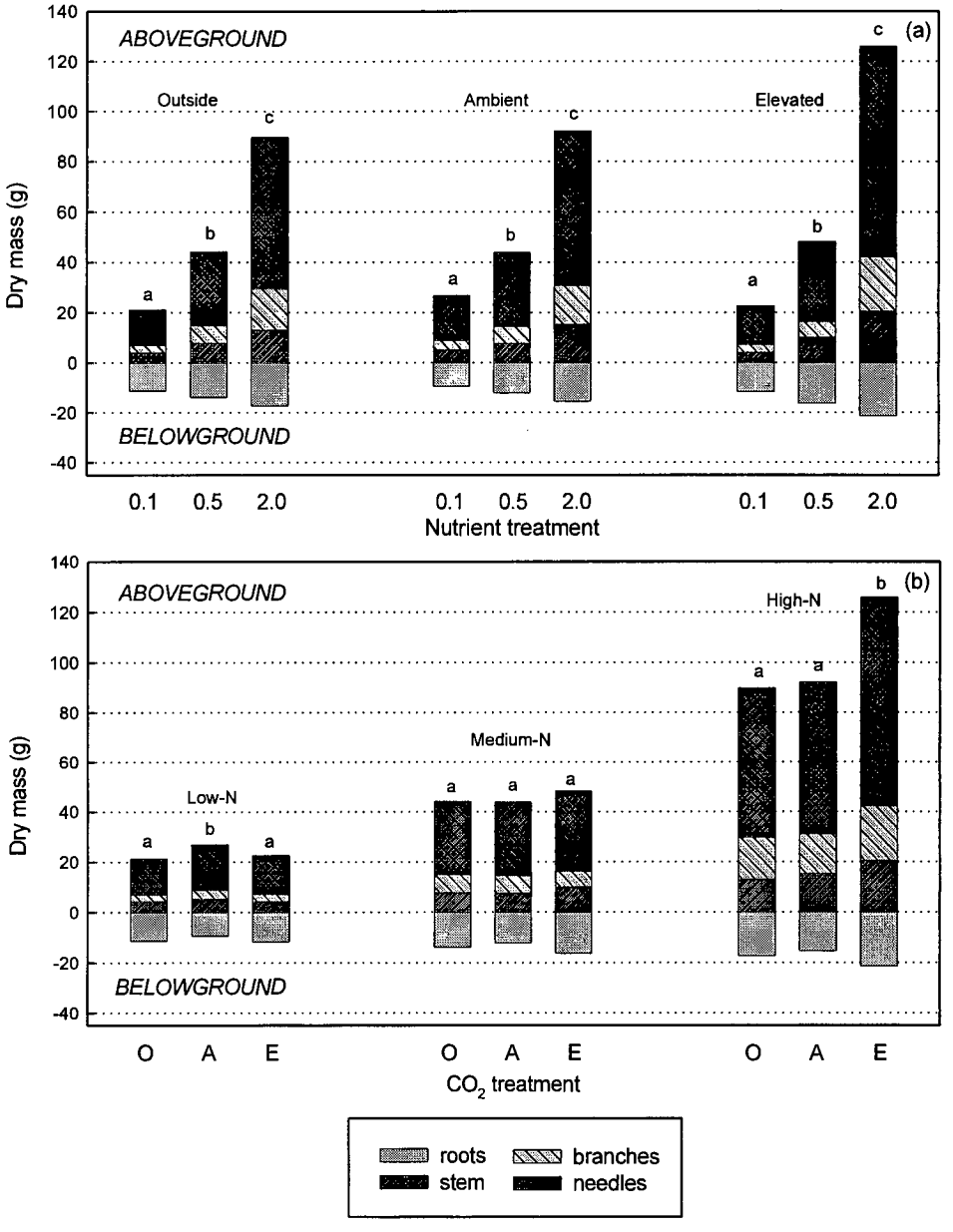

Figure 4.4: Effects of OTC, CO_2 concentration and nutrient application rate on above- and below ground- biomass allocation of Sitka spruce seedlings at the final harvest (February 1993). (a) represents the within CO_2 nutrient effects and (b) represents CO_2 treatment effects at each of the nutrient supply rates. n = 5, significant differences between [CO_2] and nutrient treatments are denoted by different letters above each column (ANOVA, LSD).

the amount of biomass in all four plant components when seedlings were supplied with the medium-N and high-N rates. There was also a significant [CO_2] and nutrient interaction on biomass allocation to the stem, branches and needles ($p < 0.001$), but not to the roots ($p = 0.219$).

Allocation of biomass to branch and stem components are presented in Figures 4.5 and 4.6, respectively. Irrespective of [CO_2] or nutrient treatment, the largest proportion of biomass within seedling branches was allocated to current year needles, then current year wood. Again nutrient treatment had a proportionally larger effect on allocation of biomass to current year needles and wood in the elevated [CO_2] treatment compared with either of the other two treatments (Figure 4.5(a)). There was no significant effect of elevated [CO_2] on branch biomass allocation in seedlings receiving the low-N or medium-N supply rates (Figure 4.5(b)). However, elevated [CO_2] significantly increased branch biomass allocation to current year needles and wood in seedlings receiving the high-N supply rate.

In all [CO_2] and nutrient treatments the largest proportion of stem biomass was held within previous years wood (>one-year old, plus secondary thickening) (Figure 4.6). Increasing nutrient supply rates increased biomass allocation to both current and previous years wood in both [CO_2] treatments (Figure 4.6(a)). Nutrient treatment had no significant effect on previous years' needle biomass. Elevated [CO_2] significantly reduced current year needle and stem wood biomass when seedlings were supplied with the low-N rate (Figure 4.6(a)and (b)). With the medium-N and high-N supply rates elevated [CO_2] increased both previous and current year stem wood biomass.

Differences in root to shoot mass ratio (R/S) are presented in Figure 4.7 for the [CO_2] treatment and outside control at each of the three nutrient treatments. There was a

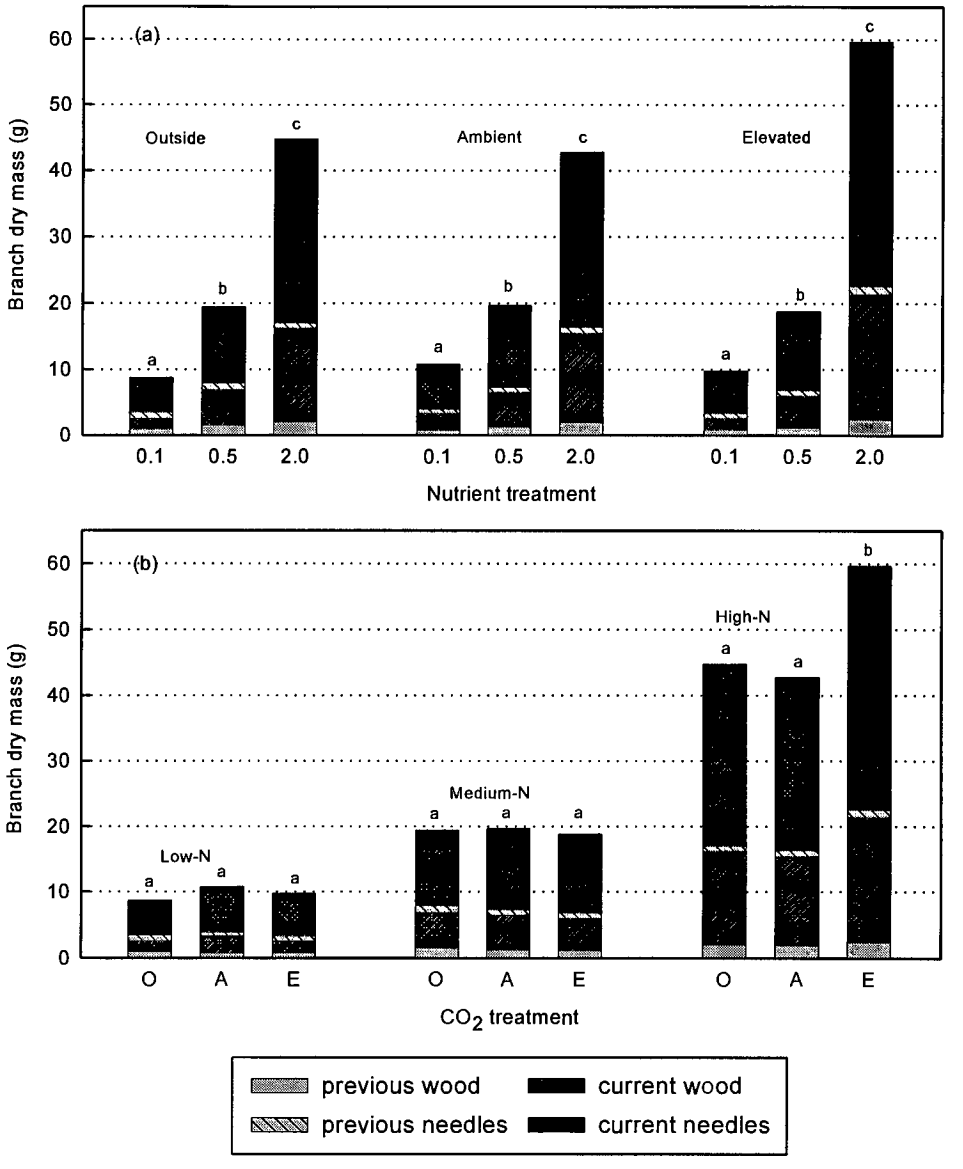

Figure 4.5: Effects of OTC, CO_2 concentration and nutrient application rate on biomass allocation within branches of Sitka spruce seedlings at the final harvest (February 1993). (a) represents the within CO_2 effects and (b) represents CO_2 treatment effects at each of the nutrient supply rates. Previous wood = wood+bark > one-year old plus secondary thickening, previous needles = needles >one-year old, current wood = wood < one-year old and current needles = needles < one-year old. n = 5, significant differences between [CO_2] and nutrient treatments are denoted by different letters above each column (ANOVA, LSD).

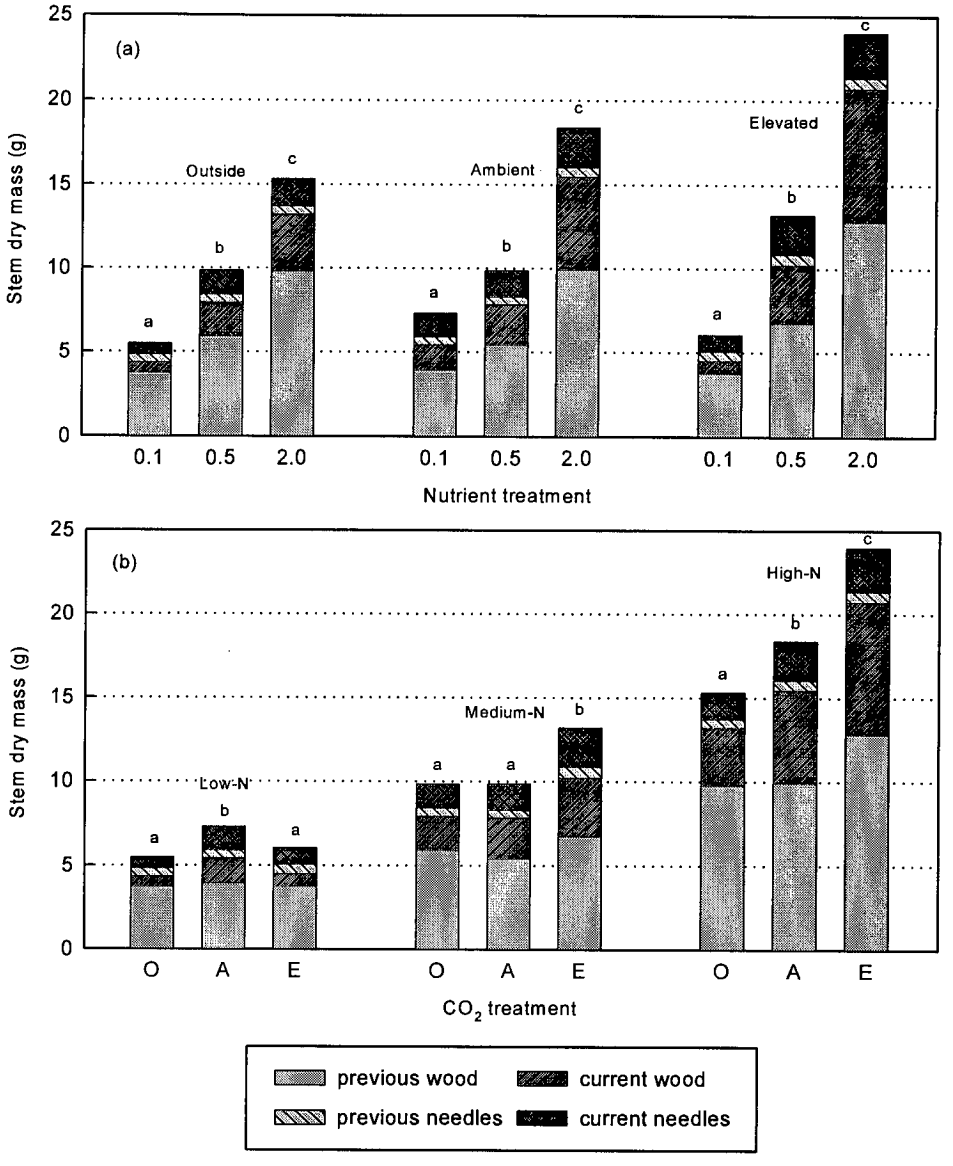

Figure 4.6: Effects of OTC, CO_2 concentration and nutrient supply rate on biomass allocation within stem of Sitka spruce seedlings at the final harvest (February 1993). (a) represents the within CO_2 nutrient effects and (b) represents CO_2 treatment effects at each of the nutrient supply rates. Previous wood = wood +bark >one-year old plus secondary thickening, previous needles = needles >one-year old, current wood = wood <one-year old and current needles = needles <one-year old. n = 5, significant differences between [CO_2] and nutrient treatments are denoted by different letters above each column (ANOVA, LSD).

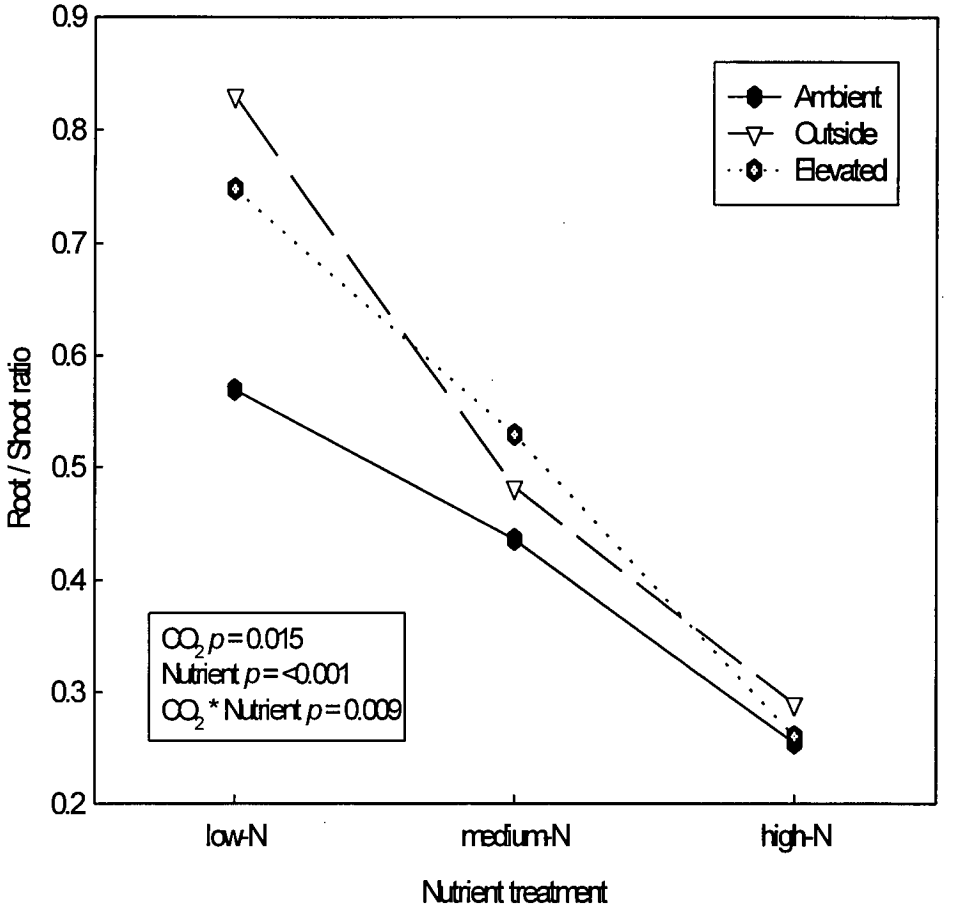

Figure 4.7: Effects of OTC, CO_2 concentration and nutrient supply rate on Root/Shoot mass ratio of Sitka spruce seedlings at the final harvest (February 1993). Values are treatment means, n = 5 and significance (ANOVA).

significant effect of both [CO_2] and nutrient treatment on R/S (p = 0.015 and <0.001, respectively). There was also a significant CO_2 x nutrient interaction on R/S (p = 0.009). In the low-N treatment, elevated [CO_2] increased R/S by 31 % compared with the equivalent chamber-grown, ambient [CO_2] seedlings. However, under less nutrient limiting conditions (i.e. the high-N treatment) elevated [CO_2] had no

significant effect on R/S. Increasing nutrient supply rate from low-N to high-N significantly decreased the R/S by 188, 124 and 186 % in the elevated, ambient and outside control treatments, respectively. Increasing nutrient supply rate from low-N to high-N had a bigger and opposite effect on R/S than doubling atmospheric CO_2 concentration.

Figure 4.8 shows the proportional change in biomass distribution between roots, stems, branches and needles for each of the [CO_2] treatments and the outside control with increasing nutrient supply rate. As shown above, in all cases as the relative proportion of biomass allocated to the roots decreased with increasing nutrition, the proportion of branches and needles increased. The biggest proportional change as a result of shifts in root allocation was in branch wood, (see exploded section Figure 4.8). There was no significant increase in the proportion of carbon allocated to stem wood in any of the treatments.

4.3.5 Foliar analysis

Total seedling leaf area (TLA), total seedling leaf mass (TLM), specific leaf area (SLA), leaf area ratio (LAR) and leaf mass ratio (LMR) obtained from the February 1993 harvest are presented in Table 4.4 for each of the [CO_2] and nutrient treatments. TLA and TLM were stimulated by both elevated [CO_2] and increasing nutrient supply rate (Table 4.4). There was also a significant CO_2 x nutrient interaction on TLA and TLM, as seedlings receiving the low-N supply rates were unaffected by [CO_2]. Elevated [CO_2] increased TLA by 43 % and TLM by 37 % when seedlings were supplied with the high-N rate. There was no significant effect of elevated [CO_2] on SLA, LAR or LMR but a highly significant effect of nutrient supply rate. Increasing nutrient supply rates from low-N to high-N reduced SLA by 12 % and 18%, increased LAR by 6 % and 5.5 % and LMR by 18 % and 22 %, for the ambient and elevated [CO_2] treatments, respectively.

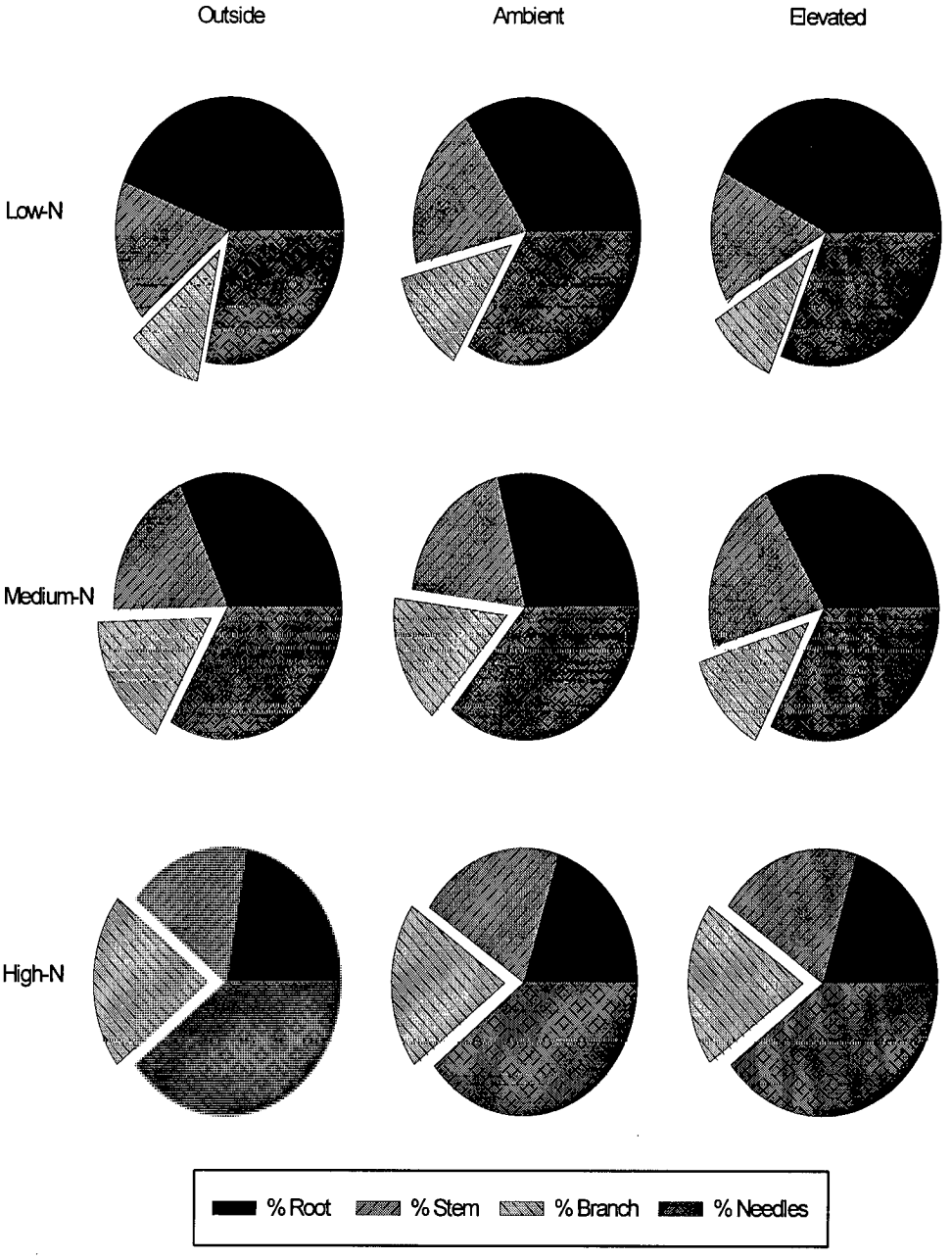

Figure 4.8: Effects of OTC, CO_2 concentration and nutrient application rate on the proportional distribution of total seedling dry mass of each of the four main plant components at the final harvest. Exploded section shows the branch wood component which was most affected by shifts in biomass allocation to roots.

Table 4.4: Effects of CO_2 concentration, nutrient application rate and open top chamber on leaf parameters of two-year-old Sitka spruce after 23 months CO_2 and 12 months nutrient treatment. Values presented are the treatment means and significance level (ANOVA). Significant differences within both the CO_2 and nutrient treatments, for each of the parameters are indicated by a different letter (LSD). Letters presented across rows are CO_2 treatment differences and within columns are nutrient treatment differences.

Parameter	x optimum nutrient treatment	OTC and ambient CO_2	OTC and elevated CO_2	Outside and ambient CO_2	*p* value		
					CO_2	nutrient	CO_2 x nutrient
TLA	0.1 a	445	452	430	0.021	<0.001	<0.001
	0.5 b	696	719	667			
	2.0 c	1300	1857	1504			
		a	b	ab			
TLM	0.1 a	9.22	8.52	7.18	<0.001	<0.001	<0.001
	0.5 b	15.03	15.68	14.31			
	2.0 c	30.09	41.34	30.48			
		a	b	a			
SLA	0.1 a	48.81	53.39	59.93	0.568	<0.001	0.118
	0.5 b	47.04	46.16	46.75			
	2.0 b	43.48	45.22	50.91			
LAR	0.1 a	16.05	16.83	16.82	0.894	0.010	0.054
	0.5 a	16.94	14.97	15.36			
	2.0 b	17.09	17.73	20.37			
LMR	0.1 a	0.33	0.32	0.28	0.091	<0.001	0.184
	0.5 b	0.36	0.33	0.33			
	2.0 c	0.39	0.39	0.40			

4.3.6 Foliar nutrient analysis

Table 4.5 shows the macronutrient concentration of current year (<one-year old) and previous year (>one-year old) foliage. Nutrient treatment significantly affected nitrogen [N], phosphorus [P] and potassium [K] concentrations in both current and previous years' foliage. The concentration of all three macronutrients decreased with lower nutrient supply rates. On average current year foliar [N], [P], and [K] were 130, 32 and 36 % lower in the low-N compared with the high-N treatment,

respectively. Foliar [N] was consistently lower in elevated [CO_2] compared with ambient [CO_2] grown plants, irrespective of age class or nutrient supply rate ($p \leq 0.001$). The total reduction in [N] as a result of elevated CO_2 was biggest under the low-N treatment, 44 %, 11 % and 8 % for the low-N, medium-N and high-N treatments, respectively. There was no significant [CO_2] effect on [P] or [K], although values were consistently lower in seedlings receiving elevated [CO_2]. Foliar [C] was roughly 51 % of dry mass in all treatments. Foliar nutrient concentrations of plants grown inside OTCs under ambient [CO_2], were not significantly different from those in the control plot, irrespective of nutrient treatment.

Reductions in [N], as a result of decreasing nutrient supply rate and elevated [CO_2], led to significant increases in foliar carbon to nitrogen mass ratios (C/N). Elevated [CO_2] increased C/N ratios by 35 and 8 % when seedlings were supplied with low-N and high-N rates, respectively. Increasing nutrient supply rate from low-N to high-N reduced the C/N ratio by 106 and 156 % for the ambient and elevated [CO_2] treatments, respectively.

The total amounts of macronutrients and carbon in both the current and previous years' foliage are presented in Figure 4.9. The current year foliar content of N, P, K and C of seedlings grown in elevated [CO_2] with the low-N supply rate, was smaller than that of their counterparts in ambient [CO_2]. But when nutrients were supplied at the high-N rate both macronutrients and carbon content were increased by elevated [CO_2]. Elevated [CO_2] also increased the amount of N, P, K and C in the previous years' foliage, irrespective of nutrient supply rate.

Table 4.5: Effects of CO_2 concentration, nutrient application rate and open top chamber on nutrient content of 2-year-old Sitka spruce [*Picea sitchensis* (Bong.) Carr.] after 23 months CO_2 and 12 months nutrient treatment. n = 5, Significance (ANOVA)

				p value		
% dry mass	x optimum nutrient treatment	OTC and ambient CO_2	OTC and elevated CO_2	CO_2	nutrient	CO_2 x nutrient
Nitrogen						
Current year foliage	0.1	1.3	0.9	0.001	<0.001	0.38
	0.5	2.1	1.9			
	2.0	2.6	2.4			
Previous year foliage	0.1	1.3	1.2	0.012	<0.001	0.665
	0.5	1.9	1.6			
	2.0	2.2	2.1			
Phosphorus						
Current year foliage	0.1	0.22	0.19	0.091	<0.001	0.605
	0.5	0.26	0.24			
	2.0	0.28	0.26			
Previous year foliage	0.1	0.18	0.18	0.942	0.003	0.545
	0.5	0.24	0.23			
	2.0	0.23	0.26			
Potassium						
Current year foliage	0.1	0.90	0.83	0.262	<0.001	0.596
	0.5	0.85	0.84			
	2.0	1.06	1.05			
Previous year foliage	0.1	0.77	0.73	0.630	<0.001	0.488
	0.5	0.83	0.84			
	2.0	1.00	1.07			
Carbon						
Current year foliage	0.1	52.8	51.4	0.325	0.318	0.937
	0.5	52.8	51.2			
	2.0	53.4	52.2			
Previous year foliage	0.1	51.2	51.8	0.518	0.217	0.031
	0.5	51.6	49.8			
	2.0	51.8	50.4			
C / N ratio						
Current year foliage	0.1	42.0	56.8	0.007	<0.001	0.003
	0.5	25.5	26.8			
	2.0	20.4	22.2			
Previous year foliage	0.1	40.5	45.7	0.019	<0.001	0.699
	0.5	27.2	34.3			
	2.0	23.7	24.1			

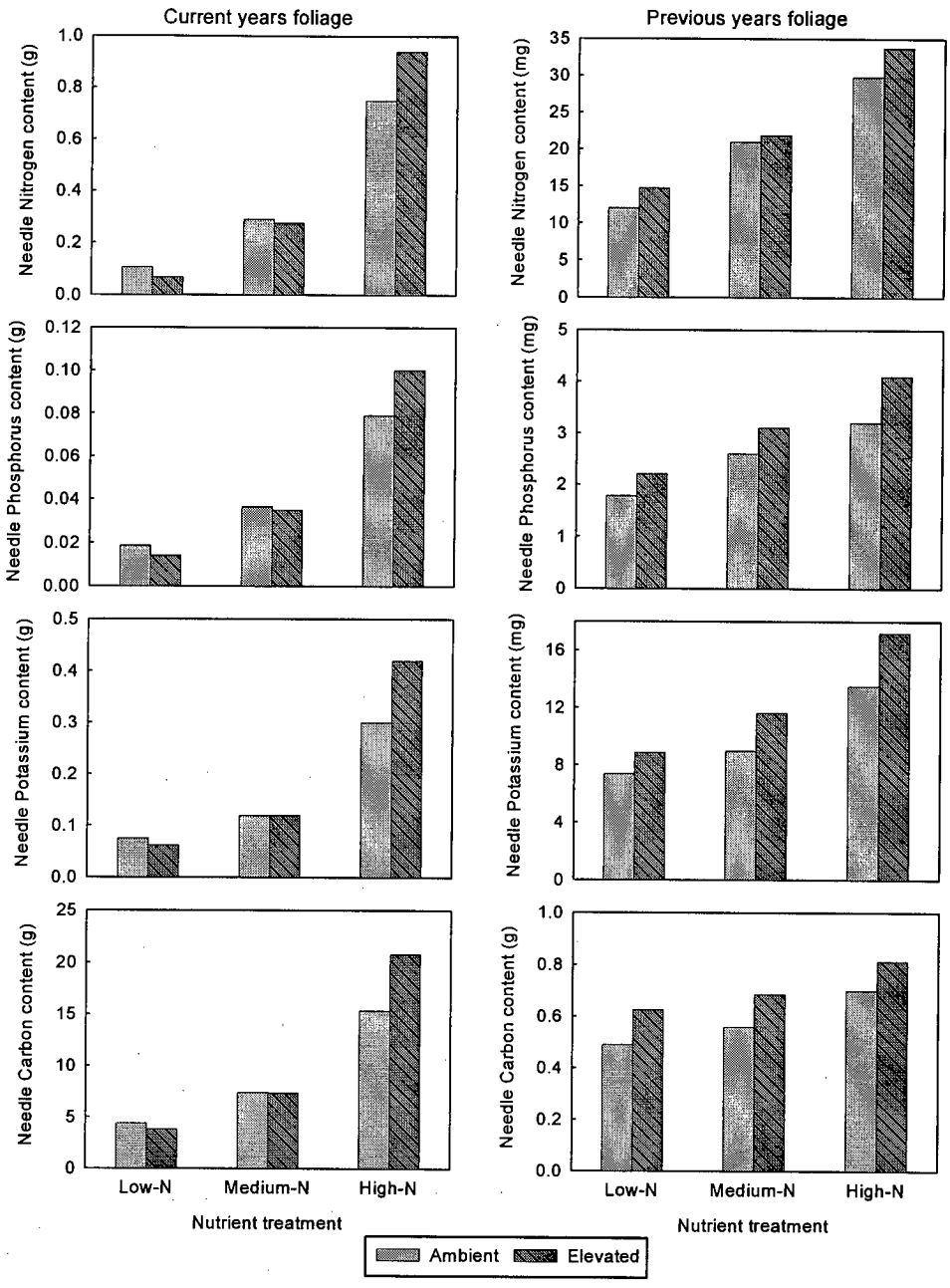

Figure 4.9: Total amount of nitrogen partitioned between current year foliage (<one-year old) (*left hand column of graphs*) and previous year foliage (>one-year old) (*right hand column of graphs*) for each of the CO_2 and nutrient treatments. n = 5, columns are means.

4.3.7 Open-Top chamber effect on biomass accumulation and partitioning

At the end of the experiment there was no significant difference in either the total dry mass, R_{dm} or leaf parameters of seedlings grown for 12 months either inside OTCs or outside in a control plot (see Figure 4.3(b) and Tables 4.2 and 4.3). However there was a significant effect of OTC on total plant height and R_{sm} (Figure 4.3 (c) and Table 4.2). Ambient [CO_2] treated seedlings grown inside OTCs were on average 25 % taller than those grown outside in the control plot, despite having a consistently lower R_{sm} across all nutrient treatments. Consequently, total stem biomass was significantly lower in the outside control vs ambient [CO_2] seedlings (Figure 4.6). In contrast, chamber grown seedlings supplied with the low-N rate had a 46 % smaller root biomass than their counterparts grown outside (see Figures 4.4 & 4.8). Thus severe nutrient limitation inside OTCs, i.e. the low-N treatment, significantly reduced the R/S ratio compared to outside OTCs (Figure 4.7). Therefore, the effect of OTCs on the growth of Sitka spruce appears to be one of a redistribution of carbon fixed rather than carbon fixation *per se*.

4.4 Discussion

4.4.1 Biomass production

Elevated [CO_2] has almost always been reported to produce an overall increase in growth and total plant biomass accumulation for the majority of C_3 tree species (El Kohen, *et al.*, 1993; Poorter, 1993; Tissue, *et al.*, 1996; Rey and Jarvis, 1997). More specifically, elevated [CO_2] has been shown to consistently stimulate biomass production of Sitka spruce (Canham and McCavish, 1981; Townend, 1993; Murray *et al.*, 1996; Barton, 1997). The results from this study support this, with elevated [CO_2] significantly increasing total biomass in two of the three nutrient treatments. The effect of elevated [CO_2] on biomass stimulation was largest when seedlings received the highest nutrient supply rate. The 37 % stimulation in dry mass

accumulation found in this study as a result of growth in elevated [CO_2] when seedlings were supplied with high-N rates, is very similar to the average values of 41 and 32 % found for a range of forest tree species by Poorter (1993) and Wullschleger, *et al.*, (1995), respectively.

The results from this study also serve to confirm 'Liebigs law' of the minimum. Total biomass accumulation was unaffected by elevated CO_2 when seedlings received the lowest nutrient supply rate. Therefore, it can be assumed that for Sitka spruce seedlings, atmospheric CO_2 concentration is limiting growth when current year foliar [N] is 1.9 % or above (medium-N and high-N treatments), while at concentrations around 0.9 % or below (low-N treatment), nutrient supply rate is the primary factor limiting growth. Despite the use of a range of nutrient application techniques; including an unbalanced regime imposed by Prior *et al.* (1997) and a modified "Hoagland solution" applied by Gebauer *et al.* (1996), results similar to those presented here have been found in *Pinus palustris* (Prior *et al.*, 1997) and *Pinus taeda* (Gebauer *et al.*, 1996). In both cases elevated [CO_2] only increased biomass production in their 'high-N' treatment regimes. The relevance of 'Liebig's Law' in determining the growth response of trees to elevated [CO_2] is discussed more fully in Appendix A.

4.4.2 Relative growth rates

Although there are three basic phenological patterns of C allocation within woody plant species, determinate (or fixed), indeterminate (or free) and semideterminate (or recurrent flushing), the majority of Temperate conifers possess a determinate growth pattern (Gower *et al.*, 1995; Kozlowski, 1992). This pattern of growth is characterised by a single, short burst of shoot growth in the late spring and early summer followed by a long lag period to budset. Distribution of the assimilate is dependent on this flushing cycle, with most of the assimilate (i.e. >90 %) allocated

to shoots during the early flushing episode, but then directed (i.e. > 95%) to the lower stem and roots during the later lag stage (Gower *et al.*, 1995). Confirmation of this growth pattern in Sitka spruce is evident from the data presented on weekly relative height growth rates (R_w) (Figure 4.2). R_w increased rapidly from late April to a peak in early June and then declined steadily until mid-September. Consequently changes in the magnitude or pattern of R_w as a response to either elevated [CO_2] or nutrient supply rate will have an effect not only on R_w directly, but also on biomass accumulation and allocation as well. Annual RGR of both biomass and leader extension (R_{dm} and R_{aw}, respectively) were significantly higher in seedlings grown in elevated [CO_2] with a high rate of N supply, but not with a low rate of N supply, thus demonstrating a clear interaction between atmospheric CO_2 concentration and nutrient supply rate on RGR. Other studies on tree species which have examined the effect of elevated [CO_2] on growth rates in response to varying nutrient supply rates have reported similar results for *Betula pendula* (Petterson *et al.*, 1993), *Pinus taeda* (Tissue et al., 1996), *Picea glauca, Populus tremuloides* (Brown and Higginbotham, 1986).

Because the response of tree seedlings differ substantially from that of mature trees, in terms of morphology, phenology and physiology (Eamus, 1996) the relevance of studies reporting increased RGR in the early stages of tree development has been questioned. However, there is much evidence in the current literature to support the importance of early stimulation in RGR in determining long term biomass productivity. Several studies have reported a decline in RGR stimulation as a result of elevated CO_2 with time (Petterson *et al.*, 1993; Norby et al., 1995; Tissue *et al.*, 1996; Rey and Jarvis, 1997), thus bringing into question the relevance of earlier findings. It has been shown, however, that this early stimulation in RGR can led to long-term biomass enhancements of trees grown in elevated [CO_2] (Kramer, 1981;

Rey and Jarvis, 1997) and is therefore of importance to future growth and productivity.

4.4.3 Biomass allocation

Significant changes in both the above- and below-ground biomass allocation were found in response to [CO_2] and nutrient treatments and there was a highly significant interaction between [CO_2] and nutrient supply rate on the response of R/S mass ratio, and branch, stem and needle dry mass allocation.

One of the most pronounced and widely published effects of elevated [CO_2] on biomass allocation patterns is enhanced supply of carbon to the roots, resulting in a stimulation of the R/S mass ratio (Norby *et al.*, 1986*a*, 1992; Rochefort and Bazzaz, 1992; Rogers *et al.*, 1992; Stulen and Den Hertog, 1993; Murray, *et al.*, 1996). There are, however, also reports of R/S ratios being unchanged (Conroy *et al.*, 1986; Hollinger, 1987; Radaglou and Jarvis, 1990) and even decreased (Tolley and Strain, 1984) in elevated CO_2.

In this study, the stimulation in R/S attributable to elevated [CO_2] was nutrient dependant. With the low N supply rate, elevated [CO_2] stimulated R/S by 31 % but there was no increase in the high-N treatment. These results are consistent with a number of studies which have reported nutrient dependancy of the root response of seedlings to elevated [CO_2] (e.g. Brown and Higginbotham, 1986; Pettersson *et al.*, 1993). Therefore, it is likely that much of the variability in the reported root responses to elevated [CO_2] is a result of differing experimental regimes and in particular different plant nutrient status. Although species differences can not be ruled out, the range of results reported just for Sitka spruce, serves to substantiate the importance of experimental regime when trying to predict R/S responses to future CO_2 concentrations. In two separate studies Murray *et al.* (1996) and Barton (1997)

reported increased R/S mass ratios in response to elevated [CO_2], while in a third study R/S was unaffected by elevated [CO_2] (Townend, 1993). The difference in the results reported by these studies was probably a result of differences in foliar [N]. In the first two studies foliar [N] were around 1.7 % while in the third it was above 2.5 %.

From the response of Sitka in the present study to increasing nutrient supply rates (R/S decreased between 124 and 186% between the low-N and high-N treatments for the ambient and elevated CO_2 treatments, respectively) it can be concluded that increasing plant nutrition from limiting to non-limiting rates has a bigger influence on R/S than doubling atmospheric CO_2 concentration. In addition, the effect of increasing nutrient supply rate on R/S is enhanced in elevated [CO_2]. Pettersson *et al.* (1993) also concluded that in general R/S ratio is thought to remain constant when plants are grown in non-limiting nutrient conditions, whereas in nutrient-limited conditions carbon is preferentially allocated to roots.

An interesting aspect of biomass allocation was the effect of elevated [CO_2] and nutrition on the proportions of biomass allocated between the roots and branches. Although all four major plant components, roots, stem, branches and needles were significantly increased by elevated [CO_2], in the medium-N and high-N treatments, the proportions of each plant component differed. Irrespective of [CO_2], as the proportion of carbon allocated to the roots decreased with increasing nutrient supply rates, the proportion of branch biomass increased. There was an increase in the proportion of needle biomass as well but its relative increase was less than that of branches. Therefore, it would appear that at this early stage in development of Sitka spruce, structural branch biomass and to a lesser extent needles are more important carbon sinks than stem biomass as nutrient uptake increased and the demand for carbon allocation to the roots decreased. Interestingly, Norby *et al.* (1992) found a

very similar result after growing yellow-poplar saplings in elevated [CO_2] for almost three growing seasons.

4.4.4 Foliar responses

In this study, both TLM and TLA were significantly increased by elevated [CO_2] when seedling were supplied with the high-N rate and by increasing nutrient supply rates *per se*. Increases in foliar biomass and area in response to elevated [CO_2] have been commonly found across a wide range of tree species; *Betula pendula* (Rey and Jarvis, 1997), *Eucalyptus grandis* (Conroy *et al.*, 1992), *Liriodendron tulipifera* (Norby *et al.*, 1992), *Picea sitchensis* (Barton, 1997), *Pinus palustris* (Prior et al., 1997), *Populus x euramericana* (Curtis *et al.*, 1995). The magnitude of this response to elevated [CO_2] is often reduced as nitrogen availability declines, a result evident from the decline in stimulation of both TLM and TLA with decreasing supply rates found in this study. However, the opposite effect has also been reported, Townend (1993) found a significant reduction in the percent of biomass allocated to needles in four clones of *Picea sitchensis*, this result was only found in the present study under low-N supply rates, where carbon was preferentially allocated to the roots.

Because specific leaf area (SLA) is a compromise between maximising instantaneous C gain and long-term C, water and nutrient use efficiency, it is not surprising that it is strongly linked to any change in atmospheric CO_2 concentrations or nutrient availability. Although decreases in SLA have often been observed in plants grown in elevated [CO_2] (Rogers *et al.*, 1983*a*), a response often attributed to foliar starch accumulation, we found no evidence of any change in SLA as a result of elevated [CO_2]. Recently several studies, although reporting increased starch contents under elevated [CO_2], have also reported no effect on SLA (Barton, 1997; Prior *et al.*, 1997; Rey and Jarvis, 1997). It has also been reported that low nutrient availability reduces SLA (Gower *et al.*, 1995), again as a result of starch accumulation. We

found no evidence of this, instead there was a highly significant effect of nutrient treatment on SLA, in the opposite direction.

4.4.5 Nutrition

When plants are grown in elevated [CO_2] compared with ambient [CO_2] a decline in tissue nutrient concentrations, in particular [N], is generally observed, this has widely become known as the nutrient 'dilution' effect (Brown, 1991; Coleman *et al.*, 1993; Luo *et al.*, 1994; Gebauer, *et al.*, 1996). In this study, both [CO_2] and nutrient treatment had significant effects on foliar [N]. Tissue [N] was between 8 and 44 % lower in elevated [CO_2] than in ambient [CO_2], depending on nutrient supply rates. The biggest effect of elevated [CO_2] on [N] was found at the lowest nutrient supply rate.

Local wet and dry deposition rates of ammonia (NH_3), ammonium (NH_4) and nitrate (NO_3) amount to an annual nitrogen deposition of 15.9 kg N ha^{-1} for the Bush site (Fowler, D. *pers comm*). This value is very close to the UK mean deposition rate of ~ 15 kg N ha^{-1}. Although such a deposition rate is not particularly small, and would have contributed to the overall amount of N available to the plant, its effect on the nutrient treatments applied would appear to be both minimal and independent of whether seedlings were grown inside or outside OTCs. The fact that the low-N treatment induced a N deficiency in the seedlings and seedlings grown inside OTCs in ambient [CO_2] had the same foliar [N], irrespective of nutrient supply rate, demonstrates the minimal input of N by natural deposition processes and lack of chamber effect on N deposition rates, respectively.

Because root biomass was always larger in elevated [CO_2] and the R/S mass ratio increased with the low nutrient supply rate, the potential for N sequestration from nutrient poor soils is liable to increase. Because increased N sequestration is likely

to lead to bigger plants, and the seedlings in this study grown in elevated [CO_2] with low-N supply rates had the same biomass and lower foliar [N] than those grown in ambient [CO_2], it is unlikely that there was any change in N uptake rates in this study. Instead it would appear that for a lower foliar [N] elevated [CO_2] produced the same amount of biomass, thereby increasing nutrient use efficiency (NUE), also evident from the increase in C/N ratios. Although it has been proposed that NUE will increase with elevated [CO_2] irrespective of soil N availability, our results show that this response is diminished when nutrients are available at high rates.

Interestingly, there was no effect of CO_2 concentration on the other macronutrients, specifically P and K, nor on total C, evidently supporting the concept that the nutrient 'dilution' effect is specific to N and that fine adjustments in either its distribution within the plant or the plants metabolic requirements have occurred. From the data presented in Figure 4.9 it would appear that nutrient acquisition rates (N, P and K) were also increased in elevated [CO_2]. Since elevated [CO_2] has a larger effect on foliar [N] compared with [P] and [K] it is likely that the biomass accumulation and allocation responses presented in this study are a result of changes in N supply rates rather than either P or K.

4.4.6 Open top chamber effect

The biggest effect of open top chamber on the growth of Sitka spruce was not on accumulation of carbon *per se*, but on its allocation within the plant. Because of the determinate nature of the growth pattern and the effect this has on sink priorities (see above), it is not surprising that chambers, and more specifically the increase in temperature attributed to them, influences growth. OTCs have a significant effect on bud phenology of Sitka spruce (Chapter 6; Murray *et al.*, 1994), essentially extending the growing season and hence the period of shoot elongation. Because shoots have a greater priority in terms of carbon sinks than roots, any extension in

the period of shoot and, in particular stem elongation can only serve to reduce the amount of carbon available for root growth.

The results from Chapter 3 show that there was no significant effect of OTC on either the photosynthetic PPFD response curve or on A_{mmax}, and therefore, it may be assumed that carbon fixation was similar both inside and outside the OTCs. This is supported by the lack of evidence of any reduction in biomass accumulation in seedlings grown outside in the control plot. Plants grown inside OTCs are taller with lower R/S ratios than those grown outside.

4.5 Conclusions

The majority of tree growth responses to elevated [CO_2] are influenced by N availability and the results presented in this study on Sitka spruce are no exception. The distribution of Temperate conifer forests, and in particular Sitka spruce plantations, is predominantly on low fertility soils, therefore, it is more than likely that soil N availability will be the controlling resource determining growth and biomass allocation and accumulation responses to elevated [CO_2] in the future.

Under nutrient poor conditions the effect of elevated [CO_2] on Sitka spruce will be to decrease aboveground biomass and hence reduce aboveground net primary productivity. However, under nutrient rich conditions elevated [CO_2] will increase aboveground net primary productivity. The effect of increasing nutrient supply rates on all biomass parameters was always bigger than that produced by increasing atmospheric CO_2 concentrations.

4.6 Final conclusions

The experiment described here is one of only a few which have examined the interaction between elevated [CO_2] and a range of nutrient supply rates.

- Elevated [CO_2] stimulated annual relative growth rates when nutrients were supplied at high enough rates. Although, there was no effect of [CO_2] on RGR at the low-N supply rate.
- Total seedling biomass was significantly increased by elevated [CO_2] when nutrients were supplied at the medium and high-N rates but not at the low-N rate. Therefore, net primary productivity will only increase in elevated [CO_2] when nutrients are not limiting growth.
- Biomass allocation was significantly affected by both elevated [CO_2] and nutrient supply rate. Increasing nutrient supply rate increased the proportion of biomass allocated to aboveground components compared to roots. Elevated [CO_2] increased the R/S ratio when seedlings were supplied with low-N rates, but not at the higher supply rates. Therefore, aboveground net primary productivity will be reduced in elevated [CO_2] under nutrient poor conditions.
- As the proportion of biomass allocated to roots decreased with increasing nutrient supply rate, the proportion of branch wood biomass and to a lesser extent branch needle biomass increased.
- Foliar [N] was decreased in elevated [CO_2] irrespective of nutrient supply rate but biomass was unaffected or increased, therefore, growth in elevated [CO_2] increased nutrient use efficiency.

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CHAPTER 5

Effect of elevated [CO_2] and varying nutrient application rates on gas exchange of Sitka spruce (*Picea sitchensis* (Bong.) Carr.)

Abstract

Sitka spruce (*Picea sitchensis* (Bong.) Carr.) seedlings were supplied with either 0.1(low-N) or 2.0 (high-N) x optimum nutrient solutions and grown outside in a control plot or inside open-top chambers and exposed to either ambient, 355 μmol mol^{-1} or elevated, 700 μmol mol^{-1} CO_2. Gas exchange measurements, chlorophyll determinations and nutrient analysis were made on current year (<one-year-old) shoots on the upper whorl after 17 months [CO_2] and six months nutrient treatment.

Nutrient treatment had a significant effect on the PPFD response, irrespective of [CO_2] or chamber treatment; seedlings supplied with high-N rates had higher net photosynthetic rates compared with those supplied with low-N rates. The degree of photosynthetic stimulation as a result of increased [CO_2], was larger in seedlings receiving the high-N compared with those receiving the low-N supply rate. PPFD-saturated net photosynthesis of seedlings grown and measured in elevated [CO_2] was *ca* 26 % higher than that of trees grown and measured in ambient [CO_2]. There was a strong linear correlation between measured dark respiration rates and foliar [N], irrespective of nutrient supply rate, [CO_2] or OTC.

There was no significant effect of [CO_2] or chamber treatment on the CO_2-response of seedlings receiving the high-N supply rate. In contrast, when seedlings were supplied with low-N, the CO_2-response of those growing in elevated [CO_2] was significantly different from those grown in ambient [CO_2], both V_{cmax} and J_{max} were lower in elevated [CO_2]. These results demonstrate that acclimation of photosynthetic processes to elevated [CO_2] occurs at low nutrient supply rates.

There was no effect of elevated [CO_2] on stomatal conductance (g_s). However when measurements were made at treatment CO_2 concentrations, elevated [CO_2] seedlings had a higher net assimilation rate for a given g_s. Stomatal conductance was highly dependant on foliar [N] ranging from ~60 mmol m^{-2} s^{-1} at ~1.5 g N m^{-2} to 200 mmol m^{-2} s^{-1} at ~5 g N m^{-2}.

Foliar [N] was 10 and 28 % lower in the elevated compared to ambient [CO_2] grown seedlings for the high-N and low-N treatments, respectively. There was no effect of [CO_2] on foliar [P] but [P] was ~20 % lower in seedlings receiving the low-N supply rate compared to those receiving the high-N supply rate. There was no apparent difference in the nutrient composition of chamber grown plants compared with plants grown outside in the control plot.

Total chlorophyll concentration increased with increasing N supply in all treatments. There was no significant effect of elevated [CO_2] on specific leaf area. Thus, chlorophyll concentration expressed either on an area or dry mass basis for a given foliar [N] was higher in seedlings grown in elevated [CO_2] compared with those grown in ambient [CO_2].

Keywords: Picea sitchensis, elevated CO_2, open-top chambers, nutrients, Ingestad, photosynthesis, stomatal conductance, dark respiration, chlorophyll

5.1 Introduction

There is a growing body of evidence supporting the theory that the early predictions of substantial stimulation in both carbon fixation and growth in elevated atmospheric CO_2 concentrations may not be sustained (e.g. Ceulemans and Mousseau, 1994; Pettersson and McDonald, 1994). Although within their natural life span, trees growing today will experience atmospheric CO_2 fertilisation up to double the current day value, other natural resources in particular soil nutrients may become limiting. Such limitations will ameliorate the influence of CO_2 fertilisation on net sequestration of atmospheric CO_2 into organic form. A range of studies of C_3 plants has shown that there is a change in both the carbon flux and the pattern of nitrogen allocation when plants are grown in enhanced [CO_2] (e.g. Norby, *et al.*, 1986*a*; Norby *et al.*, 1986*b*; Pettersson and McDonald, 1994).

A general observation across the majority of long term experiments in which plants have been grown in elevated [CO_2], is that leaf nutrient concentrations (per dry mass) decrease, especially nitrogen concentrations [N] (see reviews by Mousseau and Saugier, 1992; Rogers et al., 1993; Ceulemans and Mousseau, 1994). In addition to the direct effect of elevated CO_2 on tissue [N], there is also likely to be an indirect effect on the rate of soil nitrogen mineralisation (Gifford, 1992). Despite the optimistic view postulated by Gifford (1992), that *since the energy supply for bacterial dinitrogen fixation is derived from the oxidation of organic matter, one could hypothesize that nitrogen fixation may be carbon substrate colimited, hence artificially increasing the concentration of atmospheric CO_2 would over time, increase the fixation of nitrogen* - the time scale over which such changes will occur and stabilise is unlikely to match the shift in nitrogen requirement of vegetation growing in elevated atmospheric CO_2 concentrations.

Photosynthetic responses of plants grown in elevated [CO_2] are multifarious and essentially a result of both physiological and environmental variables. The environmental variables to which such plants are exposed are essentially determined by experimental protocol. Thus techniques used to investigate photosynthetic responses to enhanced CO_2 concentrations are responsible for confounding many of the findings. In some field based experiments (i.e. full sun and large rooting volumes), where plants had access to moderate to high nutrient supply rates little /no photosynthetic acclimation has been found (see Curtis *et al.*, 1995). In contrast, in cases where nutrients were in low supply, the response of assimilation to increase in CO_2 concentration rapidly declined (e.g. Tissue and Oechel, 1987; Curtis *et al.*, 1994). Even so, despite a reduction in photosynthetic capacity, assimilation rates may still remain significantly higher in plants growing in elevated [CO_2] compared with those in ambient [CO_2]. This phenomena has been considered partial acclimation, and is likely to be a common physiological response of woody species to elevated atmospheric CO_2 concentrations (Gunderson and Wullschleger, 1994).

One of the major environmental variables which interacts directly and indirectly with atmospheric CO_2 concentrations on plant physiological processes is nutrient availability. Of those studies which have investigated interactions between elevated [CO_2] and nutrients, the majority have concentrated on the effects of changing nitrogen supply rates (Pettersson and McDonald, 1994; Kerstiens, *et. al.*, 1995; Curtis, 1996). This is not surprising given the pivotal role nitrogen plays within photosynthetic processes. Photosynthesis and Rubisco content have been positively correlated with nitrogen content and availability (Evans, 1989; Tissue *et. al.*, 1993). Inadequate nitrate supply has been shown to impair synthesis of enzymes (e.g. RuBP carboxylase) and therefore CO_2 assimilation and can also inhibit formation of thylakoids, slowing electron transport and light-harvesting.

Despite the sparsity of literature covering the interaction of phosphorus with elevated [CO_2], there is also strong evidence to suggest that phosphorus concentration [P] is just as important to plant growth and photosynthesis (Conroy, 1992). Low phosphorus supply rate inhibits assimilation by decreasing intermediary metabolism and the formation of assimilates. Of the few studies which have examined the impact of phosphorus in elevated [CO_2] conditions, P has generally been the sole limiting nutrient, i.e. all other nutrients were held at presumed optimum levels (Conroy *et. al.,* 1986*b*; McKee and Woodward, 1994). There are only a few studies which have investigated the interactive affect of a 'balanced' nutrient deficiency with elevated [CO_2] (Pettersson and McDonald, 1994).

In contrast to nitrogen/CO_2 studies, phosphorus studies have shown that in elevated [CO_2] phosphorus is usually taken up in proportion to plant growth, and the foliar concentration required to bring about maximum productivity actually increases. For example, in *Pinus taeda*, phosphorus deficiency had a stronger impact on down regulation of photosynthesis in elevated [CO_2] than nitrogen deficiency (Tissue *et al.*, 1993; Thomas *et al.*, 1994).

There seems to be general agreement that nutrition is part of the acclimation of photosynthesis to elevated [CO_2] and that 'down-regulation' of photosynthesis in most cases can be related to low nutrient supply rate and/or lack of carbon sinks (Ceulemans and Mousseau, 1994; Pettersson and McDonald, 1994).

The present study was set up to explore the interactive effect of atmospheric CO_2 concentration and a 'balanced' nutrient availability on photosynthesis of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) seedlings. Plants were grown outside in a control plot or inside open-top chambers (OTC) where they were exposed to either ambient

or elevated CO_2 concentrations. A balanced nutrient solution was supplied at one of two addition rates according to "Ingestad principles" (Ingestad and Lund, 1986).

5.2 Materials and methods

5.2.1 Plant material

In March 1992, 220 one-year-old Sitka spruce seedlings, identity number 83(1012)LOT3, provenance 10, Queen Charlotte Islands, which had been raised under ambient or elevated CO_2 concentrations for 12 months, were potted into 2 dm^3 pots containing unfertilised composite soil. The soil consisted of sphagnum peat, 5 mm quartz and sterilised loam in the ratio 13:4:3 by volume. Twenty seedlings were placed randomly in each of the 10 open-top chambers and in an outside control plot, giving 220 seedlings in total. Within the outside plot, four seedlings were randomly placed in each of five blocks.

5.2.2 Growth conditions

Open-top chambers were used to expose the seedlings continuously to two different atmospheric CO_2 concentrations, ~355 μmol CO_2 mol^{-1} (ambient [CO_2] treatment) and ~700 μmol CO_2 mol^{-1}(elevated [CO_2] treatment). There were five OTCs per [CO_2] treatment. The chambers had a floor area of 7.0 m^2 and height of 2.3 m. Ambient air or ambient air supplemented with additional CO_2 was passed through a series of charcoal filters and pumped directly into each OTC via a polythene manifold 1.5 m above ground level. For a full description of open-top chamber properties and the CO_2 exposure facility see Chapter 2, Fowler *et al.* (1989) and Murray *et al.* (1994). CO_2 concentrations were monitored in each of the OTCs. OTCs receiving the ambient treatment had an average [CO_2] of 355 ± 15 μmol mol^{-1}, as a result of diurnal fluctuations and those receiving the elevated treatment had an

average [CO_2] of 700 ± 80 μmol mol^{-1}, as a result of ambient fluctuations and changes in external windspeed.

5.2.3 Nutrient treatments

A full description of the nutrient treatment is given in Chapter 4. Throughout the 1992 growing season 10 dm^3 from one of two balanced nutrient solutions was applied weekly to each of the plants using a micro pipette. Nutrient application rates were based on the Ingestad technique (Ingestad and Lund, 1986), which matches the addition rate of nutrients to plant growth rates. The nutrient addition rate was calculated based on previous growth measurements of Sitka spruce seedlings. Relative nutrient proportions required for optimum growth were assumed to be similar to those described for *Picea abies* by Ingestad (1979). A nitrogen concentration [N] of 2 % by mass, in current-year foliage was assumed to be optimum for Sitka spruce seedlings (Binns *et al.*, 1980). Two rates of nutrient supply were selected to give 2 x optimum (High-N) and 0.1 x optimum (Low-N) foliar nitrogen contents. Both nutrient treatments were applied to 10 of the 20 plants in each of the ten chambers and to two of the four plants in each of the five outside control blocks.

5.2.4 Gas exchange analysis

Photosynthetic rate (A), stomatal conductance (g_s), and transpiration rate (E), as a function of photosynthetic photon flux density (A/I) and intercellular leaf CO_2 concentration (A/C_i) were measured using an open gas analysis system (Jarvis and Čatský, 1971). A full description of the system is given in Chapter 3. Shoots were illuminated bi-laterally by two Wotan power-star lamps (HQI 250 W) and photosynthetic photon flux density (PPFD) varied by the use of neutral density filters (Leverenz and Jarvis, 1980). The ambient [CO_2] of the air supplied to each of the three cuvettes was varied by mixing pure CO_2 and CO_2-free air. Water vapour

density was regulated by mixing dry air and water-saturated air. All gas flows were controlled by a series of in-line rotameters and a Tylan mass flow controller. CO_2 and water vapour concentration of the inlet (reference) and outlet (analytical) air of each cuvette were monitored using a bench-top infra-red gas analyser (type 225 Mk III, ADC, Hoddesdon, Herts, UK) and cooled mirror dewpoint hygrometer (series 3000, Mitchell, UK). Leaf temperature was regulated at 19 °C by means of a water cooling system and feedback CAL 6000 PID temperature controller, resulting in a vapour pressure deficit (VPD) of 10.5 mb.

Measurements commenced on 21 September 1992. In order to prevent budset and limit the amount of variation in the rates of photosynthesis caused by differences in the previous day/night's temperatures and daylength, all measured plants received supplementary illumination and additional heating within their respective CO_2 treatments, in a heated glasshouse. Net photosynthesis has been shown to peak during mid August for pot-grown, Sitka spruce plants (Chandler and Dale; 1993). Therefore a daylength (18 hours) and temperature (minimum day/night temperature of 14/9 °C) regime simulating this time of year was selected.

One seedling from each of the 10 OTCs and 5 outside control blocks for both the low-N and high-N treatments was selected for gas exchange measurements, 30 seedlings in total. Throughout the sampling period three plants (one per cuvette) were brought into the laboratory on the evening prior to measurement and a terminal shoot from one of the upper whorl of branches was sealed in a cuvette. Dark respiration rates (R_m) were measured the following morning before switching on the lights. *A/I* and A/C_i measurements were made on the same branch of each tree on consecutive days. A minimum of 40 minutes equilibration time was allowed at each PPFD or $[CO_2]$ prior to recording the measurement. *A/I* responses were measured on seedlings grown and measured at the same $[CO_2]$. In addition, PPFD-saturated

rates of assimilation were measured at both treatment [CO_2], i.e. 355 and 700 μmol mol^{-1}. During A/C_i response measurements, PPFD was maintained at 1200 μmol m^{-2} s^{-1}. One hour of equilibration time at each PPFD or [CO_2] was allowed prior to measurement. A full calibration of the IRGA was undertaken each morning prior to that days set of measurements.

Following gas exchange measurements, the needles from each shoot were excised and the fresh mass of two sub-samples of 20 needles from each shoot was measured. The projected area of one set of 20 needles was determined using an image analysis system (IIR, Digithurst, Royston, UK) and the needles were oven dried at 70 °C to constant mass along with the remaining needles from the shoot, to provide a fresh mass/dry mass ratio. The fresh mass of the second sub-sample of 20 needles was determined and the needles were then placed in liquid nitrogen and stored for subsequent determination of chlorophyll *a* and *b* contents (see below).

5.2.5 Chlorophyll analysis

Chlorophyll was extracted from the 20 sub-sampled needles from each of the individual shoots used in the gas exchange analysis. Samples were removed from liquid nitrogen and placed in 4.5 dm^3 of N,N-dimethylformamide (DMF) for 14 days in the dark at room temperature (~20 °C) (Moran and Porath, 1980; Moran, 1982). The absorption of the resulting chlorophyll solution was measured at 647, 664 and 750 nm (A_{647}, A_{664} and A_{750}, respectively), using a spectrophotometer (Model 601, Bausch and Lomb, UK). The chlorophyll concentrations of the solutions were then calculated in $\mu g\ dm^{-3}$ from the following equations (Porra *et al.*, 1989):

$$\text{Chl } a = 12.00\ (A_{664} - A_{750}) - 3.11\ (A_{647} - A_{750})$$

$$\text{Chl } b = 20.78\ (A_{647} - A_{750}) - 4.88\ (A_{664} - A_{750})$$

Using the fresh mass / dry mass and the fresh mass / area ratios obtained on the other sample from each shoot, the concentrations of chlorophyll *a* and *b*, were expressed on a dry mass and area basis, respectively.

5.2.6 Calculation of parameters and statistical analysis

Each data set collected was analysed to yield assimilation rate, stomatal conductance and internal CO_2 concentration according to the equations of von Caemmerer and Farquhar (1981).

5.2.6.1 CO_2 response curves

Each data set collected was analysed for subsequent modelling according to the equations based on the biochemical model of Farquhar *et al.* (1980). According to this model when Rubisco catalyses the oxygenation reaction of RuBP with one mol of O_2, 0.5 mol of CO_2 are released (Farquhar and von Caemmerer, 1982), and thus net assimilation of CO_2 (A) can be expressed by the equation:

$$A = V_c - 0.5\ V_o - R_d \qquad [5.1]$$

where V_c and V_o are the rates of carboxylation and oxygenation respectively, and R_d is CO_2 evolution from the mitochondria in the light (i.e. non-photorespiratory respiration) known as "day" respiration (Brooks and Farquhar, 1985).

Because the rate of *photosynthesis* is assumed to be limited by either the rate of electron transport (RuBP regeneration-limited net photosynthesis), A_{RuBP} [5.3], or the amount, activity and kinetics of Rubisco (RuBP regeneration-saturated net photosynthesis), A_{carb} [5.4] the net rate of A can be expressed as [5.2]

$$A = \min \{A_{RuBP}, A_{carb}\} \qquad [5.2]$$

where J is the electron transport capacity, V_{cmax} is the maximum rate of

$$A_{RuBP} = J_{max} \frac{C_i - \Gamma*}{\alpha C_i + \beta\Gamma*} - R_d \quad [5.3]$$

$$A_{carb} = V_{cmax} \frac{C_i - \Gamma*}{C_i + K_c (1 + O/K_o)} - R_d \quad [5.4]$$

carboxylation, C_i and O are internal leaf concentrations of CO_2 and O_2, respectively, $\Gamma*$ is the CO_2 compensation concentration in the absence of mitochondrial respiration, α and β are constants and K_c and K_o are Michaelis constants for carboxylation and oxygenation, respectively. The CO_2 response data were then fitted to this model using a reiterative optimisation program (Proc NLIN, SAS institute Inc., Cary, NC) and the parameters V_{cmax} and J_{max} derived.

5.2.6.2 PPFD response curves

Because the PPFD response data were collected at non-saturating CO_2 concentrations, C_a = 355 and 700 μmol mol^{-1}, it could not be assumed that the electron transport rate (J) solely limited photosynthesis, and therefore a less mechanistic model than the one proposed by Farquhar *et al.* (1980) was used to analyse these data. A non-rectangular hyperbolic function outlined in Jarvis *et al.* (1985) [5.5], and a reiterative optimisation program (Genstat 5) were used to estimate the parameter values with the least mean square error. The measured input variables for the model are photon flux density (I), ambient CO_2 concentration (C_a), stomatal conductance (g_s) and assimilation rate (A). The model assumption is that photosynthesis is related to photosynthetic photon flux density (PPFD) by a non-rectangular hyperbola of the form:-

$$\theta(A + R)^2 - (\alpha I + A_{max} + R)(A + R) + \alpha I(A_{max} + R) = 0 \quad [5.5]$$

Parameters of the model are α = initial slope of the *A/I* curve, θ = convexity coefficient (which defines the degree of curvature between the initial slope and the asymptotic value of *A*), A_{max} = the rate of PPFD saturated assimilation, g_m = mesophyll conductance, and R = rate of dark respiration.

This yielded fitted assimilation versus PPFD (*A/I*) curves for each CO_2 and chamber treatment at both nutrient supply rates. The optimising routine of the Genstat package also estimated the following parameter values by the least mean square error method:- R, g_m, α and θ.

Analysis of variance was used to test the significance of difference between the measured maximum assimilation rates (A_{mmax}) and measured dark respiration rates (R_m) of chamber grown ambient and elevated CO_2 treatments and the outside control plot. These data were calculated from the *A/I* data sets where $I \geq 1200$ μmol m^{-2} s^{-1} and $I = 0$ μmol m^{-2} s^{-1} for A_{mmax} and R_m, respectively.

5.2.7 Statistical analysis

The statistical differences between the CO_2 and chamber treatments for both *A/I* and A/C_i curves for both nutrient treatments were determined by a combined curve analysis of variance (Ross, 1981). This technique tests the reduction in residual variance obtained by fitting a set of individual curves compared to the residual variance obtained from a common curve. Significant differences for all linear regressions were tested using a similar technique to that described for the combined curve analysis. Differences in the measured variables; chlorophyll concentration and maximum net assimilation rates (A_{mmax}), between the chamber grown ambient and

elevated [CO_2] treatments and the outside control plot at both nutrient supply rates were tested by analysis of variance using Genstat 5 software (Payne *et al.* 1987).

5.2.8 Nutrient analysis

[N] and [P] were measured on the foliage one month after the gas exchange measurements were completed at the Chemical Analysis Laboratory of the Institute of Terrestrial Ecology, Merlwood (see Chapter 4 and Murray *et al.*, 1996 for a full description of analytical techniques). Sub-samples of current-year-needles were taken from each tree, including some from the gas exchange trees, and bulked by nutrient treatment for each of the 10 OTCs, i.e. five samples for each nutrient treatment within both the ambient and elevated CO_2 treatments. One bulked sample for each nutrient treatment was taken from the outside control plot.

5.3 Results

5.3.1 CO_2 response

The CO_2-response of net photosynthesis for the different [CO_2] treatments is summarised in Figure 5.1(a) and (b). The mechanistic model of Farquhar *et al.* (1980), represented in Figure 5.1 by the lines, fitted all the data sets well. Pair-wise comparisons between the curves fitted to the ambient and elevated [CO_2] grown seedlings showed a significant difference between them at the low-N supply rate (Table 5.1). A list of the parameter values obtained from fitting the A / C_i response curves for the different treatments at both nutrient supply rates is given in Table 5.2. There was no effect of elevated [CO_2] on either V_{cmax} or J_{max} when nutrients were supplied at the high-N rate, but both V_{cmax} and J_{max} were lower in the elevated compared with the ambient [CO_2] treated seedlings when supplied with low-N. There was a highly significant effect of nutrient supply rate on the fitted curves for all treatments ($p = 0.001$). Increasing the nutrient supply rate from low-N to high-N

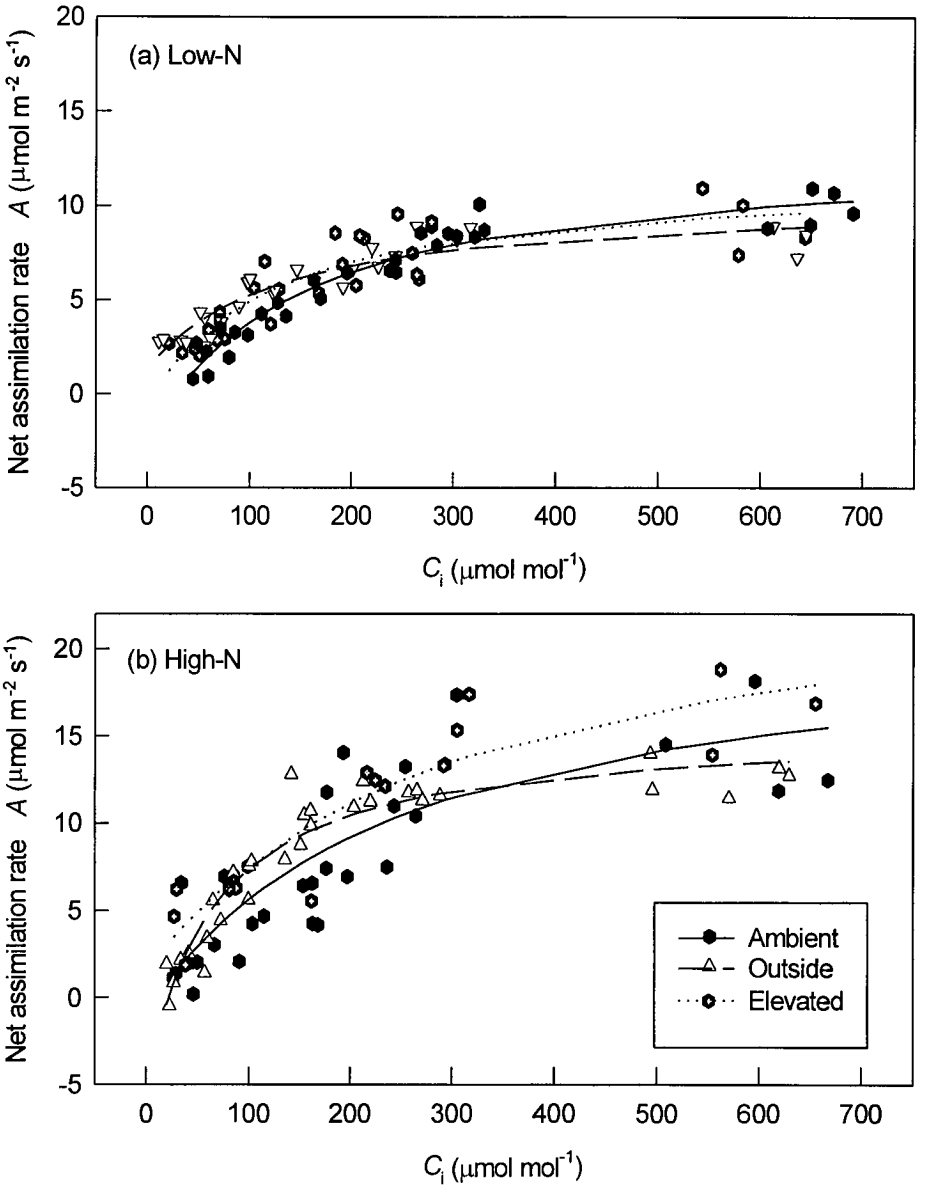

Figure 5.1: Changes in assimilation rate A of Sitka spruce as a function of C_i in (a) low-N (0.1 x optimum) and (b) high-N (2.0 x optimum) nutrient supply rates, for seedlings grown inside open-top chambers with either 355 μmol mol^{-1} CO_2 (ambient) or 700 μmol mol^{-1} CO_2 (elevated) and outside in a control plot (outside). All measurements were taken at a PPFD of 1200 μmol m^{-2} s^{-1}. The curves are the best fits to the mechanistic model of Farquhar *et al.* (1980) between treatments. Leaf temperature = 20 °C and VPD = 10.5 mb.

increased both V_{cmax} and J_{max} by a similar amount, *ca* 45 %, for the ambient [CO_2] grown seedlings. In contrast, the effect of increasing nutrient supply rate to the elevated [CO_2] grown seedlings was not the same for V_{cmax} and J_{max}, they were increased by 66 and 57 %, respectively. Elevated [CO_2] increased the ratio of J_{max} / V_{cmax} by 8 % when seedlings were supplied with low-N rates.

There was a good correlation between leaf [N] and V_{cmax} expressed on an area basis ($r^2 = 0.90$) for all treatments (Figure 5.2a). J_{max} was also well correlated with leaf [N], although the correlation was not as good as for V_{cmax} ($r^2 = 0.69$) (Figure 5.2b). There was no significant effect of OTC on either the fitted A / C_i response curves or the predicted parameter values obtained from the model at either nutrient supply rate. Again, V_{cmax} and J_{max} were increased by increasing the nutrient supply rate to the outside control plants.

Table 5.1: Combined analysis of variance tables (Ross, 1981) for curves fitted to the PPFD-saturated, CO_2 response of Sitka spruce shoots supplied with 0.1 x optimum nutrition (low-N) or 2.0 x optimum nutrition (high-N) rates and grown outside in a control plot or grown inside open top chambers in either ambient, 355 μmol mol^{-1}, CO_2 or elevated, 700 μmol mol^{-1}, CO_2.

Curve comparisons	df	ss	mean ss	F ratio	*p* value
(I) Low-N					
Ambient vs Elevated [CO_2]	3	12.17	4.06	4.05	0.05
Residuals	54	54.07	1.00		
(ii) High-N					
Ambient vs Elevated [CO_2]	3	33.01	11.0	1.31	ns
Residuals	43	360.86	8.39		
(iii) Low-N					
Ambient vs Elevated [CO_2]	3	24.16	8.05	3.91	ns
Residuals	52	106.9	2.06		
(iv) High-N					
Ambient vs Elevated [CO_2]	3	29.32	9.77	1.66	ns
Residuals	57	334.98	5.87		

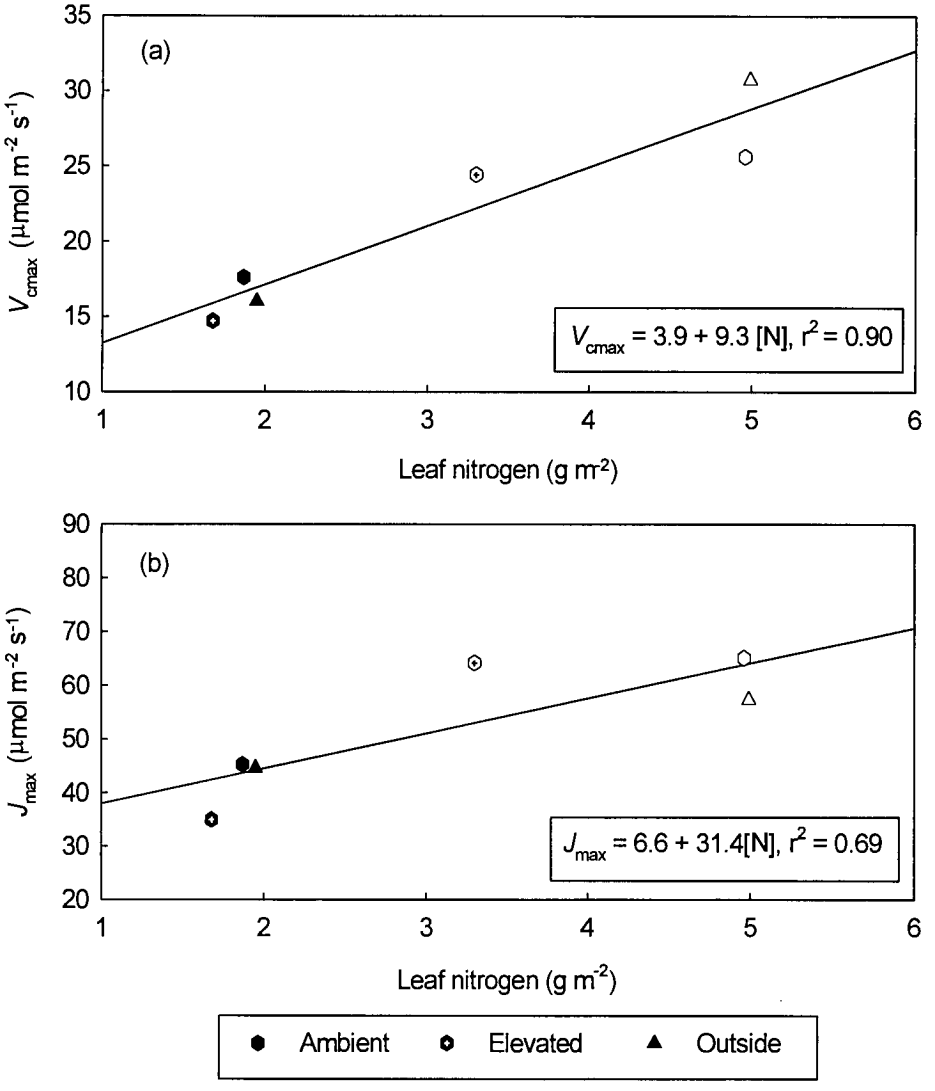

Figure 5.2: Relationship between foliar [N] and (a) V_{cmax} and (b) J_{max} for ambient [CO_2], elevated [CO_2] and outside grown seedlings. Open symbols = High-N treatment and closed symbols = Low-N treatment. PPFD = 1200 µmol m^{-2} s^{-1}, leaf temperature = 20 °C and VPD = 10.5 mb.

Although there was no significant effect on the C_i / C_a ratio of seedlings with the low-N or high-N supply rate at either 355 or 700 µmol m^{-2} s^{-1} CO_2, the ratio was generally higher in elevated CO_2 (Figure 5.3), with the exception of measurements

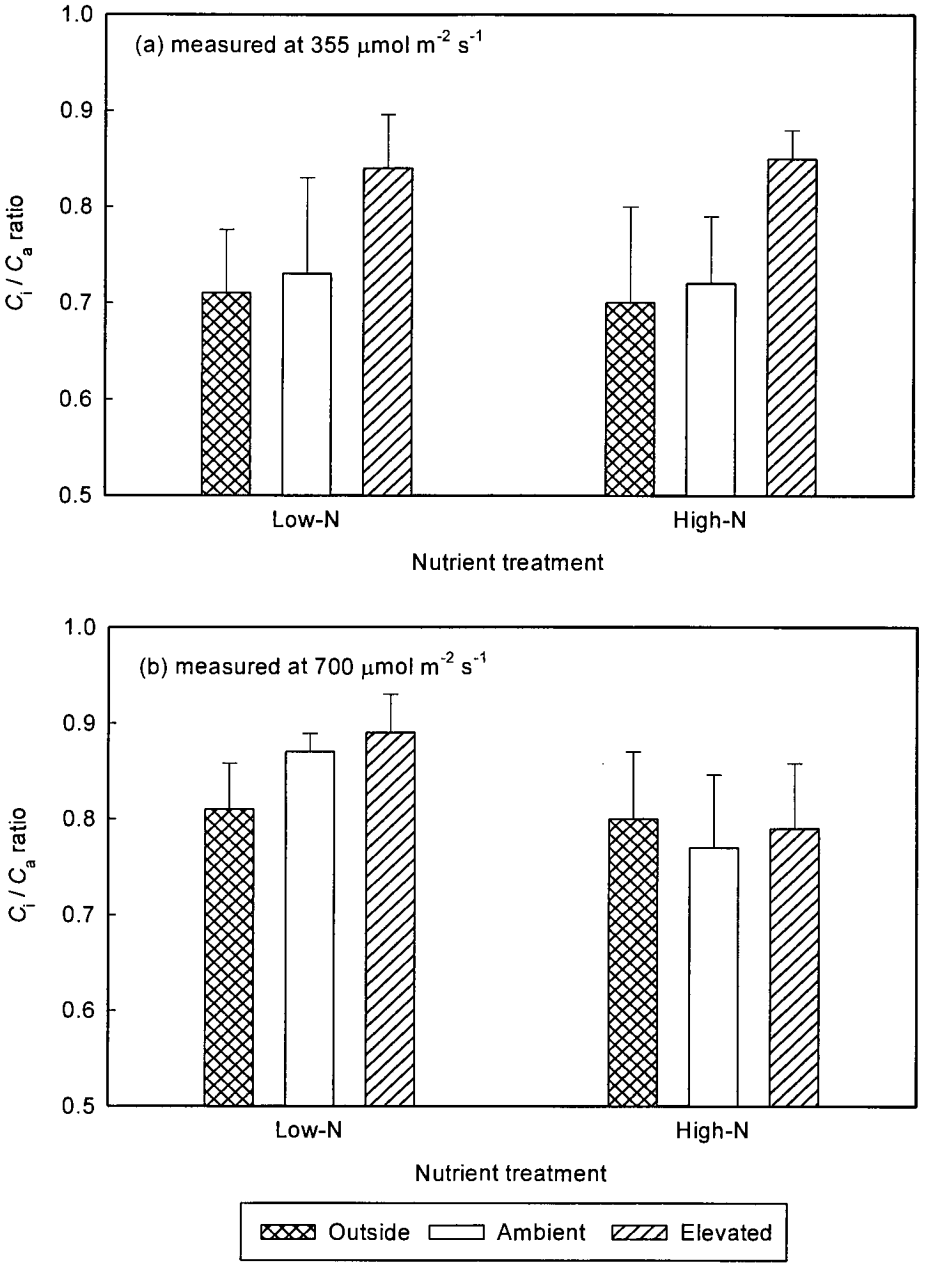

Figure 5.3: Relationship between atmospheric CO_2 concentrations (C_a) and intercellular CO_2 concentrations (C_i) for ambient [CO_2], elevated [CO_2] and outside grown Sitka spruce seedlings with two nutrient supply rates. Measurements were made at both treatment CO_2 concentrations (a) 355 μmol mol^{-1} CO_2 and (b) 700 μmol mol^{-1} CO_2. PPFD = 1200 μmol m^{-2} s^{-1}, leaf temperature = 20 °C and VPD = 10.5 mb.

made at 700 μmol m^{-2} s^{-1} on seedlings receiving the high-N supply rate, when there was no difference in C_i / C_a ratio across any of the [CO_2] treatments.

Table 5.2: Estimated parameter values, V_{cmax} and J_{max} obtained from the mechanistic Farquhar *et al.* (1980) model for Sitka spruce seedlings grown in OTCs in either ambient or elevated [CO_2] and grown outside in a control plot. PPFD = 1200 μmol $m^{-2}s^{-1}$, leaf temperature = 20 °C and VPD = 10.5 mb.

		[CO_2] Treatment		
Parameter	Nutrient supply rate	Ambient	Elevated	Outside
V_{cmax}	Low-N	17.6 ± 1.1	14.7 ± 1.6	16.0 ± 2.0
	High-N	25.6 ± 4.3	24.4 ± 3.2	30.7 ± 2.1
J_{max}	Low-N	45.2 ± 2.7	40.9 ± 2.3	42.5 ± 4.5
	High-N	65.1 ± 9.1	64.1 ± 8.25	9.3 ± 2.5
J_{max} / V_{cmax}	Low-N	2.57	2.78	2.66
	High-N	2.54	2.63	1.93

5.3.2 Response to PPFD and dark respiration rates

Changes in net photosynthetic rate as a function of PPFD are shown in Figure 5.4(a) and (b). The data and fitted curves presented were obtained from measurements made at the treatment CO_2 concentration, i.e. plants grown in ambient [CO_2] were measured at 355 μmol mol^{-1} and those grown in elevated [CO_2] were measured at 700 μmol mol^{-1}. The non-rectangular hyperbolic function fitted all the data sets well with over 95 % of the variance accounted for across all treatments receiving the low-N supply rate and *ca* 90 % of the variance in the high-N supply rate. Pair-wise comparisons between the PPFD response curves fitted to the ambient [CO_2], elevated [CO_2] and outside control plants for both nutrient regimes showed a highly significant effect of nutrient treatment ($p \leq 0.001$). Pair-wise comparisons between response curves fitted to the ambient and elevated [CO_2] treatments were significantly different for both nutrient regimes (Table 5.3, $p \leq 0.001$). There was no significant difference between the response curves fitted to the ambient [CO_2] chamber grown plants and the outside control plants in either nutrient supply regime (Table 5.3).

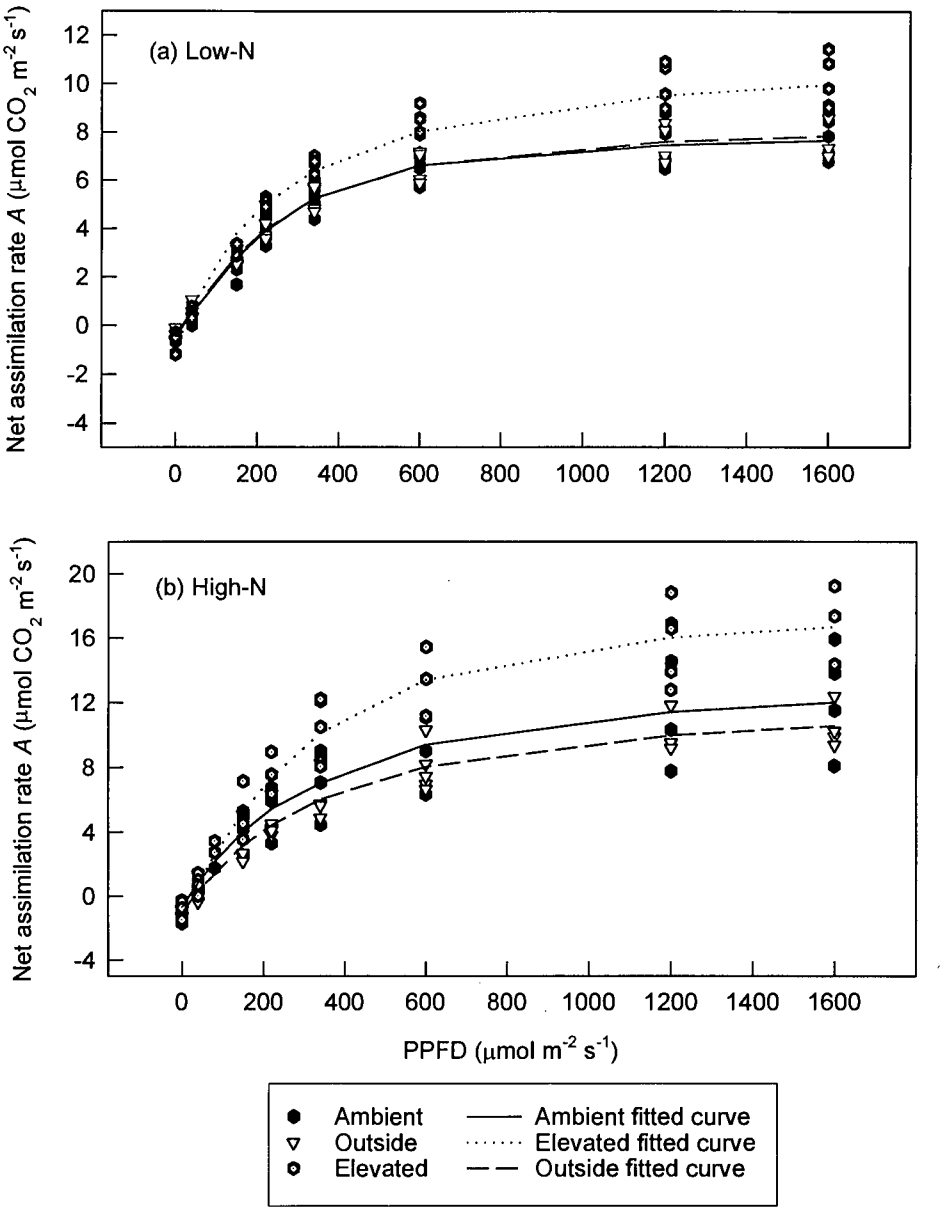

Figure 5.4: Changes in assimilation rate of Sitka spruce as a function of PPFD in (a) low-N and (b) high-N nutrient supply rates, for ambient [CO_2], elevated [CO_2] and outside grown seedlings. Measurements were made at treatment CO_2 concentrations, i.e. ambient [CO_2] and outside treatments measured at 355 μmol mol^{-1} CO_2 and elevated [CO_2] measured at 700 μmol mol^{-1} CO_2. The curves are the best fits to the non-rectangular hyperbolic equation from the model of Jarvis *et al.* (1985) for each treatment. Leaf temperature = 20 °C and VPD = 10.5 mb.

Table 5.3: Combined analysis of variance tables (Ross, 1981) for curves fitted to the CO_2 exchange rates of Sitka spruce shoots at varying PPFD after 17 months exposure to either 355 μmol mol^{-1} CO_2 (Ambient) or 700 μmol mol^{-1} CO_2 (Elevated) and supplied with 0.1 x optimum nutrition (low) and 2.0 x optimum nutrition (high) rates, using the theoretical model of Jarvis *et al.* (1985).

Curve comparisons	df	ss	mean ss	F ratio	*p* value
(I) Low-N					
Ambient vs Elevated [CO_2]	4	84.13	12.78	30.95	<0.001
Residuals	79	33.03	0.41		
(ii) High-N					
Ambient vs Elevated [CO_2]	4	314.6	35.09	13.496	<0.001
Residuals	67	174.25	2.6		
(iii) Low-N					
Ambient [CO_2] vs Outside	4	25.04	0.0385	0.114	ns
Residuals	72	24.882	0.3455		
(iv) High-N					
Ambient [CO_2] vs Outside	4	155.4	4.9775	2.315	ns
Residuals	63	135.49	2.15		

Table 5.4 shows the parameter values obtained when fitting the model to the *A/I* response curves. The photochemical efficiency (α) was higher in elevated [CO_2] seedlings compared with ambient [CO_2] seedlings, at their respective growth CO_2 concentrations (Table 5.4). There was no effect of nutrition on α in elevated [CO_2], but seedlings receiving ambient [CO_2] and the low nutrient regime had lower α than those receiving the high nutrient regime.

For both ambient and elevated [CO_2] seedlings the convexity coefficient (θ) was lower when receiving the high-N compared with the low-N supply rate.

The estimated sum of conductances of the cell wall, membranes, cytosol and chloroplast (g_m), predicted by the model was between *ca* 70 % lower in seedlings receiving the low-N compared to high-N regime (Table 5.4). Compared with plants

grown in the ambient [CO_2] treatment, elevated [CO_2] treated plants had lower values of g_m, with the effect being largest at low nutrient concentrations.

The model estimates of dark respiration (R) were always lower with the low-N supply rate compared with the high-N supply rate, and higher for elevated [CO_2] seedlings compared with ambient [CO_2] seedlings. Seedlings growing inside OTCs had lower R when supplied with high-N rates. The actual measured dark respiration rate (R_m), i.e. assimilation rates at 0 PPFD were always slightly lower than the model R values. There was a good correlation between R_m and needle [N] expressed on an area basis (Figure 5.5, $r^2 = 0.84$). The variability in R_m as a result of nutrient supply rate, growth [CO_2] and OTC can all be accounted for by changes in foliar [N].

Table 5.4: Estimated parameter values from the theoretical model for Sitka spruce seedlings grown outside in a control plot in ambient [CO_2] or in open-top chambers in either ambient, 355 μmol mol^{-1} CO_2 or elevated, 700 μmol mol^{-1} CO_2 and supplied with a high-N (2.0 x optimum) or low-N (0.1 x optimum) nutrient regime. The values are from curves obtained at the respective CO_2 treatment concentrations, i.e ambient measured at ambient [CO_2] and elevated measured at elevated [CO_2]. α = initial slope of A/I curve, g_m = mesophyll conductance (mmol m^{-2} s^{-1}), R = dark respiration (μmol m^{-2} s^{-1}), and θ = convexity coefficient.

CO_2	*Nutrient*	α	g_m	*R*	θ
Ambient [CO_2]	Low-N	0.024	27.07	0.461	0.771
Ambient [CO_2]	High-N	0.028	46.69	0.703	0.350
Elevated [CO_2]	Low-N	0.051	23.52	0.576	0.700
Elevated [CO_2]	High-N	0.050	40.09	0.791	0.621
Outside control	Low-N	0.026	28.28	0.441	0.669
Outside control	High-N	0.037	41.89	1.075	0.285

5.3.3 Maximum assimilation rate, stomatal conductance and foliar [N]

PPFD-saturated rates of photosynthesis (A_{mmax}) were stimulated on average by 26 % in elevated [CO_2] compared with ambient [CO_2] when grown and measured at the same [CO_2] (Table 5.5). Elevated [CO_2] enhanced A_{mmax} by 33 and 19 % compared

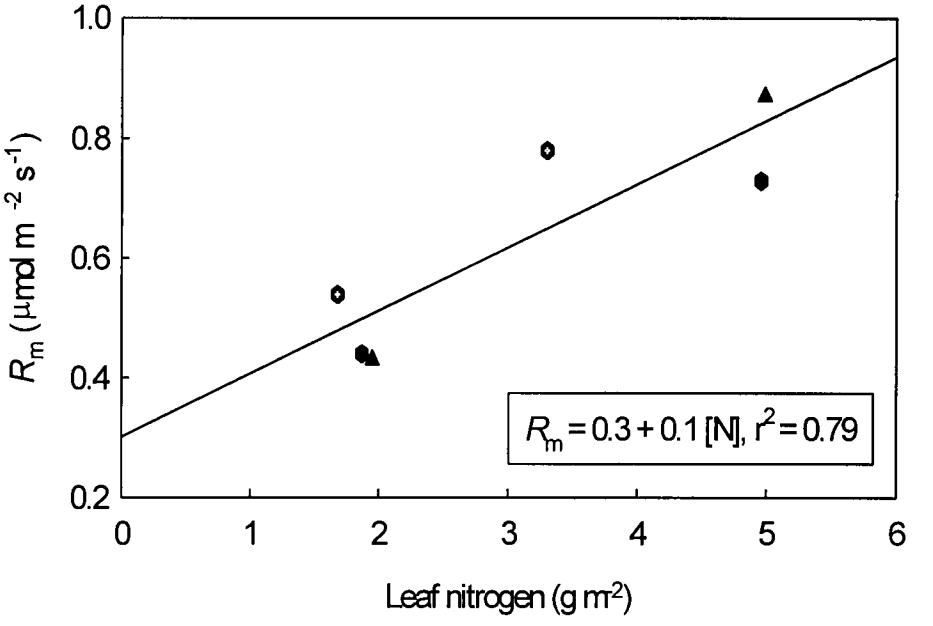

Figure 5.5: Relationship between measured shoot dark respiration rates and foliar [N] for ambient [CO_2], elevated [CO_2] and outside grown seedlings. Leaf temperature = 20 °C and VPD = 10.5 mb.

with ambient [CO_2], for the high-N and low-N supply rates, respectively. On average, for ambient [CO_2], elevated [CO_2] and outside treatments, seedlings with the low-N supply rate had a 33 % lower A_{mmax} rate compared with those receiving the high-N supply rate. When A_{mmax} was measured at a common CO_2 concentration, i.e. ambient and elevated [CO_2] treated plants measured and compared at 355 μmol^{-1} CO_2 and 700 μmol mol^{-1} CO_2, respectively, there was no significant effect of elevated [CO_2], i.e. no apparent down regulation of the photosynthetic apparatus.

There was a marked effect of elevated [CO_2] on the linear response between A_{mmax} and foliar nitrogen content when measurements were made at treatment [CO_2] ($p \leq$

0.001, Figure 5.6). The photosynthetic rate of elevated [CO_2] grown seedlings was significantly higher for a given foliar [N] compared with ambient [CO_2] seedlings.

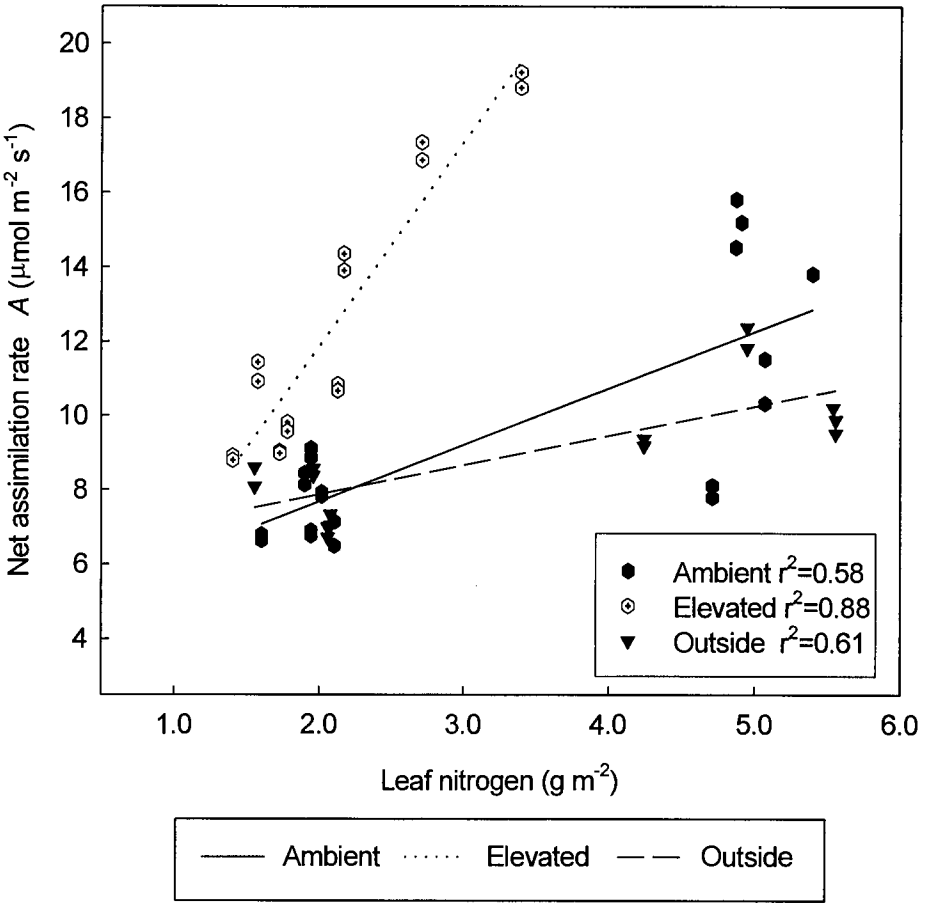

Figure 5.6: Relationship between measured PPFD-saturated net assimilation rates (A_{mmax}) and foliar [N] for ambient [CO_2], elevated [CO_2], and outside grown seedlings. Lines represent fitted linear regressions for each treatment. Photosynthetic measurements were made at treatment CO_2 concentrations i.e. ambient [CO_2] and outside treatments measured at ~355 μmol mol^{-1} CO_2 and elevated [CO_2] measured at ~700 μmol mol^{-1} CO_2. PPFD = 1200 μmol m^{-2} s^{-1}, leaf temperature = 20 °C and VPD = 10.5 mb.

There was no significant effect of elevated [CO_2] or open-top chamber on the linear response between stomatal conductance (g_s) and foliar [N] (Figure 5.7a). g_s was highly dependant on foliar [N] ranging between 60 mmol m^{-2} s^{-1} at *ca* 1.5 g N m^{-2} and 200 mmol m^{-2} s^{-1} at *ca* 5 g N m^{-2}. There was a significant difference between the linear response of A_{mmax} to g_s, between elevated [CO_2] and ambient [CO_2] seedlings when grown and measured at the same [CO_2] ($p \leq 0.01$, Figure 5.7b).

Table 5.5: PPFD-saturated rate of photosynthesis (A_{mmax}, µmol m^{-2} s^{-1}) of Sitka spruce seedlings measured at both ambient and elevated [CO_2]. Values are the means ± 1 SEM. PPFD = 1200 µmol m^{-2} s^{-1}, Leaf temperature = 19 ± 1 °C, VPD = 10.5 mb.

Light-saturated rates of photosynthesis (A_{max}, µmol m^{-2} s^{-1})			
		Nutrient treatment	
[CO_2] treatment	Measurement [CO_2]	Low-N	High-N
Ambient	355 µmol mol^{-1} CO_2	7.95 ± 1.2	11.1 ± 2.4
Ambient	700 µmol mol^{-1} CO_2	9.15 ± 1.39	14.7 ± 3.2
Elevated	355 µmol mol^{-1} CO_2	8.35 ± 0.92	15.3 ± 1.67
Elevated	700 µmol mol^{-1} CO_2	9.78 ± 0.86	16.52 ± 2.0
Outside	355 µmol mol^{-1} CO_2	8.80 ± 2.4	11.82 ± 0.53
Outside	700 µmol mol^{-1} CO_2	8.20 ± 0.7	12.62 ± 0.9

5.3.4 Foliar nutrient concentrations

There was a highly significant effect of nutrient treatment *per se* on foliar [N]. For the ambient and elevated [CO_2] treated plants and the outside control plot foliar [N] was increased by 51, 61 and 48 %, respectively, from the low-N to high-N treatment. Current year foliar [N] was significantly lower in elevated [CO_2] than in ambient [CO_2] (Table 5.6). At high-N supply rates, foliar [N] was reduced by 10 %, whereas in the low-N treatment foliar [N] was reduced by 28 %. There was no significant effect of CO_2 concentration on foliar [P] but there was a significant effect of nutrient treatment (Table 5.6). In the ambient [CO_2], elevated [CO_2] and outside treatments seedling [P] was 21, 26 and 22 % lower in the low-N compared to the high-N treatment (Table 5.6).

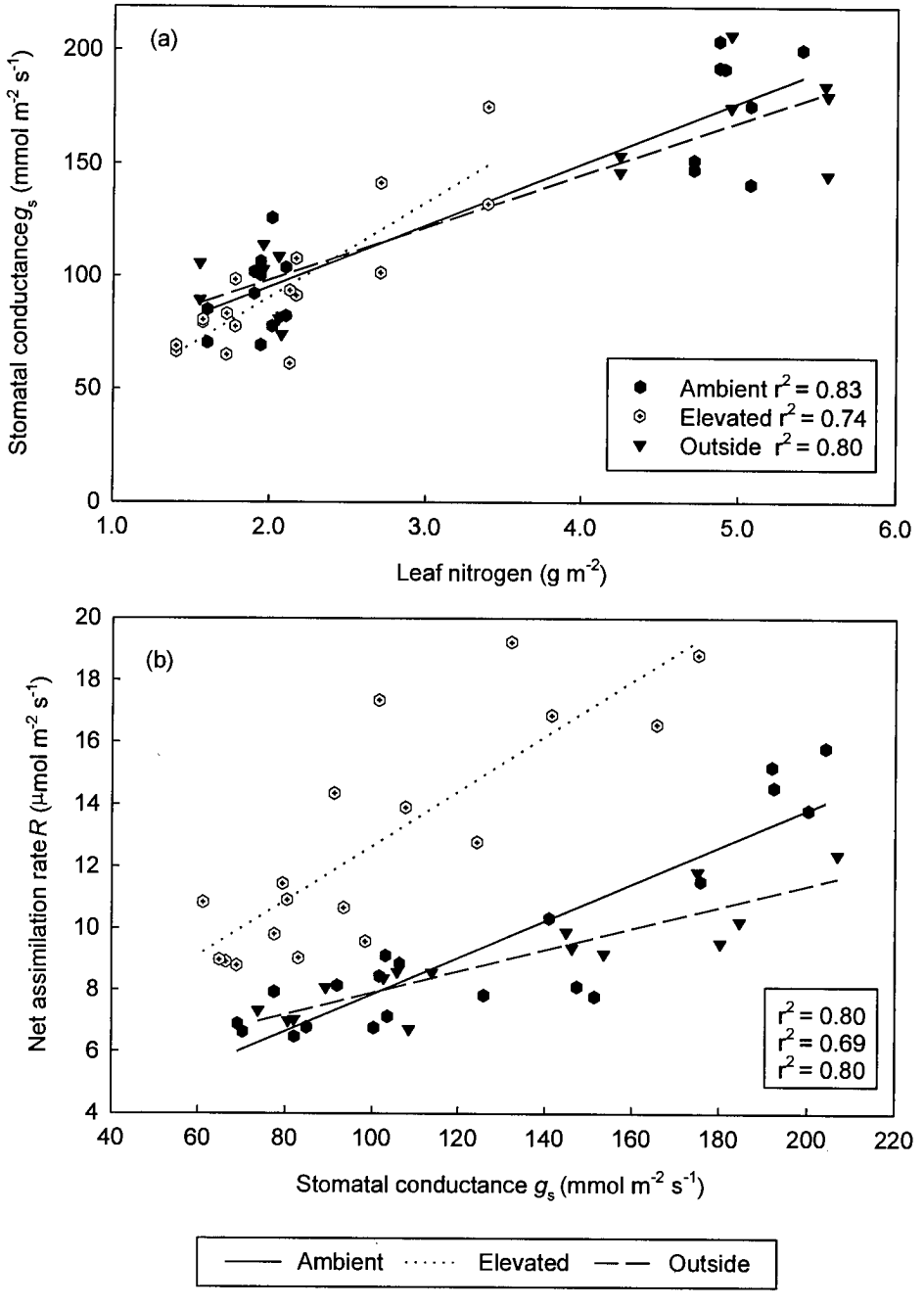

Figure 5.7: Relationship between (a) stomatal conductance and foliar [N] and (b) PPFD-saturated net assimilation rates and stomatal conductance for ambient [CO_2], elevated [CO_2] and outside seedlings. Lines represent fitted linear regressions for each treatment. Photosynthetic measurements were made at the treatment CO_2 concentrations, i.e. ambient [CO_2] and outside treatments measured at 355 μmol mol^{-1} CO_2 and elevated [CO_2] treatment measured at 700 μmol mol^{-1} CO_2.

Unfortunately, samples from the outside control plot had to be bulked, and therefore statistical analysis of these data was not possible. However, there was no apparent difference in the nutrient composition of the ambient [CO_2] grown seedlings compared to outside control seedlings.

Table 5.6: Nutrient analysis of current year needles (<one-year-old) of Sitka spruce supplied with either 0.1 x optimum nutrition (low-N) or 2.0 x optimum nutrition (high-N) and grown outside in a control plot (Outside) or inside open top chambers and exposed to either 355 μmol mol^{-1} CO_2 (Ambient [CO_2]) or 700 μmol mol^{-1} CO_2 (Elevated [CO_2]). Values are means ± 1. Significant differences between treatments (ANOVA).

Treatment		Nutrient concentration (% dry mass)	
[CO_2]	Nutrient	Nitrogen [N]	Phosphorus [P]
Ambient	High-N	2.62 ± 0.04	0.28 ± 0.024
Ambient	Low-N	1.28 ± 0.09	0.22 ± 0.012
Elevated	High-N	2.36 ± 0.10	0.27 ± 0.015
Elevated	Low-N	0.92 ± 0.05	0.20 ± 0.015
Outside	High-N	2.5	0.27
Outside	Low-N	1.3	0.21
p value			
[CO_2]		0.05	ns
Nutrient		0.001	0.01

5.3.5 Chlorophyll concentration

Total chlorophyll concentration increased linearly with increasing N supply for all three treatments, ambient [CO_2], elevated [CO_2] and outside (Table 5.7 & Figure 5.8). However the proportion of chlorophyll to leaf [N] was significantly different between the elevated [CO_2] and ambient [CO_2] seedlings (Figure 5.8, $p \leq 0.001$). For a given foliar [N], elevated [CO_2] seedlings had a higher chlorophyll concentration than either the ambient [CO_2] or outside control seedlings. There was no effect of open-top chamber *per se* on chlorophyll concentration.

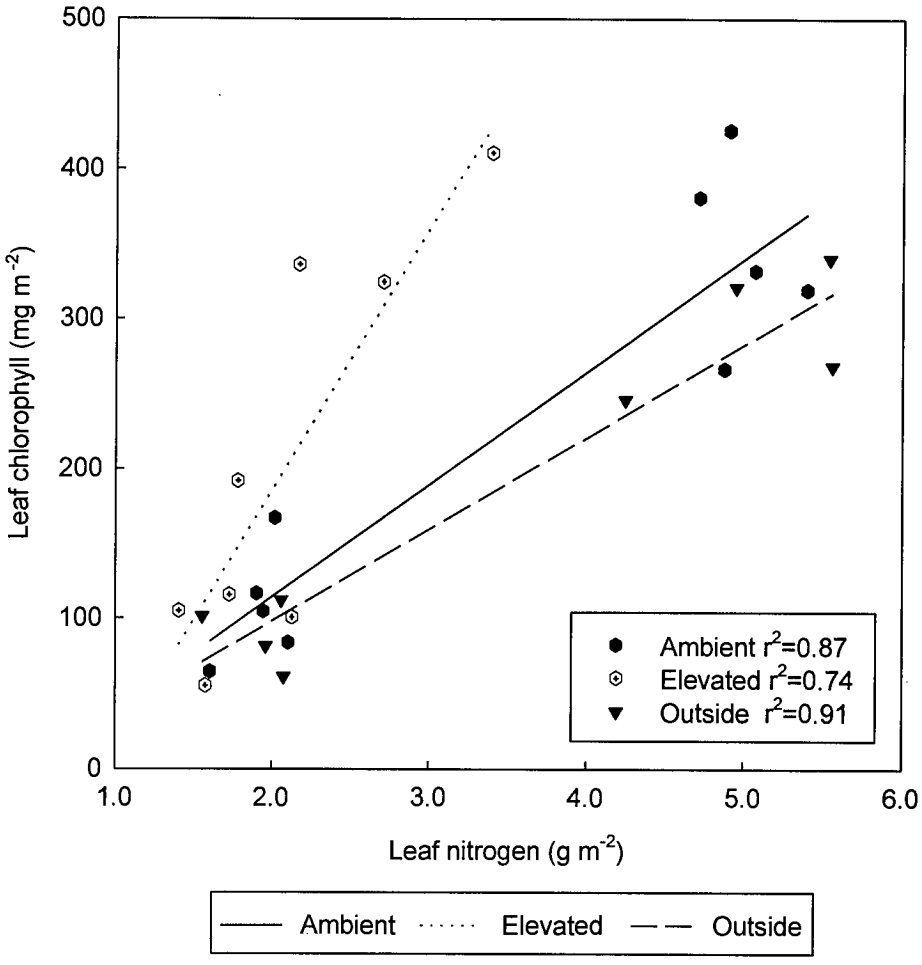

Figure 5.8: Relationship between foliar [N] and total chlorophyll content for ambient [CO_2], elevated [CO_2] and outside seedlings. Lines represent fitted linear regressions for each treatment.

The high-N supply rate led to a higher chlorophyll *a/b* ratio than the low-N, irrespective of chamber or [CO_2] treatment (Table 5.7). The lower chlorophyll concentration found in plants receiving the low nutrient addition rate was primarily the result of a reduction in the amount of chlorophyll *a* compared with the amount of chlorophyll *b*.

Table 5.7: Total chlorophyll concentration expressed per unit dry mass (μg/g), area (μg/cm^2) and ratio (chlorophyll *a*/*b*) for Sitka spruce plants supplied with either high-N (2.0 x optimum) or low-N (0.1 x optimum) nutrient supply rates. Plants were grown outside in a control plot (Outside) or inside open-top chambers and fumigated with either ~355 μmol mol^{-1} CO_2 (Ambient [CO_2]) or ~700 μmol mol^{-1} CO_2 (Elevated [CO_2]). Values are means ± 1 SEM.

	Total chlorophyll (μg/g)		Total chlorophyll (μg/ cm^2)		Ratio (chloro *a*/*b*)	
	High	Low	High	Low	High	Low
CO_2 treatment						
Ambient	1798±162	734±156	34.4±2.7	10.7±1.7	2.77±0.04	2.32±0.18
Elevated	1370±226	628±154	29.8±4.4	11.4±2.2	2.77±0.08	2.58±0.21
Outside	1507±110	586±98	29.4±1.7	8.7±0.9	2.75±0.08	2.51±0.16

5.4 Discussion

5.4.1 Nutrition and photosynthesis

The acclimation of photosynthesis to elevated [CO_2] with consequent 'down-regulation' may in most cases be related to poor nutritional conditions and/or lack of carbon sinks (Ceulemans and Mousseau, 1994; Pettersson and McDonald, 1994). Because the proteins of the Calvin cycle (PCR) enzymes and thylakoids represent the majority of leaf nitrogen, plant photosynthetic capacity is directly related to leaf nitrogen content (Evans, 1989; Kellomäki and Wang, 1997). In view of the many observations of depletion of foliar [N] with increasing atmospheric [CO_2] (Conroy, 1992; see reviews by Mousseau and Saugier, 1992; Rogers *et al.*, 1993; Ceulemans and Mousseau, 1994) and the lack of change in foliar [P] (Conroy *et al.*, 1986*b*; Norby *et al.*, 1986*a*; Conroy, 1992; Conroy *et al.*, 1992), any stimulation in photosynthesis as a result of elevated [CO_2] is likely to be modified by potential changes in foliar [N] rather than [P].

Deficiency levels of [N] and [P] for pot grown Sitka spruce seedlings have been estimated at 0.92 and 0.1 % dry mass, respectively (Chandler and Dale, 1995). The 'Ingestad' approach, in conjunction with a composite soil, adopted in this study successfully produced a range of foliar N and P concentrations but only induced N deficiency. Therefore, despite the importance of P availability for the PCR cycle, the results from this study are likely to be attributable to N-limitation within the photosynthetic mechanism rather than P.

The interaction between atmospheric [CO_2] and leaf [N] on photosynthetic rates is most evident from the stimulation by elevated [CO_2] of PPFD-saturated photosynthesis at the different nutrient supply rates: elevated [CO_2] increased A_{mmax} by 33 % and 19 % for seedlings supplied with high-N rates and low-N rates, respectively. This result for Sitka spruce is in agreement with a number of observations which show that for a range of C_3 species, assimilation rate is generally more strongly stimulated in elevated [CO_2] when plants received high nutrient supply rates (see review by Ceulemans and Mousseau, 1994). However, in contrast Kerstiens *et al.* (1995) reported no net stimulation of photosynthesis with increasing nutrient supply for four tree species, including Sitka spruce. Unfortunately, foliar nutrient concentrations were not reported and the nutrient treatments were supplied as a one-off application of slow release fertiliser, rendering it impossible to make any meaningful comparison with the present study.

There was also an apparent increase in nutrient use efficiency (NUE) as a result of elevated [CO_2]. Photosynthetic rates of seedlings grown and measured in elevated [CO_2] were higher than those of seedlings grown and measured in ambient CO_2, at the same foliar [N]; A_{mmax} and foliar [N] were 11.1mol m^{-2} s^{-1} and 2.62 % dry mass and 16.52 mol m^{-2} s^{-1} and 2.36 % dry mass, for ambient and elevated [CO_2], respectively. It is possible that the seedlings in this study have responded to growth

in elevated [CO_2] by optimising the distribution of N within the photosynthetic system, moving it away from Rubisco into the more limiting components of the light reactions. The N redistribution theory is supported by the fact that there is a proportionally larger decrease in Rubisco activity than chlorophyll content, in the elevated [CO_2] seedlings which had a lower foliar [N] than their counterparts in ambient [CO_2]. Other studies investigating the effect of elevated [CO_2] on N distribution within the photosynthetic system have also reported shifts away from Rubisco in favour of more limiting processes (Sage *et al.*, 1989; Sage, 1994; Tissue *et al.*, 1993).

Growth in elevated [CO_2] induced a substantial reduction in both carboxylation efficiency (V_{cmax}), and electron transport rate/RuBP regeneration capacity (J_{max}), in the low-N supply treatment. A decrease in both V_{cmax} and J_{max} are strong evidence of 'down-regulation' of photosynthesis in response to elevated [CO_2] in N limited conditions (von Caemmerer and Farquhar, 1981; Sage, 1994). Irrespective of the growth [CO_2], low nutrient supply rates also resulted in 'down-regulation' of photosynthesis. However, in the high-N supply treatment there was no evidence of any 'down-regulation' or acclimation of photosynthesis in elevated [CO_2]. These results are consistent with many studies which have reported changes in photosynthetic capacity with elevated CO_2 solely under nutrient limited conditions (Evans, 1989; Tissue *et al.*, 1993; Kellomäki and Wang, 1997).

5.4.2 Chlorophyll and photosynthesis

Seedlings growing in elevated [CO_2] had higher concentrations of chlorophyll per unit of foliar N compared with those growing in ambient [CO_2]. A shift in chlorophyll / [N] ratio as a result of elevated [CO_2] is consistent with observations made on *Pinus radiata* seedlings after growth in 660 μmol mol^{-1} CO_2 (Conroy *et. al.*, 1986*b*) and *Picea abies* seedlings after growth in 750 μmol mol^{-1} CO_2 (Lippert *et al.*,

1997). Assuming that the investment of nitrogen in light harvesting components is constant per unit chlorophyll (Pons et al., 1994) and that thylakoid nitrogen is proportional to the chlorophyll content (50 mol thylakoid N mol^{-1} Chlorophyll), (Evans, 1989), then these results support the theory that in this study, N was reallocated in favour of those proteins involved in electron transport. This fact and the increase in J_{max}/V_{max} ratio found in the seedlings grown in elevated CO_2 with low-N supply rates, supports the optimisation or resources theory. This states that plants tend to maximise resource-use efficiency, in particular N, by allocating resources to maintain a balance between limiting and non-limiting processes. Long and Drake (1992) calculated that a 30 per cent decrease in Rubisco activity could occur without decreasing light-saturated assimilation rates, when CO_2 concentration is elevated to 700 μmol mol^{-1}. Therefore, under nutrient limited conditions a shift in foliar N allocation from Rubisco to proteins involved in the light reactions is to be expected, and has often been reported to occur in elevated [CO_2] (Tissue *et al.*, 1993; Wullschleger, 1993; Stockfors, 1997). This form of photosynthetic acclimation in response to elevated [CO_2], is likely to be more widespread in northern coniferous tree species, which are more liable to be grown in low nutrient environments, than broadleaf species.

The processes behind and reasons for acclimation of photosynthesis in elevated [CO_2] are not fully understood, but both nutrient and sink limitations have often been proposed as likely causes (Jarvis, 1989; Arp, 1991; Stitt, 1991). Given that N accumulates in the foliage when its supply exceeds the demand placed on it by growth, the lower foliar [N] found in the elevated compared with the ambient [CO_2] treatment, at both nutrient supply rates, would indicate that seedlings in this study were not sink limited, i.e. growth rate matched nutrient supply rate. This and the fact that 'down-regulation' was only observed in the low-N supply treatment, would indicate that photosynthetic acclimation observed in this study was a result of N

limitation rather than sink limitation.

5.4.3 Nutrition and stomatal conductance

Differences in the increase in photosynthesis in response to be elevated [CO_2] may not to be solely a result of biochemical changes, but may also be attributable to differences in the response of stomata to elevated [CO_2]. A 30 to 40 per cent reduction in stomatal conductance has been estimated over a range of C_3 species for a doubling of present day atmospheric CO_2 concentration (see reviews by Cure and Acock, 1986; Eamus and Jarvis, 1989). However, recent longer term studies have predicted more moderate reductions (Johnsen, 1993; Kerstiens *et al.*, 1995; Eamus, 1996), no effect (Samuelson and Seiler, 1993*a*; Thomas, et al., 1994; Kerstiens *et al.*, 1995; Lippert et al., 1996; Heath and Kerstiens, 1997; Rey, 1997) or even a slight increase in stomatal conductance (Barton *et al.*, 1993; Dick *et al.* pers. comm.) in elevated [CO_2].

In the present study, we have found no significant effect of elevated [CO_2] on stomatal conductance, although g_s was highly correlated with foliar [N] ranging from 60 mmol m^{-2} s^{-2} at 1.5 g N m^{-2} to 200 mmol m^{-2} s^{-1} at 5.0 g N m^{-2} (see Figure 5.7). Although [CO_2] did not influence stomatal conductance in Sitka spruce directly, for a given g_s net assimilation rates were significantly higher in seedlings grown and measured in elevated [CO_2] compared with those grown and measured in ambient [CO_2]. It is also likely that the response of g_s to elevated [CO_2] is species specific, Sitka spruce is one of a number of coniferous species from northern temperate forests which show little stomatal sensitivity to [CO_2] (e.g. Beadle *et al.*, 1979; Higginbotham *et al.*, 1985).

The reported impacts of nutrient supply on the response of stomata to elevated CO_2 are variable. Although our study showed no interaction between stomatal

conductance, nutrient availability and atmospheric CO_2 concentration on Sitka spruce, a small reduction in g_s in elevated [CO_2] in nutrient-limited trees was found in *Picea mariana* (Johnsen, 1993) and *Fagus sylvatica* (Kerstiens *et al.*, 1995). The opposite has also been shown to apply, several studies have reported increases in g_s with growth in elevated [CO_2] in nutrient limited conditions, (e.g. *Liriodendron tulipifera* (Norby and O'Neill, 1991) and *Prunus avium* (Kerstiens *et al.*, 1995)), while in *Pinus taeda* (Thomas, *et al.*, 1994) and *Quercus robur* (Kerstiens *et al.*, 1995), there was no effect. Strong correlations between g_s and [N], as found in this study, have been reported recently, and may be a response to changes in biomass accumulation and allocation, in particular shifts in the root / shoot ratio (Heath and Kerstiens, 1997). However, it has been postulated by Conroy (1992) that it may be advantageous to minimise the decrease in transpiration rate caused by the response of g_s to [CO_2], because otherwise a low rate of mass flow of nutrients from soil to leaves, may constitute a strong limitation to growth, particularly when nutrients are in short supply. In view of the strong relationship we found between g_s and [N], and the absence of any interaction with [CO_2], it is difficult to marry this hypothesis with our results. However, it may be that the foliar nitrogen range (2.6 - 0.92 % dry mass) in our study did not extend to low enough [N] to trigger such a mechanism.

5.4.3 Nutrition and dark respiration

In this study, dark respiration was stimulated by high-N supply rate irrespective of [CO_2], expressed either on a leaf area or [N] basis. Increase in R_m as a result of high nutrient supply rates are a likely consequence of enhanced biomass accumulation (see Chapter 4) and thus stimulated demand for both growth and maintenance respiratory products. Interestingly, in the present study the degree of nutrient-induced R_m stimulation was less in seedlings grown in elevated [CO_2] than in ambient [CO_2] (37 and 52 per cent, respectively). The difference in foliar [N], and its strong linear correlation with R_m, accounted for much of the treatment variability

in R_m (Figure 5.5).

In this and other recent studies on Sitka spruce, dark respiration rates were generally slightly higher in, or unaffected by, elevated [CO_2] (Townend, 1993; Kerstiens *et al.*, 1995; Barton, 1997). Although it was originally supposed that respiration in elevated [CO_2] would always be stimulated, this is not invariably the case (Wullschleger *et. al.*, 1994). As yet a clear general trend in respiratory responses to elevated [CO_2] in tree species is far from established. Reviews by Ceulemans and Mousseau (1994) and Poorter *et al.* (1992) have reported responses in R_m to elevated [CO_2] for a wide spectrum of tree species, which ranged from -50 to +200 per cent. Much of the variability in R_m reported in these studies can probably be accounted for by changes in [N] resulting either directly, as in this study from varying nutrient supply rates, or indirectly from ontogenic shifts in plant [N] as a result of growth in elevated [CO_2]. For example, the apparent increase in R_m observed in this study on seedlings grown outside OTCs can be accounted for by the relative increase in foliar [N], which was a result of reduced growth rates in response to lower air temperatures.

Since between one and two thirds of the carbon fixed in photosynthesis is lost via respiratory processes (Amthor, 1991), the effect of elevated [CO_2] on both growth and maintenance demands is a crucial component in estimating both carbon gain and sequestration. Because Sitka spruce is frequently grown on nutrient poor soils and the relative increase in R_m, as a result of elevated [CO_2], is lower with low nutrient supply rates, proportionally less carbon will be lost via above ground respiratory processes than have been predicted from other studies on well fertilised seedlings.

5.5 Conclusions

Under elevated atmospheric [CO_2], changes in the basic processes of photosynthesis

may provide a mechanism for many C_3 species to utilise resources more efficiently, especially when they are limited. Nutrition plays a pivotal role in the photosynthetic response of Sitka spruce to elevated [CO_2]. For example, less nitrogen was required to maintain higher rates of net carbon uptake in elevated [CO_2] compared with ambient [CO_2].

Down-regulation of photosynthetic rates in response to elevated [CO_2] occurred only in seedlings growing with low-N supply rates. In elevated atmospheric [CO_2] foliar [N] was lower than in ambient [CO_2] and therefore became more limiting. This reduction in foliar [N] resulted in its preferential partitioning in favour of proteins involved in electron transport when foliar [N] fell below 1 %, i.e. the low-N supply treatment.

Growth without nutrient limitation in elevated atmospheric [CO_2] does not lead to any apparent photosynthetic down-regulation and becomes more nutrient efficient. However, the Rubisco activity of nutrient-deficient Sitka spruce is reduced in elevated [CO_2] compared to be ambient [CO_2] although photosynthetic rates remain higher, indicating a significant nitrogen effect on photosynthetic acclimation.

As no evidence was found for a reduction in stomatal conductance in response to elevated [CO_2] it is unlikely that any increase in water use efficiency will occur. Further, in view of the increase in total leaf area as a result of growth in elevated [CO_2], a significant rise in water use per tree is likely to occur.

Despite dark respiration rates being slightly higher in elevated [CO_2] for a given foliar [N], the effect of [CO_2] on R_m was less than that of [N]. Because of the large proportion of carbon consumed in respiration it is likely that the effect of elevated [CO_2] on net carbon sequestration will be mediated through interactions between

nutrient supply, [CO_2] and dark respiration rates.

5.6 Summary conclusions

This study has shown that elevated [CO_2] and nutrient supply rate will affect the physiology of seedling Sitka spruce interactively.

- The rate of photosynthetic enhancement by elevated [CO_2] was nutrient dependant. Photosynthetic rates of seedlings growing in elevated [CO_2] were on average 33 and 19 % higher than in ambient [CO_2] for the low-N and high-N supply rates, respectively.
- Net photosynthetic rates were positively correlated with foliar [N], as was V_{cmax} and J_{max}.
- Down-regulation of photosynthesis only occurred as a result of growth in elevated [CO_2] when seedlings were nutrient deficient, i.e. in the low-N supply treatment. Both V_{cmax} and J_{max} were lower in elevated [CO_2] when seedling were supplied with low-N rates.
- Differences in the ratio of J_{max} to V_{cmax} as a result of growth in elevated [CO_2] with low-N supply rate, suggests that N was redistributed within the photosynthetic apparatus. The increased J_{max} to V_{cmax} ratio and larger amount of chlorophyll to foliar [N] found in the low-N treated seedlings grown in elevated [CO_2] indicate that N was partitioned in favour of RuBP regeneration (thylakoid/chlorophyll proteins).
- Both elevated [CO_2] and higher foliar [N] increased dark respiration rates of shoots. The response of dark respiration to nutrition was bigger than that of [CO_2].
- In this study, stomatal conductance was not significantly affected by elevated [CO_2]. However, when measurements were made at treatment [CO_2] elevated [CO_2] seedlings had a higher photosynthetic rate for a given g_s.

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CHAPTER 6

Effects of elevated [CO_2], nutrition and climatic warming on bud phenology in Sitka spruce (*Picea sitchensis*) and their impact on the risk of frost damage

Abstract

Effects of elevated [CO_2], clone and plant nutrition on bud dormancy of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) were examined. Sitka spruce seedlings were fumigated with ambient or elevated (ambient + 355 μmol mol^{-1}) concentrations of CO_2 in open-top chambers for three growing seasons. In 1991 and 1992, elevated [CO_2] delayed bud burst in the spring and advanced bud set in the autumn. The effect of the open-top chamber on the thermal requirement for bud burst was greater than the effect of elevated [CO_2] (50 and 30 day degrees (D_d), respectively). In a second study, four clones of Sitka spruce taken from two provenances at 43 and 45° N, were fumigated with ambient or elevated [CO_2]. There was a large natural variation in the timing of bud burst and bud set among the clones. Elevated CO_2 had no effect on bud dormancy of the Skidegate a clone, but it reduced the growing season of the North Bend b clone by 20 days. In a third study, Sitka spruce seedlings growing in ambient or elevated [CO_2], were supplied with one of three nutrient regimes, low (0.1 x potential), medium (0.5 x potential) or high (2.0 x potential), using a method and solution based on the Ingestad technique. Elevated [CO_2] did not affect bud dormancy in the high-nutrient treatment, but it reduced the growing season of plants in the low-nutrient treatment by 22 days. Increasing plant nutrient supply lengthened the growing season, plants flushed earlier in the spring and set bud later in the autumn.

The effects of elevated [CO_2] plus a 0, 2 or 4°C climatic warming on the timing of bud burst and the subsequent risk of late spring frost damage were assessed using a simulation model and meteorological data from three sites, Edinburgh, Braemar and Masset. The model predicted that (i) doubling the CO_2 concentration in the absence of climatic warming, will delay the onset of bud burst at all three sites, (ii) climatic warming in ambient [CO_2] will hasten bud burst and (iii) climatic warming in elevated [CO_2] will hasten bud burst at Edinburgh and Braemar but to a lesser extent than climatic warming alone. At Masset, a 4 °C warming was required to advance the date of bud burst of seedlings in the elevated [CO_2] treatment. At all three sites, elevated [CO_2] and climatic warming increased the mean daily temperature on the date of bud burst, thus reducing the risk of subsequent frost damage.

Keywords: *bud burst, bud dormancy, bud set, bud phenology model, clone, growing season length, mineral nutrition, thermal requirement*

6.1 Introduction

Synchronisation of plant dormancy with annual temperature cycles is important, especially in cool temperate regions. In these regions, premature onset of vegetative growth in the spring and delayed growth cessation in the autumn will extend the duration of shoot growth, but may result in frequent frost damage. Conversely, the delayed onset of growth in the spring and premature dormancy in the autumn will under-utilize site resources, and may result in reduced competitiveness. Bud phenology, which is the study of the timing and duration of bud dormancy, especially in relation to climatic conditions, must therefore be considered when assessing the impact of climate change on plant productivity and survival.

Physiological and phenological responses of buds to environmental variables, such as air temperature and daylength, have an underlying genetic basis (Perry, 1971; Dunlap *et al.*, 1992), reflecting the adaptive significance of these characteristics (Worrall and Mergen, 1967; Kramer, 1992). Thus, phenological characteristics of native trees are generally well coupled with local climatic conditions, with little or no risk of frost damage. However problems may arise if predicted climatic changes occur at a rate that is faster than the adaptive ability of most tree species (Gates *et al.*, 1992), or when exotic species are introduced to regions with less favourable climatic conditions than in their natural range. Sitka spruce (*Picea sitchensis* (Bong.) Carr.) in the UK is an exotic species whose natural range extends along a narrow coastal strip in northwestern North America. Reports of spring and autumn frost damage to Sitka spruce at various sites emerged soon after the species' introduction to Britain (Macdonald, 1927; Day and Peace, 1946; Day, 1957). It became clear that there was a problem of synchronization between bud dormancy and the local climate (Cannell and Sheppard, 1982; Cannell and Smith, 1984; Cannell *et al.*, 1985).

This problem may be exacerbated or ameliorated by climatic change, which may either delay or advance the timing of bud burst depending on the degree of chilling required by Sitka spruce (Cannell and Smith, 1983b; Murray *et al.*, 1989). Spring bud burst in woody perennials is regulated by temperature, whereas autumn bud set is controlled by both temperature and day length (Koski and Selkäinaho, 1982; Cannell and Smith, 1983; Koski and Sievänen, 1985; Falusi and Calmassi, 1990; Hänninen, 1990; Hänninen *et al.*, 1990). Thus, any change in air temperature will affect the timing and duration of bud dormancy and consequently the plants' performance, competitiveness and survival. In addition, changes in the timing of the onset and cessation of growth could increase or decrease the probability of frost damage, depending on the degree of climatic warming and the likelihood of late spring frosts (Cannell and Smith, 1983b; Murray *et al.*, 1989).

Increased atmospheric CO_2 concentrations may also affect bud phenology directly through changes in biochemistry and physiology. For example, changes in starch or hormonal concentrations may alter dormancy status and growth patterns, by shifting the timing and duration of the vegetative season (Powell, 1969; Waring, 1969; Zimmerman *et al.*, 1980; Lanner and Connor, 1988; Cannell, 1990). In addition, increased atmospheric CO_2 concentrations have been shown to change the C/N ratio within trees (Eamus and Jarvis, 1989; Conroy, 1992). Increasing plant nutrition generally results in an increased relative growth rate and extended growing season (Ågren, 1985; Ingestad and Kahr, 1985; Dewald *et al.*, 1992).

In temperate regions, a better understanding of the relationship between bud phenology and the many biotic and abiotic factors affecting it is essential to predict the growth, competitiveness and survival of native and exotic tree species, in response to increasing CO_2 concentrations and global warming. In this study the phenological responses of bud burst and bud set of Sitka spruce to a doubling of the

present day atmospheric CO_2 concentration, over a range of nutrient treatments and in four clones covering a latitudinal gradient were evaluated. The data were used to parameterise a bud phenology model. The model was then used to predict the timing of bud burst and the mean minimum temperature on the date of bud burst at three sites, two in Scotland and one in North America, at ambient and elevated CO_2 concentrations, with and without climatic warming.

6.2 Materials and methods

Experiments were performed in open-top chambers at the Bush Estate near Edinburgh, Scotland (55°51'N, 198 m altitude), from 1 June 1990 to 31 May 1993. Measurements were made on (i) the timing of bud burst in the spring and bud set in the autumn over three growing seasons on Sitka spruce seedlings growing in ambient or elevated [CO_2], (ii) the genetic variability in the bud phenological response to elevated [CO_2] on four clones of Sitka spruce and (iii) the effect of nutrition on bud burst and bud set in elevated and ambient [CO_2].

6.2.1 Open-top chambers

Eight octagonal open-top chambers (OTC), with a floor area of 7.0 m^2 and height of 2.3 m were used in 1990. Four of the OTCs received ambient [CO_2] and four received elevated [CO_2]. In March 1991, the number of open-top chambers was increased to 10, giving five replicates per [CO_2] treatment. The mean daily temperature was 1.4 ± 0.98 °C higher inside the OTC than outside (Figure 6.1). For a fuller description of chamber properties see Fowler *et al.* (1989).

6.2.2 CO_2 exposure facility

Before injection into all chambers, ambient air was passed through a series of activated charcoal filters to remove ozone, sulfur dioxide and nitrogen dioxide. The

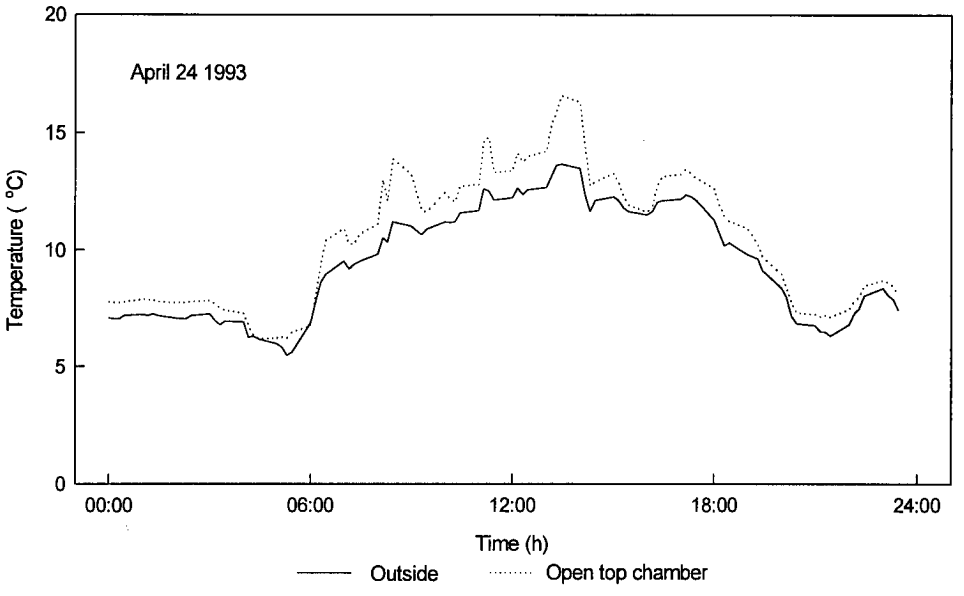

Figure 6.1: The diurnal temperature fluctuations inside and outside the open top chambers (OTC), on a typical day during spring bud burst.

ambient [CO_2] chambers then received this air directly through a polyethylene manifold 1.5 m above ground level. The CO_2 concentration in these chambers fluctuated diurnally around a mean daily value of 355 μmol mol^{-1}. The elevated [CO_2] chambers received air supplemented with pure CO_2 (Distillers MG, UK) to raise the ambient concentration by 355 μmol mol^{-1}, i.e. to double present day ambient concentrations. Pure CO_2 was injected directly into the ambient air stream in the chamber fan units at preset flow rates, where it was mixed thoroughly before being released into the chambers. The CO_2 concentration in the elevated chambers varied around 700 ± 80 μmol mol^{-1}, depending on the ambient concentration and external wind speeds which affected ambient air incursion through the open top.

6.2.3 Potted seedlings

In June 1990, 2000 unflushed two-year-old (1+1) bare-rooted Sitka spruce (*Picea sitchensis* (Bong.) Carr.) seedlings, Forestry Commission identity number 83(2015)S LOT 2, provenance 20, origin Queen Charlotte Islands, were taken from cold storage and potted in 2-dm^3 pots containing a composite soil consisting of sphagnum peat, 5 mm quartz and sterilized loam in the ratio 13/4/3 by volume. Vitax Q_4 fertilizer (N,P,K; 5.3/7.5/10) was added at 4 g per dm^3 to the soil. The plants were then randomised and 250 were selected and evenly distributed among 10 randomized blocks in each of the eight open-top chambers. In March 1991, 30 plants from each chamber were repotted in 4.5-dm^3 pots. An additional 30 plants were randomly selected from each of the [CO_2] treatments, repotted and placed in two additional chambers, increasing the replicate number of chambers per treatment to five. The remaining plants were discarded because of insufficient space in the OTCs. In March 1992, 15 plants from each of the 10 chambers were repotted in 18-dm^3 pots. These plants are the same as those used in the experiments described in chapters 2 and 3.

6.2.4 Clonal plants

Cuttings were taken from physiologically mature Sitka spruce trees growing in a clonal provenance trial near Edinburgh, Scotland, in March 1990. Cuttings from two clones were selected from each of the North Bend (41.3° N) and Skidegate (53.2° N) provenances, and immediately transferred to a mist propagation bench in a greenhouse. In July 1990, when the cuttings had rooted, they were potted in 1-dm^3 pots containing composite soil. They remained in the greenhouse until March 1991, when 60 plants from each of the four clones were repotted in 4.5-dm^3 pots and randomly placed in each of six open-top chambers, 10 plants per clone per chamber, three chambers per [CO_2] treatment.

6.2.5 Nutrient-treated seedlings

In March 1992, 330 one-year-old Sitka spruce seedlings, which had been raised in

ambient (355 μmol mol^{-1}) or elevated (700 μmol mol^{-1}) CO_2 conditions, were potted in 2-dm^3 pots containing composite soil and randomly placed in each of 10 open-top chambers and an outside control plot, giving 30 plants per chamber and 30 plants outside. Balanced nutrient solutions were applied on a weekly basis to each of the trees based on the Ingestad technique (Ingestad and Lund, 1986), which matches the addition rate of nutrients to plant growth rates. The nutrient addition rate was calculated based on previous growth measurements of Sitka spruce seedlings. A potential nitrogen concentration of 2% in current-year foliage was assumed and three rates of nutrient supply were selected to give 2 x potential (High), 0.5 potential (Medium) and 0.1 x potential (Low) foliage nitrogen content. Each of the nutrient treatments were applied to 10 of the 30 plants in the chambers and outside. The three nutrient treatments significantly affected plant growth rates and biomass allocation. These plants are the same as those used in the experiments described in chapters 4 and 5.

6.2.6 Spring bud phenology

Spring bud burst was measured on the potted seedlings in 1991 and 1992, on the clonal plants in 1992, and on the nutrient-treated seedlings in 1993. The leader buds were scored every second day during the spring flushing period. The buds were scored on a scale of 1 to 4, where 1 - slight swelling, 2 - swollen bud, 3 = green needle clearly showing through the bud scales, and 4 - needle elongation. For each plant, the date of bud burst was taken to be the date on which the leader bud reached Stage 3.

6.2.7 Autumn bud phenology

Bud set in the autumn was measured in 1991 on the potted seedlings, and in 1992 on the potted seedlings, clonal plants and nutrient -treated seedlings. Leader buds were scored several times weekly from August to October, as either growing or dormant. The date of bud set of each plant was taken to be the date on which the bud became dormant, i.e. dark in colour and firm to touch.

6.2.8 Statistical analysis

Data for the numbers of plants that had achieved either bud burst or bud set by each recording date were analysed by a modification of the cumulative distribution analysis formally presented by Hunter *et al.* (1984) and developed by Brain and Butler (1988). Analysis of the data was difficult because there was serial correlation between values at successive recording times and the counts were not normally distributed. However, the underlying variable (the time to bud burst or bud set) was analyzed by fitting its cumulative distribution function to the empirical cumulative distribution derived from the observed data. A maximum likelihood analysis was performed with the Genstat 5 software programme.

The time to bud burst was normally distributed with no transformation of the time axis. If the time to bud burst is t, the cumulative distribution function (F) is

$$F(t) = N(z)$$

$$z = b(t - b), \qquad [6.1]$$

where b is the mean time to bud burst, b is the inverse of the standard deviation of time to bud burst and N is the cumulative normal distribution function with zero mean and unit variance.

The data on bud set were more difficult to model. It appeared that the distribution fitted should have been

$$F(t) = N(zl)$$

$$zl = b(\log(t - l) - m), \qquad [6.2]$$

where l was the lag period before initiation of bud set, m was the mean adjusted time to bud set ($\log(t - l)$) and b the inverse standard deviation of the adjusted time to bud set. However there were computational problems in fitting this model, because every treatment had a different lag period. A modification of the fitting procedure used for the bud burst model gave reasonable results for bud set except for the plants in the

outside treatments. Therefore, the first approach was used on this occasion and the outside treatments were only included when they fitted within this model. The statistical difference between treatments was determined by testing the goodness of fit of individual treatment parameters with the goodness of fit of parameters obtained from the combined data (Ross, 1981).

6.2.9 Spring bud phenology model simulations

The Cannell and Smith (1983) bud burst model was chosen to predict the likely consequences of increased global warming on the timing of bud burst in the spring, and the minimum temperature on that date. The assumption of this model is that the timing of bud burst is a function of the non-linear relationship between the number of chill days (C_d) and the thermal requirement (D_d) to bud burst,

$$D_d = a + b \exp(rC_d), \qquad [6.3]$$

where D_d is the thermal requirement to bud burst, C_d is the number of chill days and a, b and exp*r* are parameters with values of -56, 602 and 0.991, respectively (Murray *et al.*, 1989). The number of chill days to bud burst was taken as the number of days since 1 November when the mean air temperature was $\leq$ 5 °C. The thermal time required to the date of bud burst was taken as the accumulated day degrees (°C) above the mean daily base temperature of 5 °C from 1 January. The numbers of chill days and day degrees received to the date of bud burst were calculated for the potted seedlings in 1991, 1992 and the nutrient-treated seedlings in 1993. Plants in the intermediate nutrient treatment were selected, because they most closely matched the nutritional status of the 1991 and 1992 seedling plants. The temperature records used to calculate D_d and C_d were obtained from screened sensors placed inside and outside the OTC, with readings recorded every 15 minutes and stored on a data logger (21x Campbell Scientific Ltd., Leicestershire, England). These data were

then used to parameterize the model for Sitka spruce growing in both ambient and elevated [CO_2].

The model, using both sets of parameters, was then used to simulate the effects of climatic warming and elevated [CO_2] on the timing of spring bud burst at three meteorological stations. Two sites in Scotland were chosen, a central lowland site at Edinburgh (55°48' N, 26m) and an upland site at Braemar (57°00' N, 339m) and one native coastal Sitka spruce site, in northwest North America, at Masset, Queen Charlotte Islands (54°02' N, 3m). A simulation was run at each of the above sites for the years 1897-1978, at ambient and elevated CO_2 concentrations, and for 0, 2 and 4 °C uniform warming, using daily maximum and minimum temperatures recorded in Stevenson screens.

Table 6.1: The dates of 50% bud burst in the spring, for plants growing inside OTCs receiving 350 μmol mol^{-1} CO_2, (Ambient CO_2), or 700 μmol mol^{-1} CO_2, (Elevated CO_2) and outside receiving 355 μmol mol^{-1} CO_2, (Outside), in three experiments.

Treatment	CO_2 and chamber treatment		
	Ambient CO_2	Elevated CO_2	Outside
Potted seedlings			
1991	April 24a[1]	May 1b	-
1992	May 4a	May 8b	May 11c
Clonal plants			
Skidegate a (54° N)	April 26a	April 29a	-
Skidegate b (54° N)	April 21a	April 25b	-
North Bend a (43° N)	April 29a	May 4b	-
North Bend b (43° N)	May 7a	May 10b	-
Nutrient-treated seedlings			
High	April 27a	April 28a	May 4b
Medium	April 25a	April 30b	May 2b
Low	April 29a	May 4b	May 12c

[1] Dates followed by the same letter within each row indicate that the fitted cumulative distribution functions were not significantly different (p = 0.05; using Chi-squared tests on the differences in deviance).

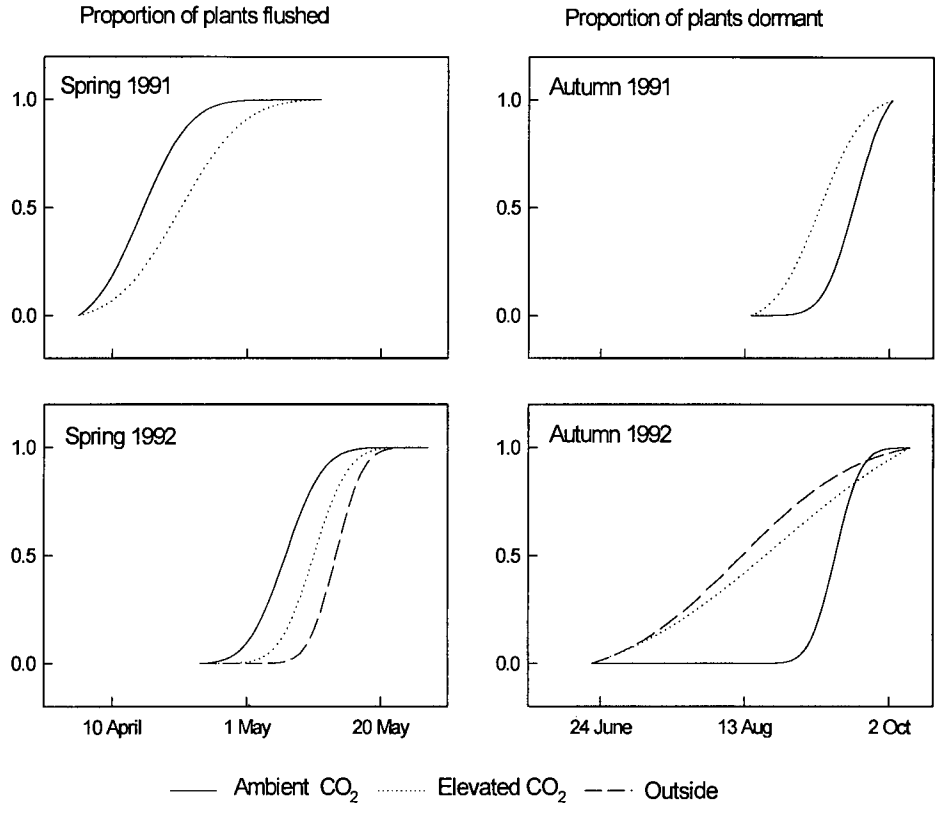

Figure 6.2: The fitted cumulative distribution function of bud burst and bud set, for the Sitka spruce potted seedlings in 1991 and 1992. Ambient = plants raised inside OTC, receiving ambient [CO_2] (355 μmol mol^{-1}), Elevated = plants raised inside OTC, receiving elevated [CO_2] (700 μmol mol^{-1}) and Outside = plants raised outside, receiving ambient [CO_2] (355 μmol mol^{-1}).

6.3 Results

6.3.1 Bud phenology of potted seedlings

Bud phenology of plants in the elevated [CO_2] and outdoor treatments was significantly different from that of plants in the ambient [CO_2] treatment in the spring and autumn of both years (Figure 6.2, spring 1991 $p < 0.001$, 1992 $p < 0.01$ and autumn, 1991 and 1992 $p < 0.001$). In the spring of both 1991 and 1992, seedlings subjected to elevated [CO_2] had a significantly higher thermal requirement to the date on which 50% of the plants had burst bud than seedlings in the ambient [CO_2]

treatment (Table 6.1). This resulted in the elevated-CO_2-treated plants flushing seven and four days later than the ambient-CO_2-treated plants, in 1991 and 1992, respectively.

Table 6.2: The dates of 50% bud set in the autumn, for plants growing inside OTCs receiving 355 μmol mol^{-1} CO_2, (Ambient CO_2), or 700 μmol mol^{-1} CO_2, (Elevated CO_2) or outside receiving 355 μmol mol^{-1} CO_2, (Outside), in 3 experiments.

Treatment	CO_2 and chamber treatment		
	Ambient CO_2	Elevated CO_2	Outside
Potted seedlings			
1991	Sept 20a[1]	Sept 5b	-
1992	Sept 13a	Aug 22b	Aug 6b
Clonal plants			
Skidegate a (54° N)	Sept 11a	Sept 7a	-
Skidegate b (54° N)	Sept 19a	Sept 15b	-
North Bend a (43° N)	Sept 24a	Sept 15b	-
North Bend b (43° N)	Oct 15a	Sept 28b	-
Nutrient-treated seedlings			
High	Oct 1a	Sept 29a	Sept 25a
Medium	Sept 27a	Sept 16b	Oct 2b
Low	Sept 18a	Sept 1b	July 22[2]

[1] Dates followed by the same letter within each row indicate that the fitted cumulative distribution functions were not significantly different ($p = 0.05$; using Chi-squared tests on the differences in deviance).

[2] The cumulative frequency function could not be fitted, the date of bud set was calculated from the raw data.

6.3.3 Bud phenology of mineral-nutrient-treated seedlings

Plants growing in the OTC in elevated [CO_2] and receiving low (10% of the potential rate) or medium (50% of the potential rate) nutrient supply rates, had significantly different dates of bud burst and bud set (Low = $p < 0.001$, Medium = $p < 0.01$) to plants growing in the OTC in ambient [CO_2] (Figure 6.4). The elevated [CO_2] treatment

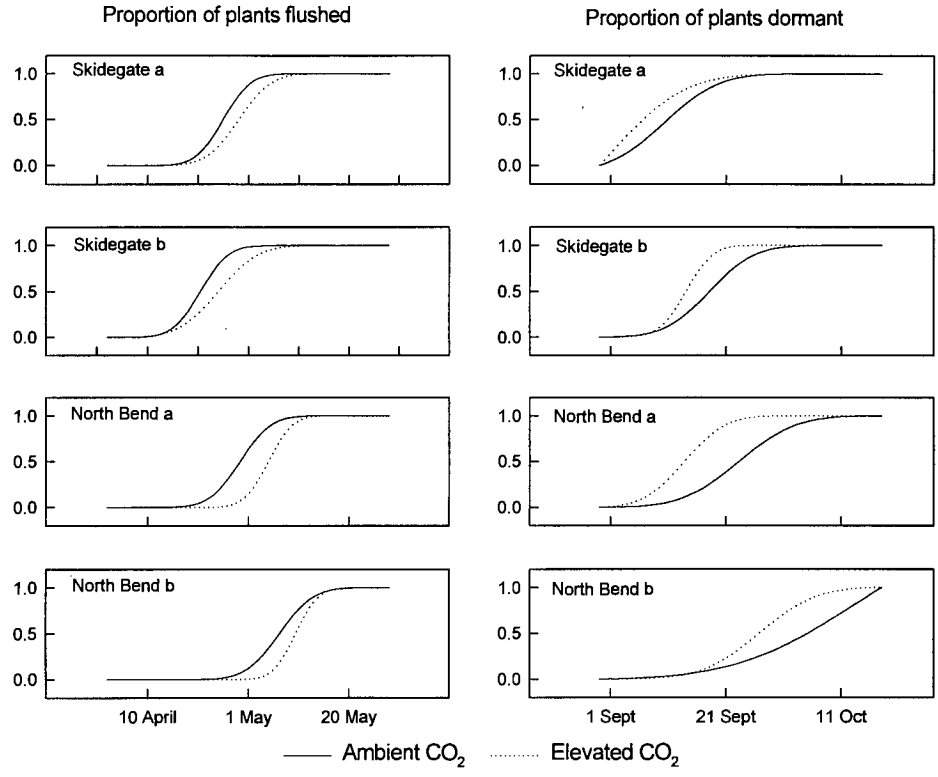

Figure 6.3: The fitted cumulative distribution of bud burst and bud set for the Sitka spruce clonal plants, from North Bend (41.3 °N) and Skidegate (53.2 °N) provenances.

delayed bud burst and advanced bud set. The effect of [CO_2] on bud dormancy was ameliorated by high rates of nutrient supply: there was no significant difference in the timing of either bud burst or bud set in plants receiving the high nutrient supply rate (200% of the potential rate).

Plants receiving the low nutrient supply rate showed the biggest dormancy response to both elevated [CO_2] and OTC. The elevated [CO_2] treatment delayed bud burst by five days in the low and medium nutrient regimes and one day in the high nutrient regime (Table 6.1). Bud set was advanced in the elevated [CO_2] treatment by 17, 11 and 2 days at low, medium and high nutrient supply rates. respectively (Table 6.2).

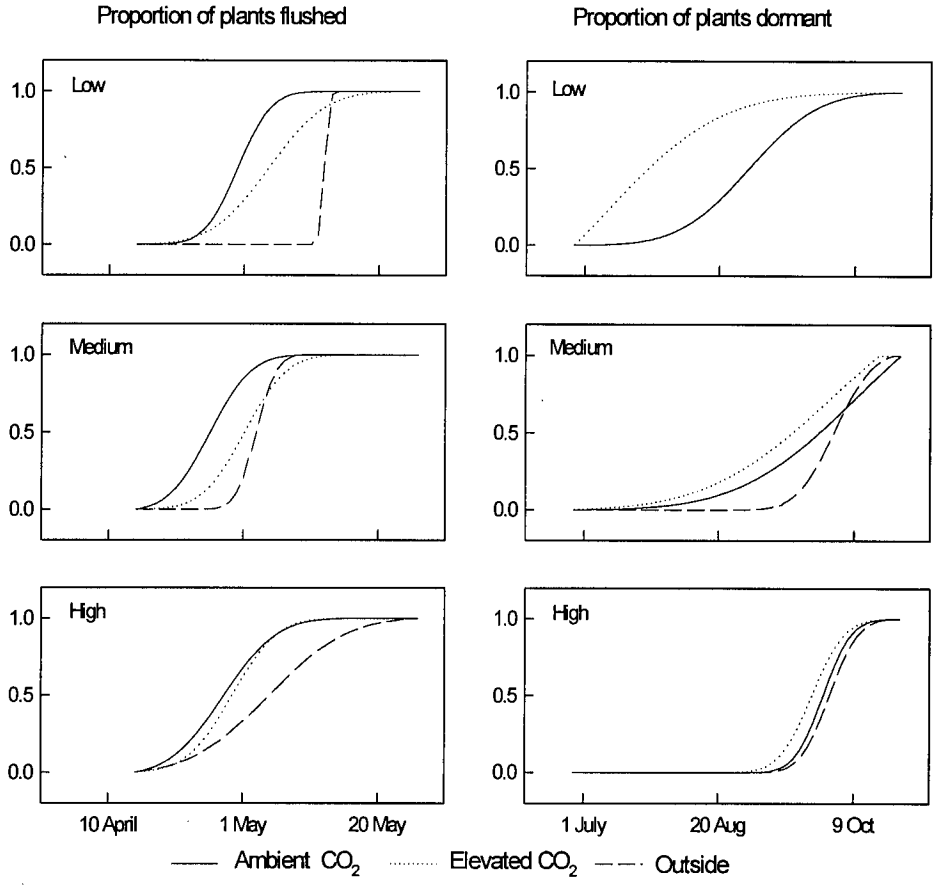

Figure 6.4: The fitted cumulative distribution function of bud burst and bud set for the Sitka spruce nutrient-treated seedlings. The optimum nutrient application rate was that required to sustain a 2% nitrogen concentration in the current-year foliage. The treatment application rates were, Low = 10%, Medium = 50%, and High = 200% of the optimum rate.

The effect of increasing the nutrient application rate from Low to High was to advance the timing of bud burst and delay bud set, resulting in an increased growing season of 15 days in ambient [CO_2], 34 days in elevated CO_2 and 65 days outside.

6.3.4 Simulation model

A non-linear regression was produced using Equation 6.3 and values of a = -56, b = 602 and exp$r$ = 0.991 (Murray *et al.*, 1989) for Sitka spruce seedlings growing

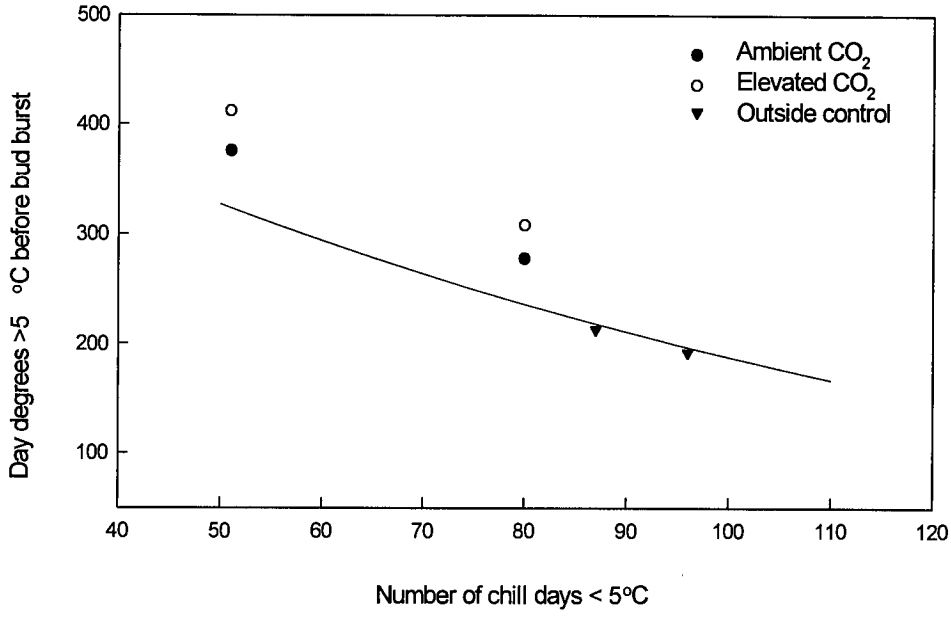

Figure 6.5: The relationship between day degrees to bud burst and number of chill days, given by the equation: $D_d = a + b \exp(rC_d)$, where is the thermal time to bud burst, C_d is a number of chill days and *a, b* and *r* are parameters for Sitka spruce in ambient CO_2 (Murray *et al.*, 1989). The symbols indicate where each of the treatments lie in relation to this line.

outside in ambient [CO_2] (Figure 6.5). The accumulated D_d required to bud burst for the plants growing in elevated [CO_2] inside the OTC and in ambient [CO_2] both inside and outside the OTC are shown in Figure 6.5. The number of chill days (C_d) received by the outside control plants, before bud burst in 1991, 1992 and 1993, was 87, 96 and 96, respectively. The parameterized model accurately predicted the thermal requirements (D_d) to bud burst for the ambient [CO_2] outside-grown control plants, given 87 and 96 chill days. As a result of the chamber warming effect, plants grown in the OTCs received fewer chill days before bud burst than the outside control plants (51 and 80 days), (Figure 6.1). The predicted values of D_d for both the ambient- and elevated-CO_2-treated plants growing in OTCs were lower than the observed values (Figure 6.5). The thermal requirement to bud burst was 50 and 80

day degrees higher than the predicted values at both levels of chilling, for the chamber-grown ambient- and elevated-CO_2-treated plants, respectively. Thus, the separate effects of chamber and elevated [CO_2] on thermal time to bud burst were assumed to be a uniform increase of 50 D_d and 30 D_d across all chilling levels.

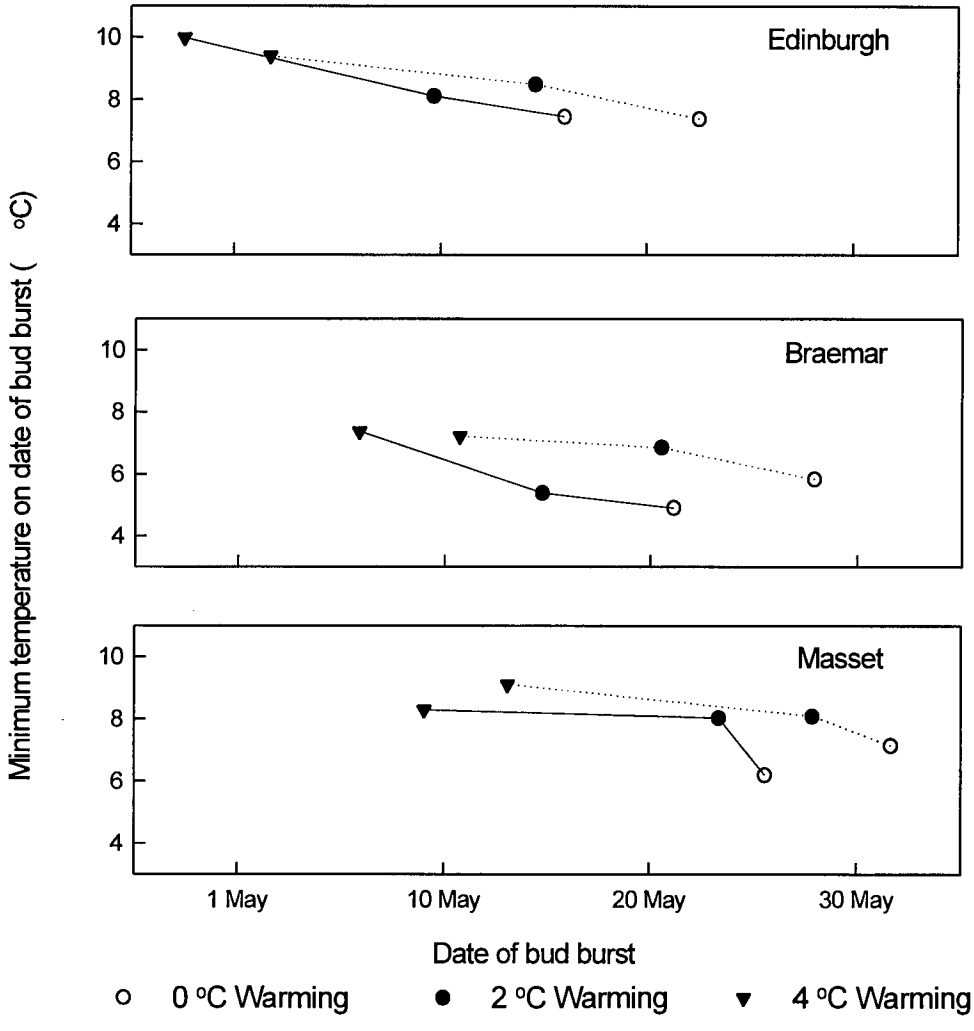

Figure 6.6: The predicted mean dates of bud burst, and the mean minimum daily temperature on that date, for Edinburgh, Braemar and Masset, for O, 2 and 4 °C uniform warming, with (- - - -) and without (—) elevated [CO_2].

To simulate the effect of elevated [CO_2] on spring bud phenology, the model parameter *a* estimated by Murray *et al.* (1989) was adjusted to account for the uniform increase in thermal requirement (30 D_d) of Sitka spruce growing in elevated CO_2. A model simulation was then run using both values of *a* (-56 and -26 for ambient and elevated [CO_2] treatments, respectively) and the temperature records for Edinburgh, Braemar and Masset. Predictions of the date of bud burst and the minimum temperature on that date for 0, 2 and 4 °C uniform warming, were obtained for each site (Figure 6.6). The effect of elevated [CO_2] alone (0 °C) was to delay bud burst at all three sites and to increase the minimum temperature on the date of bud burst at the cooler Braemar and Masset sites. The effect of climatic warming alone (ambient [CO_2]) was to advance the date of bud burst and to increase the temperature on that date at all three sites. The combined effect of elevated [CO_2] and a 2 °C uniform warming was to bring forward the date of bud burst by 2 days at Edinburgh and 1 day at Braemar and delay flushing by 2 days at Masset; the mean minimum temperature on the date of bud burst was increased at all three sites. Elevated [CO_2] and a 4 °C warming advanced bud burst by 14 days at Edinburgh, 10 days at Braemar and 12 days at Masset. The increase in the minimum temperature on each of those days was 1.6, 2.3 and 3.2 °C, respectively.

6.4 Discussion

We observed a pronounced effect of elevated [CO_2] on bud dormancy. Sitka spruce grown in elevated [CO_2] had a growing season that was, on average, 24 days shorter than that of Sitka spruce grown in ambient [CO_2]. This was a result of plants grown in elevated CO_2 flushing later in the spring and setting bud earlier in the autumn than plants grown in ambient [CO_2].

6.4.1 Genetic influence on bud phenology

Our results support the theory that a genetic factor is involved in the regulation of bud dormancy (Worrall and Mergen, 1967; Perry, 1971). The timing of bud burst and bud set was strongly influenced by provenance and clone, with the two more southerly clones from the North Bend provenance having longer growing seasons than either of the Skidegate clones. Maximization of the length of the growing season is expected to be more beneficial at more southerly locations, where there is a reduced risk of late spring and early autumn frosts, which would result in damage of non-dormant frost-sensitive tissue. This study also showed that there was the potential to select clones that would not be adversely affected by increases in atmospheric [CO_2]. Although only four clones were studied, the Skidegate *a* clone showed no significant effect of elevated [CO_2] on bud dormancy, whereas the elevated [CO_2] treatment significantly reduced the growing season of the other three clones.

6.4.2 Nutritional influence on bud phenology

There was a major interaction between CO_2 concentration and nutrient supply rate on the timing and duration of bud dormancy. Bud dormancy of plants receiving the high nutrient supply rate was unaffected by [CO_2] treatment, whereas plants receiving the low nutrient treatment had a significantly shorter growing season in elevated [CO_2] (Tables 6.1 and 6.2). Therefore, Sitka spruce growing in enhanced atmospheric CO_2 concentrations (700 $\mu mol\ mol^{-1}$) may produce a larger phenological response to increasing nutrient supply rates, than Sitka spruce presently growing in ambient CO_2 concentrations. Silvola and Ahlholm (1993), who studied the effects of CO_2 concentration and nutrient status of *Salix phylicifolia* found that the length of the growing period varied by as much as 30% depending on the [CO_2]/nutrient ratio. In accordance with our findings, they found that CO_2 concentration and nutrient regime also affected bud dormancy. Therefore it is likely that increased

atmospheric CO_2 concentrations in the future, will have a bigger effect on the timing of bud burst and bud set of Sitka spruce growing on nutrient-poor sites than on fertile sites. Sitka spruce on nutrient-poor sites will experience shorter growing seasons which may decrease its annual primary productivity, unless elevated [CO_2] causes a comparative increase in relative growth rate. However, if trees growing on nutrient-poor sites are currently subject to frost damage, delayed bud burst and advanced bud set will decrease the risk of late spring and early autumn frosts. Elevated [CO_2] would therefore have a net benefit on productivity. Increasing the nutrient supply rate lengthened the growing season, suggesting that the effect of elevated [CO_2] on Sitka spruce bud dormancy at nutrient-poor sites would be reduced by the addition of fertilizer. Increasing the nutrient supply rate also ameliorates other plant responses to elevated [CO_2], including reducing the effect of [CO_2] on root-shoot partitioning (Eamus and Jarvis, 1989).

6.4.3 Open-top chamber effect on bud phenology

The large effect of the chamber on plant phenology was probably due to the increased temperatures within the chambers (Figure 6.1). Plants growing outside, at lower temperatures, had a significantly shorter growing season compared with chamber-grown plants. Figure 6.5 highlights a discrepancy between estimating thermal time to bud burst inside and outside OTCs. In this study, the thermal requirement to bud burst was calculated from daily mean air temperatures. The mean wind speed inside the OTC was 3 m s^{-1}, whereas it was 1 m s^{-1} outside in the control, resulting in a difference in the boundary layer resistance between the two environments (Monteith, 1981). Thus as a result of heat convection, the difference between plant temperatures inside and outside the chambers will have been smaller than the difference between air temperatures. This could account for the apparent increase in thermal requirement (50 D_d) of the plants growing inside the OTCs. The chamber effect (50 D_d) on the thermal requirement to bud burst was larger than the

$[CO_2]$ effect (30 D_d), in this study. Therefore, it is important to quantify the chamber effect when attempting to extrapolate results obtained from experiments in OTCs to the field.

6.4.4 Model predictions

Many factors control bud phenology and will have a major impact in determining the future survival and competitiveness of temperate tree species. To date, model simulations used to predict the effect of climatic warming on temperate tree species have not taken into account the direct impact of $[CO_2]$ on bud dormancy (Murray *et al.*, 1989; Hänninen, 1991), even though atmospheric CO_2 concentrations are rising at an increasing rate. We evaluated the direct impact of elevated $[CO_2]$ on bud dormancy and then simulated a 0, 2 and 4 °C uniform warming using the model of Cannell and Smith (1983) parameterized for ambient and elevated $[CO_2]$. This bud burst model is one of a range that exist for woody perennials (Sarvas, 1972; Sarvas, 1974; Fuchigami *et al.*, 1982; Cannell and Smith 1983*b*; Hänninen, 1990; Thornley and Johnson, 1990). Hunter and Lechowicz (1992) included this model in an evaluation of simulation models designed to predict the timing of spring bud burst in temperate trees. They concluded that the Cannell and Smith model was one of the models best suited to predict the date of bud burst in temperate trees. In addition, it has been used successfully to predict spring bud burst in a range of woody perennials, including Sitka spruce growing in Scotland (Murray *et al.*, 1989). Our simulation results show that plants subjected to elevated $[CO_2]$ without any climatic warming will flush later in the spring at higher temperatures than at present (Figure 6.5). The extent of the effect depends on local site conditions, such as climate and soil nutrient status. Climatic warming alone, will advance the date of bud burst and increase the temperature on that date. However, when $[CO_2]$ and temperature change simultaneously the effect of elevated $[CO_2]$ on the timing of bud burst is reduced, or even reversed, depending on the local site climate. At Edinburgh, the mildest site in

this study, the date of bud burst was predicted to advance, with temperatures warmer than at present; this would be beneficial to Sitka spruce, in terms of primary productivity and plant competitiveness. In contrast, at Masset, even with a 2 °C warming, spring bud burst was delayed by elevated [CO_2].

Cannell and Smith (1983*b*) demonstrated that the likelihood of damaging spring frost occurring around the time of bud burst is inversely proportional to the mean minimum temperature on the date of bud burst. Therefore, the warmer the temperature on the date of bud burst the lower the subsequent risk of frost damage. In each of the above cases the mean minimum temperature on the date of bud burst was predicted to increase, therefore the incidence of spring frost damage under elevated [CO_2] and climatic warming will decrease at all three sites.

Previous model simulations, which have not accounted for the direct effect of elevated [CO_2] on bud phenology, will over-estimate the advance in the timing of bud burst, and under-estimate the temperature on that date. Hänninen (1991) predicted that bud burst of trees growing in central Finland would occur in midwinter and that the trees would be subsequently exposed to temperatures between -27 and -10 °C. By including the ameliorating effect of elevated [CO_2] these model predictions may have appeared less devastating. Our results demonstrate the importance of including the effect of elevated [CO_2] when predicting phenological responses to climatic warming.

6.5 Conclusion

In conclusion, increasing atmospheric CO_2 concentrations in conjunction with climatic warming is likely to improve the survival of Sitka spruce in Britain, by

reducing the risk of spring and autumn frost damage and lengthening the potential growing season.

6.6 Summary conclusions

This study is the only one in which both the effect of elevated [CO_2] and climatic warming on bud phenology have been researched.

- There was a significant effect of elevated [CO_2] on bud phenology, with seedlings flushing later and setting bud earlier when grown in elevated [CO_2]. This had the overall effect of reducing the growing season by an average of 24 days.
- The results presented in this study confirm that there is a strong genetic influence on bud phenology, with clones from more southerly provenances having longer growing seasons. This will allow the forester to select for particular provenances most suited to future [CO_2] and climatic conditions.
- Nutrient supply rate significantly affected the influence of elevated [CO_2] on bud phenology. Seedlings with a foliar nitrogen concentration over 2 % were unaffected by elevated [CO_2]. The lower the nutrient supply rate the larger the [CO_2] effect on bud phenology. Artificially increasing nutrient supply rates will allow foresters to will potentially alter the phenological response of Sitka spruce to elevated [CO_2], at a given site.
- Probably the biggest effect of OTCs on Sitka spruce was observed on bud phenology. In fact, the effect of OTC on spring bud burst was bigger than that of elevated [CO_2] *per se*. This was probably a result of the increased ambient temperature found inside OTCs.
- The model simulations predict that in future elevated CO_2 concentrations with climatic warming the timing of spring bud burst and the likelihood of subsequent

frosts will be site dependant. At the mild Edinburgh site, budburst will be earlier than at present and the likelihood of spring frosts reduced, thereby potentially increasing net primary productivity. However, at the cooler Masset site with a 2 °C warming, bud burst will be delayed, with a decreased risk of subsequent frost damage decreased. The effect this will have on net primary productivity will depend on the balance between the advantage gained from increased frost hardiness to the disadvantage of a reduced growing season.

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CHAPTER 7

7.1 Introduction

The aim of this chapter is to summarise the main results found in each of the previous experimental chapters, particularly in respect to use of OTCs as an experimental technique, duration of the experiment and the interaction between nutrient availability and elevated atmospheric CO_2 concentrations. The discussion will be focused on a synthesis of the main responses to elevated [CO_2] and the potential interactions between them. In addition, recommendations for possible future work are made.

7.2 Effects of open top chambers

It is widely recognised that the environment within open-top chambers differs from that outside (i.e., the non-chambered plots), principally with respect to temperature, radiation, and atmospheric turbulence (Taylor *et al.*, 1994). This phenomenon has become know as the 'chamber effect'. Consequently, trees grown inside chambers may differ from those in non-chambered plots as a direct result of changes in their physical environment (Olszyk *et al.*, 1992). The physiological and ecological significance of these chamber effects depends on species specificity, and experimental duration and design. A common experimental design difference between studies using OTCs, is the flow rate through the OTC. Flow rates have to be sufficiently high in order to maintain leaf and air temperatures as close as possible to those of outside, but there is a temptation to reduce flow rates in order to conserve expensive CO_2. The consequence of this is a much higher undesirable differential occurring between inside and outside air temperatures. To facilitate the extrapolation of elevated [CO_2] results obtained from OTC studies, to the 'real world' it is essential

to quantify the direct impact of OTCs *per se* on the particular species in question, and for that particular design set up. Interestingly, the increase in temperature within an OTC is comparable with that forecast for a doubling of atmospheric [CO_2] (IPCC 1995). Therefore, responses attributed to growth inside OTCs could potentially be similar to those which will be found in the future.

One of the objectives of this thesis was to determine if phenology, growth, biomass allocation or physiology of seedlings grown in OTCs and exposed to ambient [CO_2] differed from their counterparts growing in outside control plots.

The primary impact of OTCs on seedling growth, phenology and physiology in this study are outlined in Table 7.1. Although the OTCs modify solar radiation including UV-B), rainfall and pollution levels, the principle factor contributing to the chamber effect in this study would appear to be the concomitant rise in air temperature. In the present study, the main impact of higher air temperature inside OTCs was on bud phenology. Spring bud burst and autumn bud set are both regulated by temperature (Cannell and Smith, 1983; Murray *et al.*, 1994), and the increased thermal heat found within OTCs directly affected both. Seedlings growing outside OTCs had a significantly shorter growing season than their counterparts inside OTCs (Chapter 6; Murray *et al.*, 1994).

Because of Sitka spruce's determinate growth pattern any variable affecting bud phenology will have a major impact on growth and the allocation of assimilates. Because distribution of assimilate is dependent on the flushing cycle, with most of the assimilate allocated to shoots during the early spring flushing period, but then directed to the roots during the later summer / autumn lag stage (Chapter 4), more carbon was partitioned to the roots of the outside seedlings. These seedlings had a

Table 7.1: The main effects of open-top chamber on the phenology, growth, allocation, physiology and nutrition of seedling Sitka spruce. All effects reported are significant at p=0.5.

	Variable	OTC effect	Magnitude
PHENOLOGY	Growing season	Increased	10-100% High-Low-N
GROWTH	RGR_{sm}	Decreased	
	RGR_{am}	ns	
	Height	Increased	25 %
	Root collar diameter	ns	
	SLA	ns	
	Total mass	ns	
ALLOCATION	Stem mass	Increased	31 %
	Branch mass	Increased	22%
	TLA / TLM	ns	
	R/S mass ratio	Decreased	11-45 % High-Low-N
PHYSIOLOGY	Photosynthesis	ns	
	Respiration	Decreased	32 %
	Stomatal conductance	Increased	35 %
NUTRITION	[N] / mass	ns	
	[P] / mass	ns	
	[K] / mass	ns	

longer period when they were photosynthetically active but shoot growth had ceased. The ultimate result of this was seedlings growing outside OTCs were shorter, had less branch mass and bigger root systems. Interestingly, an almost identical chamber effect was found on young birch trees growing in the field, where the climate inside the chambers produced bigger trees with longer growing seasons (Rey, 1997).

The magnitude of response of each variable to growth inside OTCs was dependant on nutrient supply rate. In general, the higher the nutrient supply rate the bigger the chamber effect, with the biggest differential observed on bud phenology.

As a general technique for exposure to elevated $[CO_2]$, OTCs are probably the best compromise currently available. In the present study, OTCs have proved a successful tool in exposing Sitka spruce to elevated $[CO_2]$, minimising the increase in ambient air temperature and hence their impact on growth and physiology, while

also minimising the high costs of [CO_2] fumigation. However, as a cautionary note it is important both to understand and bear in mind the uncertainties inherent in data collected from within OTCs in order to make any accurate assessment of the impact of future [CO_2].

7.3 Experimental duration

Needle primordia of Sitka spruce are laid down during the previous growing season, therefore it is particularly important to conduct long term experiments when studying the response of this species to elevated [CO_2]. Results obtained from experiments exposing seedlings to elevated [CO_2] for less than one growing season will invariably be confounded by the impact of the growth conditions during the previous season. Because of the longevity and size of mature trees, a study of the long term response of saplings and mature trees to elevated [CO_2] would be prohibitively expensive and technically difficult, so invariably some level of compromise is inevitable.

In the first instance, the minimum duration of CO_2 experiments should encompass both budset and budburst enabling the primordia to be laid down and extend in the experimental [CO_2] conditions. In this study, all seedlings used in the experiments were germinated and grown in their respective [CO_2] for at least 12 months prior to the start of the experiments. Results obtained from short-term studies should be interpreted with great care. In this study, the duration of the experiment tended to affect the magnitude of the biomass response to elevated [CO_2] rather than the direction. For example, roots were always bigger in elevated [CO_2]-grown seedlings, but the level of stimulation ranged from 35 % after the first year to 10 % after the third (Chapter 2; Murray *et al.*, 1996). Similar results were found for physiological responses; both dark respiration rates and photosynthetic rates were consistently higher in elevated [CO_2], irrespective of the treatment period. Light-saturated A_{mmax}

rates although higher in both ambient and elevated [CO_2] after three season's, compared with two season's exposure, was stimulated in elevated [CO_2]-grown seedlings when measured in their treatment [CO_2]. The level of stimulation of A_{mmax} was apparently the same in both years, i.e. *ca* 50 % (Chapter 3).

The results presented in Chapters 2 and 3 indicate that the true test of whether an environmental variable has affected seedling physiology and hence growth is not whether that particular parameter is altered, but whether the allometric coefficient relating to it has changed. A treatment that alters plant growth rate resulting in allocation changes, e.g. shifts in R/S ratio, might merely be detecting an unchanged ontogenetic shift, resulting from the change in plant size or age. This explains the apparent downward shift in root stimulation found with time in this study, since when the correlation coefficient (K) is < 1, R/S ratio will fall with ontogeny, and when K > 1, R/S will rise.

Ultimately, the objective of this and the majority of elevated [CO_2] experiments on tree seedlings, is to gain insight into the responses of trees to future increased atmospheric [CO_2] and to attempt to evaluate which traits will be affected and likely to be continued throughout the trees natural life span. Although it has often been observed that the early enhancement in relative growth rates of young tree seedlings as a result of elevated [CO_2] is not continued after longer exposure periods, the impact of this early stimulation on long term biomass accumulation has been shown to be is extremely important (Petterson and McDonald, 1992; Tissue *et al.*, 1996; Rey and Jarvis, 1997). In these three studies, the early enhancement of annual relative growth rate, although not sustained over the entire duration of the experiment, was still evident after five years, i.e. plants growing in the elevated [CO_2] treatment were significantly larger than their counterparts growing in ambient [CO_2].

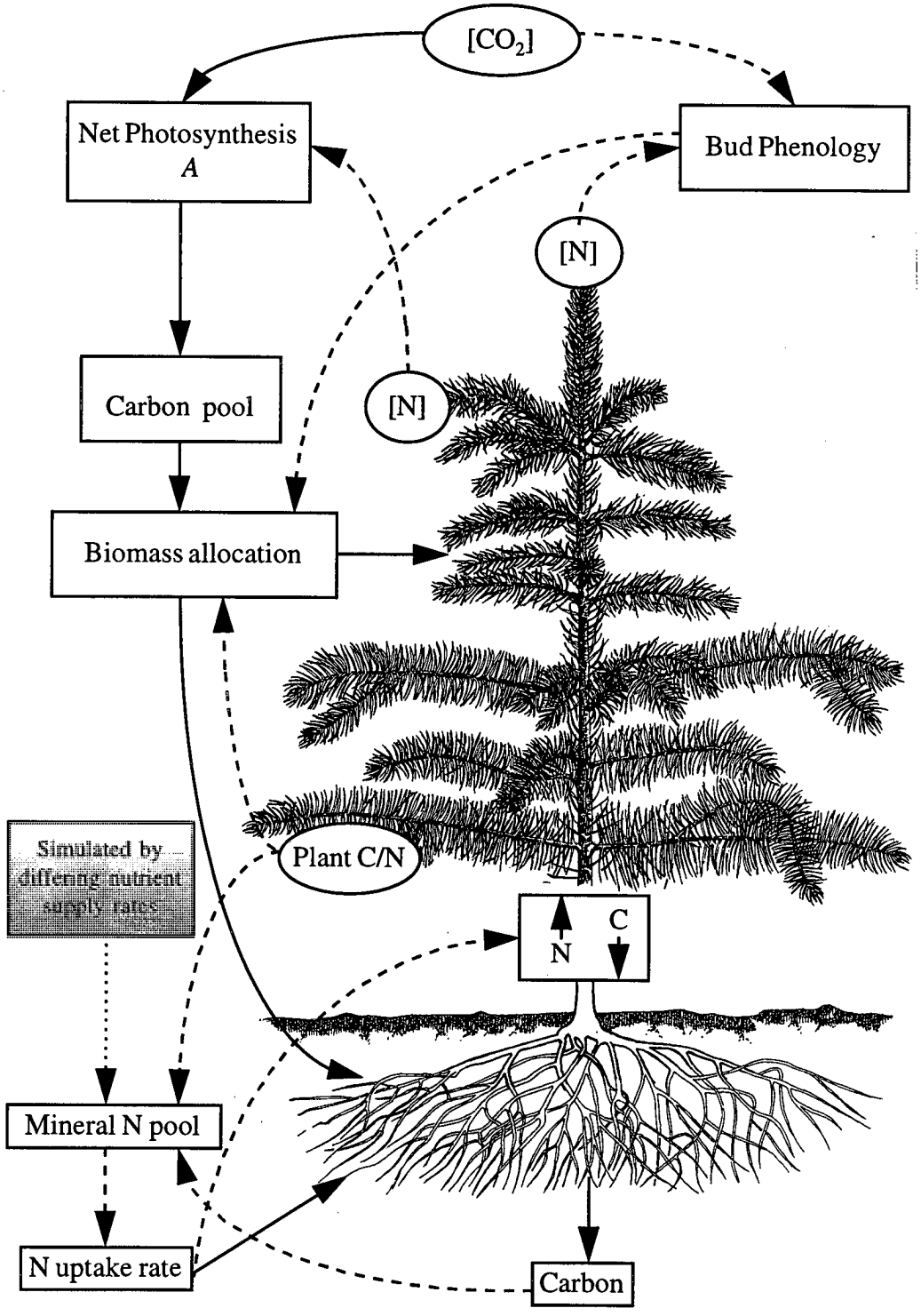

Figure 7.1: Schematic representation of carbon and nitrogen flow to and from trees, and potential feedback mechanisms in response to growth in elevated [CO_2]. Solid lines represent fluxes of C and N and dashed lines represent processes influencing them.

7.4 Effects of varying nutrient supply rates and elevated CO_2

The responses of Sitka spruce to elevated [CO_2] presented earlier in this thesis, are inextricably linked with nutrient availability. Therefore, this final discussion is focused on the impact of an atmospheric [CO_2] of 700 μmol mol^{-1} in relation to plant nutrition.

Figure 7.1 shows the fluxes of both carbon (C) and nitrogen (N) to and within a Sitka spruce seedling. Currently, atmospheric [CO_2] limits photosynthesis therefore, any increase in [CO_2] directly stimulates photosynthetic rates and hence increases the C pool (Chapter 3 and 5). Carbon held within this pool is then differentially allocated between shoot or roots depending on whether assimilate production or nutrient uptake is limiting growth, i.e. the functional balance model (Chapters 2 and 4). Photosynthesis and stomatal conductance are the only biological processes directly affected by [CO_2], but other processes such as phenology and biomass allocation are indirectly affected as a result of changes in plant C/N ratios (Chapters 2, 4 and 6).

Nutrient availability within a forest is site specific, although generally Sitka spruce plantations in Britain are restricted to poorer quality soils in upland areas. However for a given soil type, nutrient pools and hence availability within the soil ecosystem will be indirectly affected by the influence of [CO_2] on shoot and root litter C/N ratios, carbon exudation from the roots and possible changes in amounts and types of mycorrhizal colonisation (Norby *et al.*, 1987; O'Neill *et al.*, 1987; Rey and Jarvis, 1997).

Because the experiments in this study were on pot grown seedlings it has not been possible to study the direct effects of elevated [CO_2] on the soil mineral N pool. Therefore, a range of soil nutrient pools were simulated by applying three different

nutrient supply rates directly into the pots. This is an artificial situation which could be avoided in the future, in longer term field grown experiments.

7.4.1 Phenology

Unlike the effects of temperature on phenology, which are fairly well documented (Ong and Baker 1985), effects of elevated [CO_2] on both bud and growth phenology have received little attention to date (Murray *et al.* 1994). The phenological response to elevated [CO_2] in this study was modified by nutrient supply rate (Chapter 6). When compared with similar nutrient 'limited' seedlings receiving ambient [CO_2] (i.e. the low-N and medium-N nutrient treatments), those grown in elevated [CO_2] flushed later in the spring and senesced earlier in the autumn, significantly reducing the overall growing season. Under nutrient 'limited' conditions, the impact this had on seedling growth and biomass allocation was similar to that described above for seedlings grown inside and outside OTCs. Seedlings grown in elevated [CO_2] under low-N supply rates were shorter, had less above-ground biomass and larger root systems compared with their counterparts in ambient [CO_2]. However, under nutrient-rich conditions (i.e. High-N) elevated [CO_2] had no effect on bud phenology. This allowed seedlings growing in elevated [CO_2] without nutrient limitations to maximise their potential growing season.

Because shifts in seedling C/N ratios were a result of changes in [N] rather than [C], phenological responses to elevated [CO_2] will interact with site nutrient status. Plants growing on nutrient-poor sites will set bud earlier and, therefore, invest more carbon into root biomass, while those growing on nutrient rich sites will maximise the length of the growing season.

This study has also demonstrated the potential to select genetically for particular CO_2 response traits in order to maximise particular site resources. For example, if at a

given site spring frost damage poses a potential hazard, then selection of a clone, which responded to elevated [CO_2] by delaying bud burst, would minimise any risk of subsequent frost damage. However, the disadvantage to this could potentially be an under-utilisation of site resources. It has been shown that provenances of Sitka spruce differ considerably in their response to [CO_2] (M. Centritto, pers. Comm.)

7.4.2 Physiology

The importance of photosynthesis and that of carbon allocation should not be under estimated as they determine both the efficiency with which substrate is used and the extent of its productive investment, and thus the future photosynthetic potential of the whole plant.

In this study, irrespective of experimental duration (Chapter 3) or nutrient supply rate (Chapter 5) elevated [CO_2] enhanced net photosynthesis. The degree by which light-saturated photosynthesis was enhanced was nutrient dependent, rates being 19 and 33 % higher with the low-N and High-N supply rates, respectively.

Shoots from seedlings receiving the high-N supply rate for 6 months and elevated [CO_2] for 24 months did not show any down-regulation of photosynthesis. In contrast, those with the low-N supply rates did show down-regulation, of both V_{cmax} and J_{max} (Chapter 5). Sage (1994) found a similar result in his review of 40 long-term studies which focused on the effect of elevated [CO_2] on the short-term response of photosynthesis to intercellular CO_2. He concluded that, the effect of elevated CO_2 on the A/C_i response was either sink or nutrient dependent, with plants grown with low nutrients or in small pots exhibiting down-regulation, while those grown without nutrient deficiency in large pots or in the field exhibiting no effect or even up-regulation.

Acclimation of the photosynthetic apparatus in this manner is not necessarily detrimental to the plant, indeed the opposite is likely. Under nutrient-poor conditions nitrogen is liable to be the variable most limiting to growth, and therefore any coordinated biochemical adjustment, i.e. down-regulation of photosynthesis, that improves growth, competitiveness and resource use efficiency in elevated [CO_2] will ultimately increase the chance of survival in the future (Sage, 1994).

Down-regulation of the photosynthetic apparatus has also been described as a 'negative' response to carbohydrate accumulation in leaves. This has been attributed to either a feedback inhibition of photosynthesis as C supply exceeds demand, or to direct interference with chloroplast or thylakoid membrane function or by acting as a physical barrier to gas diffusion (Conroy *et al.*, 1986; Arp, 1991; Stitt, 1991; Baker and Allen, 1994; Sage, 1994). However, in this study although carbohydrates and, in particular, starch were not measured directly, there is no evidence of its accumulation in the needles. If starch had accumulated in the needles of seedlings growing in elevated [CO_2] with low-N supply rates, one would have expected a change in SLA when compared with similar plants grown in ambient [CO_2]. There was no change in SLA as a response to elevated [CO_2] for any of the nutrient supply rates imposed in this study (Chapter 4). Therefore, the down-regulation observed in this study as a result of growth in elevated [CO_2] is likely to be a response to nutrient limitation, resources being shifted to the most limiting process, in this case nutrient acquisition.

The ability of a plant to distribute nitrogen optimally between RuBP regeneration and RuBP carboxylation has been demonstrated in conditions of low PPFD (Sharkey, 1985; Sage, 1994). In low PPFD plants tend to adjust their nitrogen partitioning in favour of those proteins involved in light-harvesting and electron transport thus maintaining a balance between the light reactions and Rubisco activity,

with the result that nitrogen in plant canopies is distributed in such a way as to maximise the ratio of canopy carbon gain to canopy nitrogen content. Seedlings grown with the low-N supply rate exhibit this ability to re-partition N within the photosynthetic apparatus. Although, both V_{cmax} and J_{max} were lower in elevated [CO_2], proportionally there was a bigger reduction in V_{cmax}. This was probably as a result of the increased efficiency of Rubisco as the carboxylation/oxygenation ratio increased in favour of carboxylation.

Thus, we may conclude that photosynthetic acclimation of seedling Sitka spruce growing on nutrient poor soils under future elevated [CO_2] will involve both down-regulation and reallocation of resources within the photosynthetic apparatus.

7.4.3 Stomatal conductance

There was no significant acclimation response of stomatal conductance to elevated CO_2 (Chapter 3). However, there was a strong correlation with foliar [N] and, in addition, seedlings growing in elevated [CO_2] had a slightly higher net assimilation rate for a given g_s (Chapter 5). In contrast to the widely held hypothesis that stomatal conductance will decrease with rising [CO_2] and consequently plant water use efficiency (WUE) will increase, the effect of elevated CO_2 on WUE of Sitka spruce seedlings is unclear.

Stomatal conductance increased with increasing foliar [N] and total leaf area increased with both foliar [N] and elevated [CO_2], and therefore total plant transpiration rates are likely to increase as a response to elevated [CO_2] in nutrient rich conditions. However, it is still unclear as to whether WUE of Sitka spruce growing on nutrient rich sites in elevated [CO_2] conditions will change. Despite the lack of any decrease in g_s and an increase in leaf area, WUE may still increase as a result of the increased photosynthetic rates. However, on nutrient poor sites the

picture is complicated by shifts in C allocation in favour of roots. The small leaf area coupled with the bigger proliferation of roots found in seedlings grown in elevated [CO_2] will increase capacity to take up water. Therefore, despite net photosynthetic rates being higher, and both leaf area and g_s unchanged, the potential for increased water use will be enhanced and therefore no conclusions as to changes in WUE can be drawn.

7.4.4 Growth, biomass accumulation and allocation

Despite a universal stimulation of photosynthesis, elevated [CO_2] only increased seedling growth when N availability was high, i.e. foliar [N] > 1.9 % of dry mass (Chapters 3 and 5). Figure 7.2 summarises the proportional distribution of biomass accumulation and allocation of seedlings growing in either ambient or elevated [CO_2] with one of three nutrient supply rates (area = dry mass (g) and height of stem column = seedling height (cm)). Seedlings growing in elevated [CO_2] with low-N supply rate had a larger proportion of biomass allocated to their roots compared with their counterparts in ambient [CO_2], and because there was no concurrent increase in total biomass accumulation, this resulted in reduced above ground biomass. Thus, aboveground net primary productivity (ANPP), that is the harvestable timber, will be lower in elevated [CO_2] on nutrient poor sites. Similar findings have been reported for other coniferous tree species (Griffin *et al.*, 1993; Prior *et al.*, 1997). Despite these findings on low-N seedlings, other experimental observations suggest that the [CO_2] growth response of nutrient-limited plants can be proportionately similar to that of nutrient-rich plants (Wullschleger et al., 1993; Idso and Idso, 1994; Johnson *et al.*, 1995; Lloyd and Farquhar, 1996). In addition, current mechanistic models used to predict the long-term impact of elevated [CO_2] on net primary productivity (NPP) estimate enhanced biomass production under both nutrient-rich and nutrient-poor conditions, but only after an initial decline during the first 10 years or so (Cannell and Thornley, 1997; Thornley and Cannell, 1996). The impact that

the results presented in this thesis have on such model findings will be discussed later.

The lack of an apparent increase in biomass accumulation in nutrient-poor conditions after two to three years exposure to elevated [CO_2] (Chapters 2 and 4) can be attributed to changes in the balance between total canopy photosynthesis and the amount of C lost via respiration and fine root turnover. In this study, elevated [CO_2] resulted in changes in growth and bud phenology which led to increased root production, and likely higher rates of fine root turnover. In addition, seedlings growing in elevated [CO_2] had higher dark respiration rates and hence increased C losses. The loss of C as a result of respiration is significant. Together, construction and maintenance respiration are estimated to cost the plant between 18 % and 34 % of annual photosynthesis for coniferous species (Kinerson, et al., 1977). These findings on nutrient poor seedlings are important in terms of NPP of coniferous forests in temperate and boreal regions where currently, N commonly limits NPP (Ballard, 1984).

The potential to match increased carbon fixation rates with that of nutrient availability, and hence to stimulate NPP in future atmospheric [CO_2], is simply a matter of supplying additional fertiliser or of growing Sitka spruce on nutrient rich sites! Figure 7.2 shows the response of seedlings growing in elevated [CO_2] to a range of nutrient supply rates. When nutrients were supplied at levels which resulted in foliar [N] of 2.0 % and above, i.e. the medium-N and high-N treatments, a positive growth response to elevated [CO_2] was observed. The magnitude of this response to elevated [CO_2] was nutrient dependent: that is the higher the N supply rate the bigger the relative stimulation in biomass and hence increase in NPP.

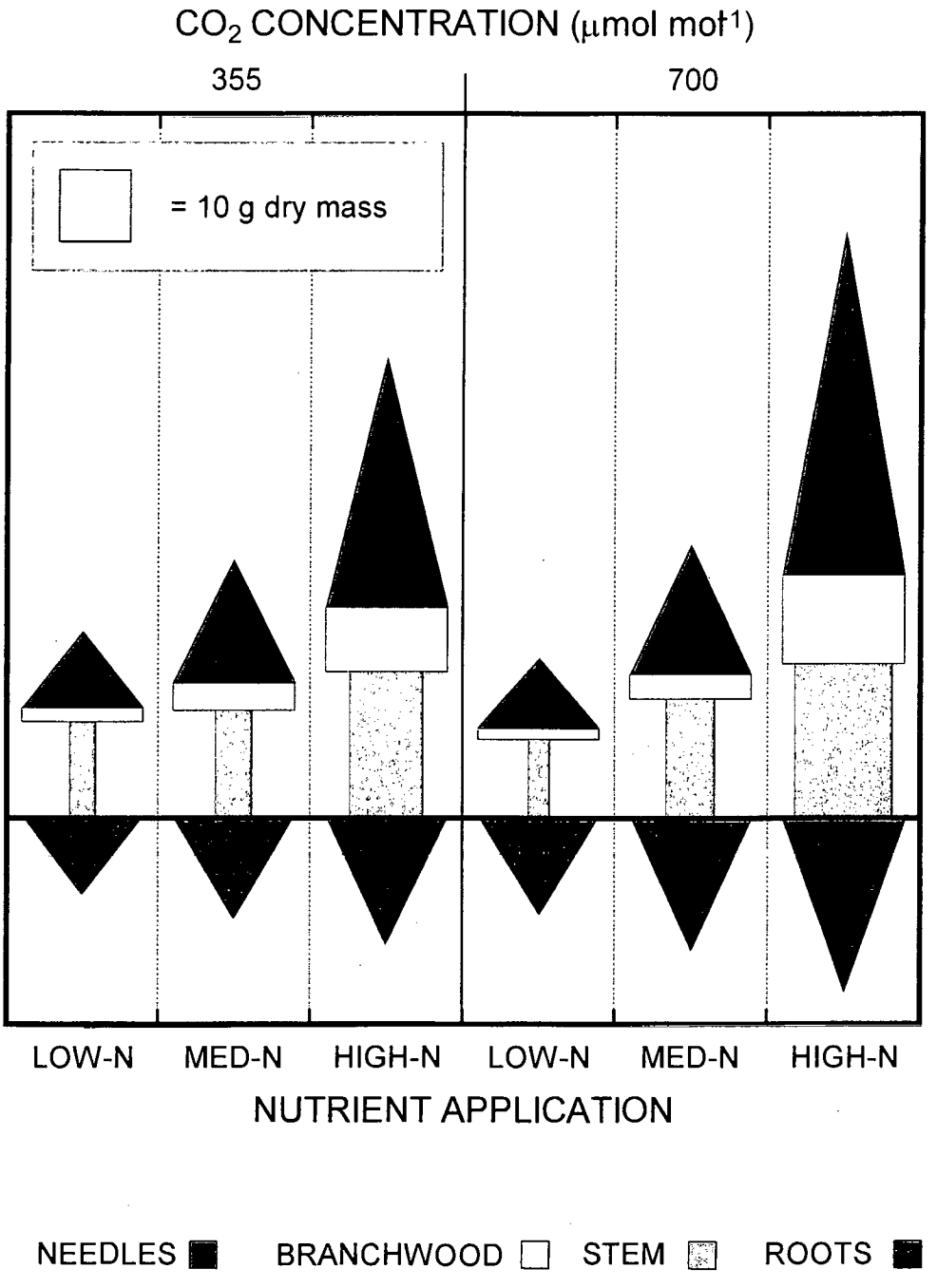

Figure 7.2: A schematic representation of the proportional allocation of biomass to needles, branchwood, stem and roots for Sitka spruce growing in ambient and elevated [CO_2] with three varying nutrient supply rates. LOW-N = 0.1 x optimum nutrition, MED-N = 0.5 x optimum nutrition, and HIGH-N = 2.0 x optimum nutrition. (area = dry mass (g), and height of stem column = seedling height (cm)).

The discrepancy between the findings presented in this study in nutrient poor conditions with those of model predictions are a result of the techniques used to predict future responses in elevated [CO_2]. The modellers attribute anomalies between their findings and those from experiments to failures on behalf of the experimenters, particularly with respect to experimental duration, and the use in the majority of cases of pot-grown seedlings (e.g. Thornley and Cannell, 1996). It is true that limitations occur when conducting [CO_2] experiments for various reasons already discussed, but it is also true that models, and their predictions, are only as good as the assumptions and mechanisms built into them. It can not be ruled out that given time and rooting volume, seedlings grown as in these experiments would increase NPP via feedbacks involving N dynamics. Potentially, the increased release of C into the soil and larger rooting volume observed in elevated [CO_2] may result in increased non-symbiotic N_2 fixation and increased N availability as a result of the greater potential to explore a large soil fraction. This hypothesis was proposed by Cannell and Thornley (1997) to explain results from their model, which show that N-poor ecosystems respond relatively more to elevated [CO_2] than N-rich ones over the long term (Cannell and Thornley, 1997). The only way to test this definitively, is to conduct really 'long-term' elevated [CO_2] experiments and make comparisons with estimated predictions of over 100 years for ecosystems equilibrated to 700 μmol mol^{-1} [CO_2], but this is impractical. Therefore, some level of balance, compromise and close collaboration between modellers and experimentalists in the design of experiments and assumptions in the models is essential. No one technique or approach will enable us to answer the question "How will our future forests respond to elevated [CO_2]?".

7.5 Synthesis of interactions between nutrient supply rates and elevated CO_2

Because phenological and physiological influences on the flow of C are strongly

correlated with plant nutrient status two different nutrient scenarios are represented by flow diagrams which summarise and explain the effect of [CO_2] under nutrient limited and non-limited conditions (Figures 7.3 and 7.4). The explanation for each of the two nutrient scenarios is based on the results obtained in this study from the nutrient experiments conducted on seedlings supplied with low-N and high-N nutrient supply rates (Chapters 4 and 5).

In essence, the magnitude of plant responses to elevated [CO_2] are dependent upon the extent to which plant C:N ratios are altered, ultimately relating to shifts in nutrient use efficiency (NUE) (Kirschbaum et al., 1994; Medlyn and Dewar, 1996).

7.5.1 Response of Sitka spruce to elevated [CO_2] when grown on nutrient-poor sites

In the low-N scenario (Figure 7.3), growth responses are a result of elevated [CO_2] leading to seedlings with larger root systems. This is a product of shifting growth patterns and a response to changing C/N ratios. As seedling C/N ratios change, knock on effects occur on both physiological and phenological processes. Lower plant [N] results in down-regulation of photosynthesis and re-partitioning of N within the photosynthetic apparatus and re-allocation of carbon to organs responsible for N acquisition. This results in a more nutrient efficient plant but with a lower ANPP, i.e. lower stem/root ratio.

However, in the longer term the larger root mass, and higher C substrate levels, may effectively withdraw more N from the soil pool and if the increase in carbon supply is also followed by a slow increase in total ecosystem N as a result of increased non-

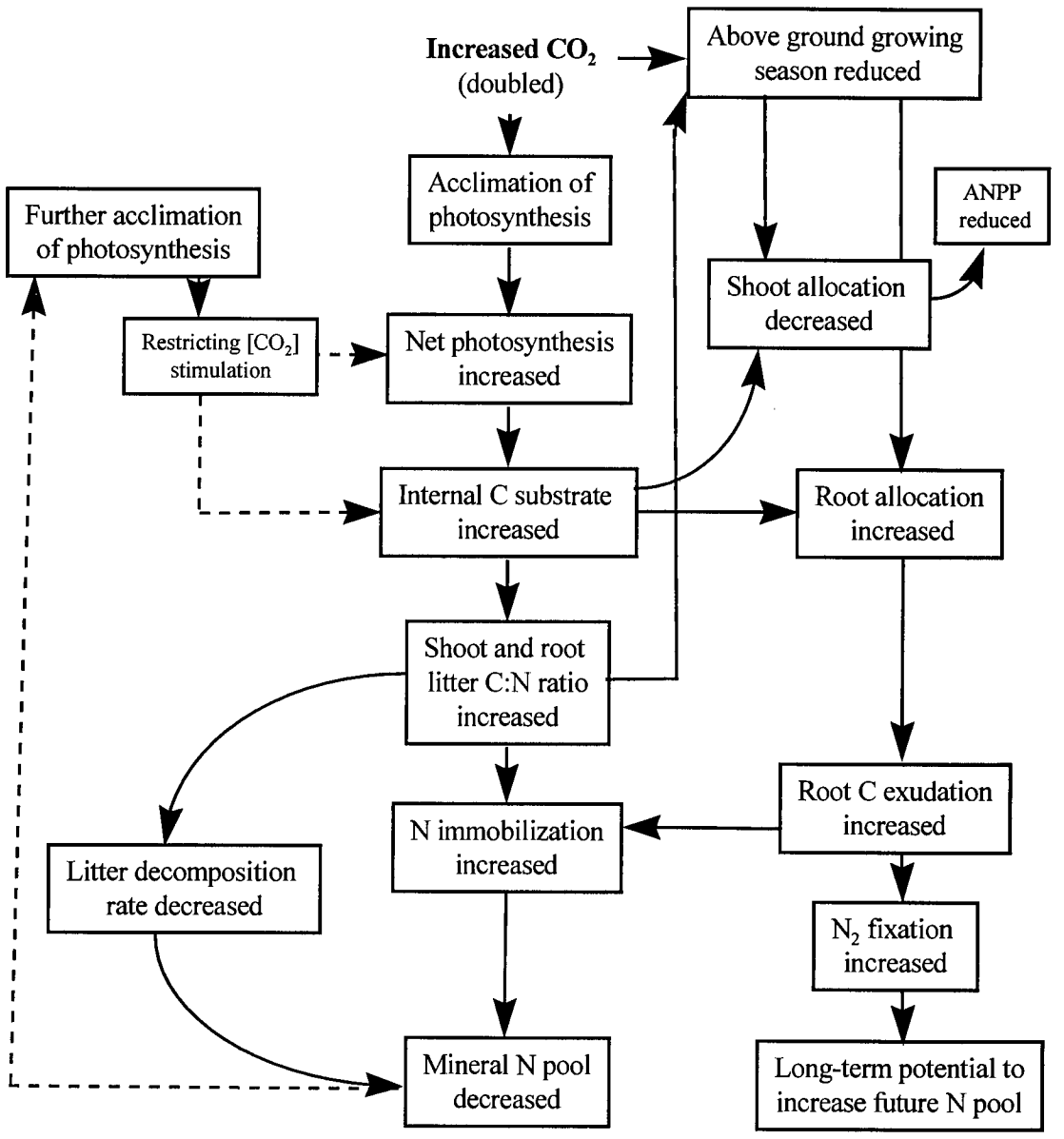

Figure 7.3: Schematic representation of the main growth and photosynthetic consequences of growing Sitka spruce with low nutrient supply rates in elevated [CO_2]. Solid lines represent main effects of growth in elevated [CO_2] and dashed lines represent possible feedback mechanisms.

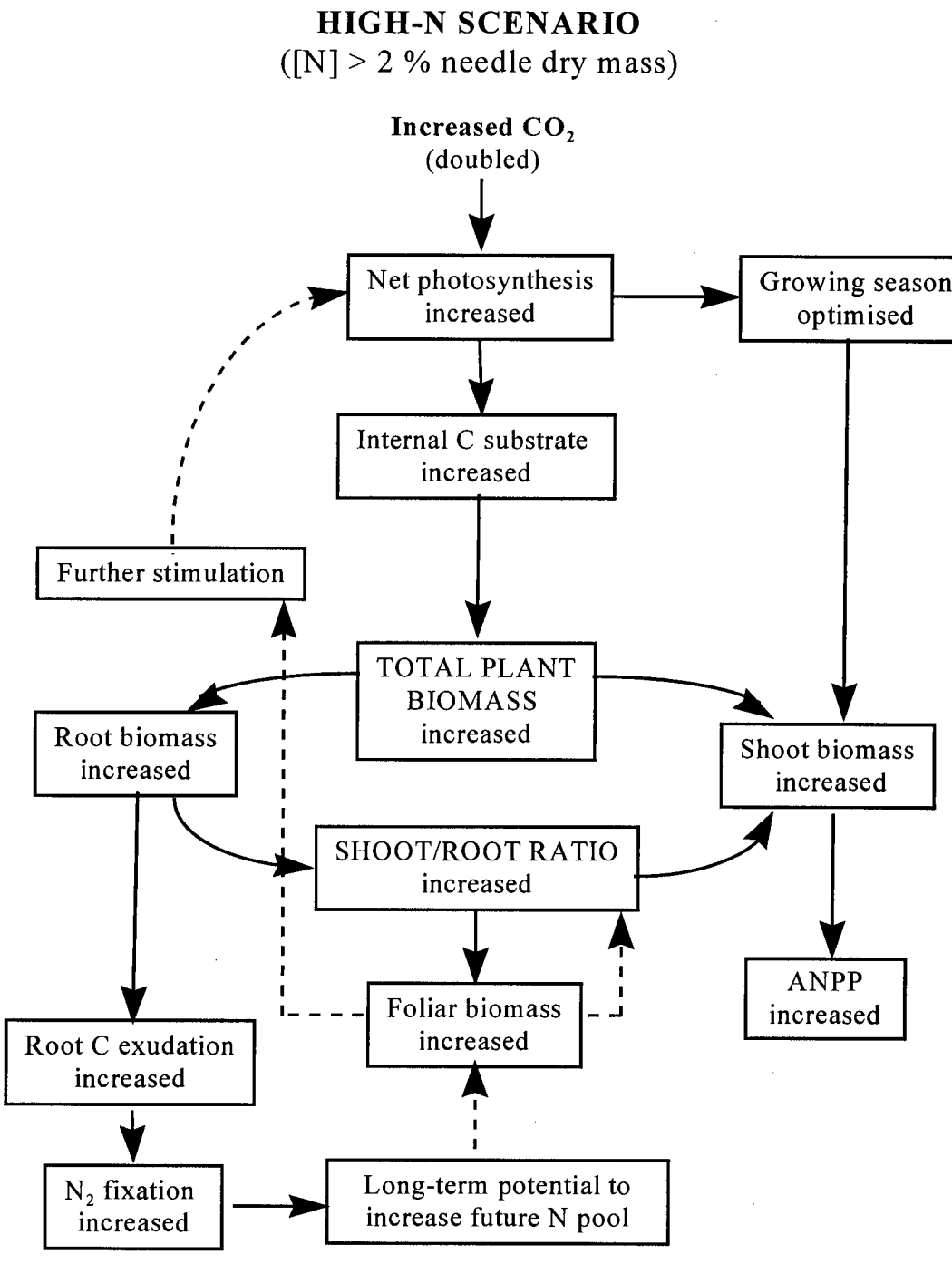

Figure 7.4: Schematic representation of the main growth and photosynthetic consequences of growing Sitka spruce with high nutrient supply rates in elevated [CO_2]. Solid lines represent main effects of growth in elevated [CO_2] and dashed lines represent possible feedback mechanisms.

symbiotic N_2 fixation then ANPP may be increased in nutrient poor conditions. However, the timescale over which such events might occur is long and the likely short-term impact of elevated [CO_2] on Sitka spruce seedlings growing on nutrient poor soils is a reduction in ANPP.

7.5.2 Response of Sitka spruce to elevated [CO_2] when grown on nutrient-rich sites.

In the high-N scenario (Figure 7.4), elevated [CO_2] increases C allocation to all plant organs. Photosynthetic rates are increased, which in turn stimulate net C accumulation. There is no effect of [CO_2] on bud phenology, and hence the above ground growing season is not reduced. Because [N] is not limiting to growth, proportionally more carbon is allocated to organs directly involved in carbon fixation, i.e. needles, and indirectly involved in their support, i.e. branches and stem. This results in a higher shoot/root ratio and hence increased ANPP. The proportion of biomass allocated to the stem in elevated [CO_2] is not as large as that in ambient [CO_2]. This is because, more carbon is invested in branch wood probably as a direct response to the increase in needle biomass. Therefore, the increase in harvestable ANPP as a result of elevated [CO_2] on seedlings growing on nutrient rich soils may not be as large as might be predicted. However, the long term effects are still uncertain, changes in branching pattern may influence the efficiency of light interception. Given that PAR is often regarded as the most limiting factor in plant photosynthesis (Sage, 1994) this might ultimately stimulate C fixation rates and hence biomass production.

7.6 Future research

As a consequence of the findings and gaps in both this study and those of the current literature, the following areas of research would greatly enhance our knowledge and

understanding of how trees will respond to increasing [CO_2] in the future.

- Despite the nearly universal stimulation in net photosynthesis as a result of elevated atmospheric [CO_2], many of the mechanisms and controlling factors behind this response remain elusive. Only by a clear understanding of such processes, will we be able to understand why the photosynthetic response between species differs, and if such differences in the ability to utilise enhanced C substrate will confer a competitive benefit to those species best able to adapt physiologically to future [CO_2]?

- Our understanding of the mechanism behind the various responses of stomatal conductance to elevated [CO_2] is poor. Why do some species reduce g_s under elevated [CO_2], while others appear to be unaffected? Is this somehow linked to the drought resistance of the species, i.e. are the stomata of species adapted to dry regions more sensitive to [CO_2] than those from wet regions?

- How will growth and bud phenology respond over the long term to elevated [CO_2]? Will the reduction in the growing season observed in this study on Sitka spruce with a low nutrient supply rate still hold true when atmospheric [CO_2] rises slowly? More phenological studies across a wide range of species are required as the response of bud phenology to elevated [CO_2] appears to be species specific (Murray and Ceulemans, 1997). Will any effect of [CO_2] on bud phenology confer an overall benefit to the plant in terms of increased frost hardiness or will its competitiveness be reduced by being unable to maximise the growing season?

- Of interest and significant importance are studies investigating the differing response to elevated [CO_2] between species with determinate and indeterminate

growth patterns. Can species with an indeterminate growth pattern respond more rapidly to increasing [CO_2]? Does the difference in species growth patterns help explain some of the discrepancies found in the current literature concerning growth and biomass allocation responses?

- Probably, the most important aspect of tree responses to elevated [CO_2] are those connected to below ground processes. Detailed studies investigating the long term effect of increased atmospheric [CO_2] on C flow to the roots and its consequence on fine root turnover, C exudation, microbial populations, soil organic matter and litter C/N ratios and hence decomposition are all of great importance.

- Results from this study have clearly demonstrated the importance of nutrients on the response of seedling Sitka spruce to elevated [CO_2]. The concentration of atmospheric CO_2 is just one of many environmental factors which control plant partitioning and productivity, for example local climate, soil and plant nutritional levels, competition and solar radiation will all directly influence carbon fixation and partitioning (Bazzaz and Miao 1993; Rogers and Runion 1994). Therefore, future [CO_2] research into more complex scenarios, which include one or more of the environmental variables outlined above is required. The possible inclusion of pollution studies, such as ozone or increased N deposition in the form of acid rain will also significantly enhance our ability to predict future responses.

- The results presented in this paper clearly demonstrate the importance of long term experimental studies, which allow plants to "acclimatise" for several growing seasons. The development of FACE and large OTC facilities across a range of environmental conditions and including the major species types is

essential. These should be developed in a world wide coherent framework with world resources being invested in long term (decades) studies which are available to and integrated within the scientific community.

- There should also be closer links between model development and experiments. Because of the timescale involved it is impossible to make failsafe predictions as to future tree responses to a doubling of present day [CO_2]. It is also true to say that models can only predict future responses based on our current knowledge of those processes most likely to be affected. Therefore, it is essential to continue funding high quality research which can feed current models and aid their development. The use of both techniques will then allow us to make predictions as accurately as possible, concerning the long term response of trees to elevated [CO_2].

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APPENDIX A

Relative importance of Liebig's Law in elevated [CO_2] studies

The law of limiting factors is an extremely important concept in terms of being able to separate growth responses fully attributable to increasing atmospheric CO_2 concentrations from other environmental or interactive growth responses. Sinclair (1992) confused this concept by interpreting 'limiting-factor' as 'nearly limiting-factor', and thus stating that several experimental results were "not consistent with the limiting-factor law". Sinclair (1992) gave examples from the current literature, where two CO_2 concentrations were applied in conjunction with a range of nutrient supply rates and where increasing the supply of either stimulated growth. It was therefore concluded that because both increasing CO_2 concentration and nutrition stimulated growth, both factors must be limiting and 'Liebig's Law' must be wrong. In fact the opposite must have been the case, in the studies reported by Sinclair (1992) neither CO_2 concentration nor nutrient availability can have been completely limiting growth. The limiting-factor concept is simple stating that if an environmental variable (in this case nutrient availability) is limiting (not just restricting) growth, then no amount of additional resources (e.g. increasing atmospheric CO_2 concentration) will stimulate carbon accumulation. In fact, in the same report the author goes on to describe exactly this phenomena, by pointing out that the biomass response of plants to "addition rates of nutrients is non-linear, with the largest increases in biomass resulting from increased nitrogen supply at the initially low supply rates of nitrogen", eventually a point is reached where some other biological or environmental variable becomes the 'limiting-factor', so no amount of additional nitrogen will stimulate growth. The pertinent question is not the questionability of 'Liebig's law' but under what range of environmental conditions will atmospheric CO_2 concentration stimulate growth, and what will be the interactive responses of plants across a range of CO_2 concentrations and environmental variables, in particular nutrient availability.

APPENDIX B

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Effects of elevated CO_2, nutrition and climatic warming on bud phenology in Sitka spruce (*Picea sitchensis*) and their impact on the risk of frost damage

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Received September 10, 1993

Summary

Effects of elevated CO_2, clone and plant nutrition on bud dormancy of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) were examined. Sitka spruce seedlings were fumigated with ambient or elevated (ambient + 350 μmol mol^{-1}) concentrations of CO_2 in open-top chambers for three growing seasons. In 1991 and 1992, elevated CO_2 delayed bud burst in the spring and advanced bud set in the autumn. The effect of the open-top chamber on the thermal requirement for bud burst was greater than the effect of elevated CO_2 (50 and 30 day degrees (D_d), respectively). In a second study, four clones of Sitka spruce taken from two provenances, at 43 and 54° N, were fumigated with ambient or elevated CO_2. There was a large natural variation in the timing of bud burst and bud set among the clones. Elevated CO_2 had no effect on bud dormancy of the Skidegate a clone, but it reduced the growing season of the North Bend b clone by 20 days. In a third study, Sitka spruce seedlings growing in ambient or elevated CO_2, were supplied with one of three nutrient regimes, low (0.1 × potential), medium (0.5 × potential) or high (2.0 × potential), using a method and solution based on the Ingestad technique. Elevated CO_2 did not affect bud dormancy in the high-nutrient treatment, but it reduced the growing season of plants in the low-nutrient treatment by 22 days. Increasing plant nutrient supply lengthened the growing season, plants flushed earlier in the spring and set bud later in the autumn.

The effects of elevated CO_2 plus a 0, 2 or 4 °C climatic warming on the timing of bud burst and the subsequent risk of frost damage were assessed using a simulation model and meteorological data from three sites, Edinburgh, Braemar and Masset. The model predicted that (i) doubling the CO_2 concentration in the absence of climatic warming, will delay the onset of bud burst at all three sites, (ii) climatic warming in ambient CO_2 will hasten bud burst and (iii) climatic warming in elevated CO_2 will hasten bud burst at Edinburgh and Braemar but to a lesser extent than climatic warming alone. At Masset, a 4 °C warming was required to advance the date of bud burst of seedlings in the elevated CO_2 treatment. At all three sites, elevated CO_2 and climatic warming increased the mean daily temperature on the date of bud burst, thus reducing the risk of subsequent frost damage.

Keywords: bud burst, bud dormancy, bud set, bud phenology model, clone, growing season length, mineral nutrition, thermal requirement.

Introduction

Synchronization of plant dormancy with annual temperature cycles is important, especially in cool temperate regions. In these regions, premature onset of vegetative growth in the spring and delayed growth cessation in the autumn will extend the duration of shoot growth, but may result in frequent frost damage. Conversely, the

delayed onset of growth in the spring and premature dormancy in the autumn will underutilize site resources, and may result in reduced competitiveness. Bud phenology, which is the study of the timing and duration of bud dormancy, especially in relation to climatic conditions, must therefore be considered when assessing the impact of climate change on plant productivity and survival.

Physiological and phenological responses of buds to environmental variables, such as air temperature and daylength, have an underlying genetic basis (Perry 1971, Dunlap et al. 1992), reflecting the adaptive significance of these characteristics (Worrall and Mergen 1967, Kramer 1992). Thus, phenological characteristics of native trees are generally well coupled with local climatic conditions, with little or no risk of frost damage. However problems may arise if predicted climatic changes occur at a rate that is faster than the adaptive ability of most tree species (Gates et al. 1992), or when exotic species are introduced to regions with less favorable climatic conditions than in their natural range. Sitka spruce (*Picea sitchensis* (Bong.) Carr.) in the U.K. is an exotic species whose natural range extends along a narrow coastal strip in northwestern North America. Reports of spring and autumn frost damage to Sitka spruce at various sites emerged soon after the species' introduction to Britain (Macdonald 1927, Day and Peace 1946, Day 1957). It became clear that there was a problem of synchronization between bud dormancy and the local climate (Cannell and Sheppard 1982, Cannell and Smith 1984, Cannell et al. 1985).

This problem may be exacerbated or ameliorated by climatic change, which may either delay or advance the timing of bud burst depending on the degree of chilling required by Sitka spruce (Cannell and Smith 1983*b*, Murray et al. 1989). Spring bud burst in woody perennials is regulated by temperature, whereas autumn bud set is controlled by both temperature and day length (Koski and Selkäinaho 1982, Cannell and Smith 1983, Koski and Sievänen 1985, Falusi and Calmassi 1990, Hänninen 1990 and Hänninen et al. 1990). Thus, any change in air temperature will affect the timing and duration of bud dormancy and consequently the plants' performance, competitiveness and survival. In addition, changes in the timing of the onset and cessation of growth could increase or decrease the probability of frost damage, depending on the degree of climatic warming and the likelihood of late spring frosts (Cannell and Smith 1983*b*, Murray et al. 1989).

Increased atmospheric CO_2 concentrations may also affect bud phenology directly through changes in biochemistry and physiology. For example, changes in starch or hormonal concentrations may alter dormancy status and growth patterns, by shifting the timing and duration of the vegetative season (Powell 1969, Waring 1969, Zimmerman et al. 1980, Lanner and Connor 1988, Cannell 1990). In addition, increased atmospheric CO_2 concentrations have been shown to change the C/N ratio within trees (Eamus and Jarvis 1989, Conroy 1992). Increasing plant nutrition generally results in an increased relative growth rate and extended growing season (Ågren 1985, Ingestad and Kahr 1985, Dewald et al. 1992).

In temperate regions, a better understanding of the relationship between bud phenology and the many biotic and abiotic factors affecting it is essential to predict the growth, competitiveness and survival of native and exotic tree species, in

response to increasing CO_2 concentrations and global warming. We evaluated the phenological responses of bud burst and bud set of Sitka spruce to a doubling of the present day atmospheric CO_2 concentration, over a range of nutrient treatments and in four clones covering a latitudinal gradient. The data were used to parameterize a bud phenology model. The model was then used to predict the timing of bud burst and the mean minimum temperature on the date of bud burst at three sites, two in Scotland and one in North America, at ambient and elevated CO_2 concentrations, with and without climatic warming.

Materials and methods

Experiments were performed in open-top chambers at the Bush Estate near Edinburgh, Scotland (55°51′ N, 198 m altitude), from June 1, 1990 to May 31, 1993. We measured (i) the timing of bud burst in the spring and bud set in the autumn over three growing seasons on Sitka spruce seedlings growing in ambient or elevated CO_2, (ii) the genetic variability in the bud phenological response to elevated CO_2 on four clones of Sitka spruce and (iii) the effect of nutrition on bud burst and bud set in elevated and ambient CO_2.

Open-top chambers

Eight octagonal open-top chambers (OTC), with a floor area of 7.0 m^2 and height of 2.3 m were used in 1990. Four of the OTCs received ambient CO_2 and four received elevated CO_2. In March 1991, the number of open-top chambers was increased to 10, giving five replicates per CO_2 treatment. The mean daily temperature was 1.4 ± 0.98 °C higher inside the OTC than outside (Figure 1). For a fuller description of chamber properties see Fowler et al. (1989).

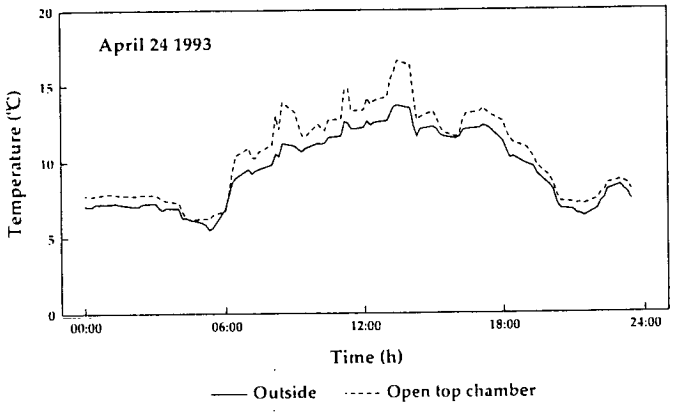

Figure 1. The diurnal temperature fluctuations inside and outside the open top chambers (OTC), on a typical day during spring bud burst.

CO_2 Fumigation facility

Before injection into all chambers, ambient air was passed through a series of activated charcoal filters to remove ozone, sulfur dioxide and nitrogen dioxide. The ambient CO_2 chambers then received this air directly through a polyethylene manifold 1.5 m above ground level. The CO_2 concentration in these chambers fluctuated diurnally around a mean daily value of 350 μmol mol^{-1}. The elevated CO_2 chambers received air supplemented with pure CO_2 (Distillers MG, UK) to raise the ambient concentration by 350 μmol mol^{-1}, i.e., to double present day ambient concentrations. Pure CO_2 was injected directly into the ambient air stream in the chamber fan units at preset flow rates, where it was mixed thoroughly before being released into the chambers. The CO_2 concentration in the elevated chambers varied around 700 ± 80 μmol mol^{-1}, depending on the ambient concentration and external wind speeds which affected ambient air incursion through the open top.

Potted seedlings

In June 1990, 2000 unflushed two-year-old (1+1) bare-rooted Sitka spruce (*Picea sitchensis* (Bong.) Carr.) seedlings, Forestry Commission identity number 83(2015)S LOT2, provenance 20, origin Queen Charlotte Islands, were taken from cold storage and potted in 2-dm^3 pots containing a composite soil consisting of sphagnum peat, 5 mm quartz and sterilized loam in the ratio 13/4/3 (v/v). Vitax Q4 fertilizer (N,P,K; 5.3/7.5/10) was added at 4 g per dm^3 to the soil. The plants were then randomized and 250 were selected and evenly distributed among 10 randomized blocks in each of the eight open-top chambers. In March 1991, 30 plants from each chamber were repotted in 4.5-dm^3 pots. An additional 30 plants were randomly selected from each of the CO_2 treatments, repotted and placed in two additional chambers, increasing the replicate number of chambers per treatment to five. The remaining plants were discarded because of insufficient space in the OTCs. In March 1992, 15 plants from each of the 10 chambers were repotted in 18-dm^3 pots.

Clonal plants

Cuttings were taken from physiologically mature Sitka spruce trees growing in a clonal provenance trial near Edinburgh, Scotland, in March 1990. Cuttings from two clones were selected from each of the North Bend (41.3° N) and Skidegate (53.2° N) provenances, and immediately transferred to a mist propagation bench in a greenhouse. In July 1990, when the cuttings had rooted, they were potted in 1-dm^3 pots containing composite soil. They remained in the greenhouse until March 1991, when 60 plants from each of the four clones were repotted in 4.5-dm^3 pots and randomly placed in each of six open-top chambers, 10 plants per clone per chamber, three chambers per CO_2 treatment.

Nutrient-treated seedlings

In March 1992, 330 one-year-old Sitka spruce seedlings, which had been raised in ambient (350 μmol mol^{-1}) or elevated (700 μmol mol^{-1}) CO_2 conditions, were

potted in 2-dm^3 pots containing composite soil and randomly placed in each of 10 open-top chambers and an outside control plot, giving 30 plants per chamber and 30 plants outside. Balanced nutrient solutions were applied on a weekly basis to each of the trees based on the Ingestad technique (Ingestad and Lund 1986), which matches the addition rate of nutrients to plant growth rates. The nutrient addition rate was calculated based on previous growth measurements of Sitka spruce seedlings. A potential nitrogen concentration of 2% in current-year foliage was assumed and three rates of nutrient supply were selected to give 2 × potential (High), 0.5 × potential (Medium) and 0.1 × potential (Low) foliage nitrogen content. Each of the nutrient treatments were applied to 10 of the 30 plants in the chambers and outside. The three nutrient treatments significantly affected plant growth rates and biomass allocation.

Spring bud phenology

Spring bud burst was measured on the potted seedlings in 1991 and 1992, on the clonal plants in 1992, and on the nutrient-treated seedlings in 1993. The leader buds were scored every second day during the spring flushing period. The buds were scored on a scale of 1 to 4, where 1 = slight swelling, 2 = swollen bud, 3 = green needle clearly showing through the bud scales, and 4 = needle elongation. For each plant, the date of bud burst was taken to be the date on which the leader bud reached Stage 3.

Autumn bud phenology

Bud set in the autumn was measured in 1991 on the potted seedlings, and in 1992 on the potted seedlings, clonal plants and nutrient-treated seedlings. Leader buds were scored several times weekly from August to October, as either growing or dormant. The date of bud set of each plant was taken to be the date on which the bud became dormant, i.e., dark in color and firm to touch.

Statistical analysis

Data for the numbers of plants that had achieved either bud burst or bud set by each recording date were analyzed by a modification of the cumulative distribution analysis formally presented by Hunter et al. (1984) and developed by Brain and Butler (1988). Analysis of the data was difficult because there was serial correlation between values at successive recording times and the counts were not normally distributed. However, the underlying variable (the time to bud burst or bud set) was analyzed by fitting its cumulative distribution function to the empirical cumulative distribution derived from the observed data. A maximum likelihood analysis was performed with the Genstat 5 software program.

The time to bud burst was normally distributed with no transformation of the time axis. If the time to bud burst is t, the cumulative distribution function (F) is

$$F(t) = N(z)$$

$$z = b(t - m), \tag{1}$$

where m is the mean time to bud burst, b is the inverse of the standard deviation of time to bud burst and N is the cumulative normal distribution function with zero mean and unit variance.

The data on bud set were more difficult to model. It appeared that the distribution fitted should have been

$$F(t) = N(z_l)$$

$$z_l = b(\log(t - l) - m), \tag{2}$$

where l was the lag period before initiation of bud set, m was the mean adjusted time to bud set ($\log(t - l)$) and b the inverse standard deviation of the adjusted time to bud set. However there were computational problems in fitting this model, because every treatment had a different lag period. A modification of the fitting procedure used for the bud burst model gave reasonable results for bud set except for the plants in the outside treatments. Therefore, the first approach was used on this occasion and the outside treatments were only included when they fitted within this model. The statistical difference between treatments was determined by testing the goodness of fit of individual treatment parameters with the goodness of fit of parameters obtained from the combined data (Ross 1981).

Spring bud phenology model simulations

The Cannell and Smith (1983) bud burst model was chosen to predict the likely consequences of increased global warming on the timing of bud burst in the spring, and the minimum temperature on that date. The assumption of this model is that the timing of bud burst is a function of the non-linear relationship between the number of chill days (C_d) and the thermal requirement (D_d) to bud burst,

$$D_d = a + b \exp(rC_d), \tag{3}$$

where D_d is the thermal requirement to bud burst, C_d is the number of chill days and a, b and exp$r$ are parameters with values of −56, 602 and 0.991, respectively (Murray et al. 1989). The number of chill days to bud burst was taken as the number of days since November 1 when the mean air temperature was ≤5 °C. The thermal time required to the date of bud burst was taken as the accumulated day degrees (°C) above the mean daily base temperature of 5 °C from January 1. The numbers of chill days and day degrees received to the date of bud burst were calculated for the potted seedlings in 1991, 1992 and the nutrient-treated seedlings in 1993. Plants in the intermediate nutrient treatment were selected, because they most closely matched the nutritional status of the 1991 and 1992 seedling plants. The temperature records used to calculate D_d and C_d were obtained from screened sensors placed inside and outside the OTC, with readings recorded every 15 minutes and stored on a data logger (21x, Campbell Scientific Ltd., Leicestershire, England). These data and model parameters previously obtained for Sitka spruce by Murray et al. (1989), were used to parame-

terize the model for Sitka spruce growing in both ambient and elevated CO_2.

The model, using both sets of parameters, was then used to simulate the effects of climatic warming and elevated CO_2 on the timing of spring bud burst at three meteorological stations. Two sites in Scotland were chosen, a central lowland site at Edinburgh (55°48′ N, 26 m) and an upland site at Braemar (57°00′ N, 339 m) and one native coastal Sitka spruce site, in northwest North America, at Masset, Queen Charlotte Islands (54°02′ N, 3 m). A simulation was run at each of the above sites for the years 1897–1978, at ambient and elevated CO_2 concentrations, and for 0, 2 and 4 °C uniform warming, using daily maximum and minimum temperatures recorded in Stevenson screens.

Results

Bud phenology of potted seedlings

Bud phenology of plants in the elevated CO_2 and outdoor treatments was significantly different from that of plants in the ambient CO_2 treatment in the spring and autumn of both years (Figure 2, spring, 1991 $P < 0.001$, 1992 $P < 0.01$ and autumn, 1991 and 1992 $P < 0.001$). In the spring of both 1991 and 1992, seedlings subjected to elevated CO_2 had a significantly higher thermal requirement to the date on which 50% of the plants had burst bud than seedlings in the ambient CO_2 treatment (Table 1). This resulted in the elevated-CO_2-treated plants flushing seven and four days later than the ambient-CO_2-treated plants, in 1991 and 1992, respectively

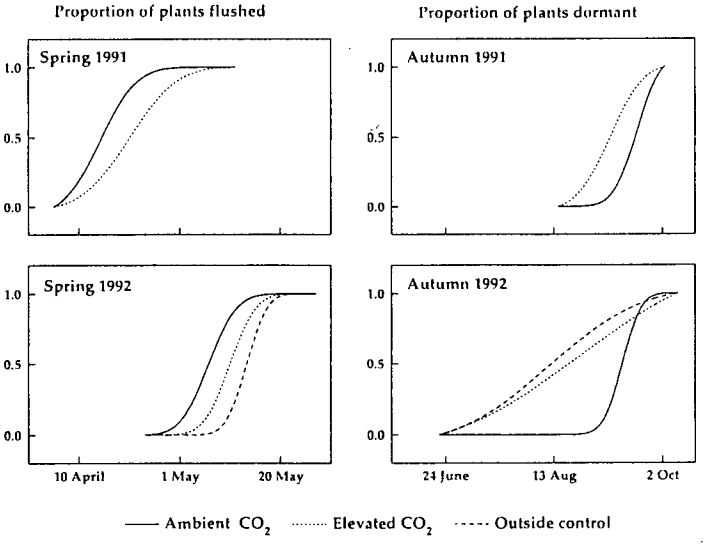

Figure 2. The fitted cumulative distribution function of bud burst and bud set, for the Sitka spruce potted seedlings in 1991 and 1992. Ambient = plants raised inside OTC, receiving ambient CO_2 (350 µmol mol^{-1}), Elevated = plants raised inside OTC, receiving elevated CO_2 (700 µmol mol^{-1}) and Outside = plants raised outside, receiving ambient CO_2 (350 µmol mol^{-1}).

Table 1. The dates of 50% bud burst in the spring, for plants growing inside OTC's receiving 350 μmol mol^{-1} CO_2, (Ambient CO_2), or 700 μmol mol^{-1} CO_2, (Elevated CO_2) and outside receiving 350 μmol mol^{-1} CO_2, (Outside), in three experiments.

Treatment	CO_2 and chamber treatment		
	Ambient CO_2	Elevated CO_2	Outside
Potted seedlings			
1991	April 24a[1]	May 1b	–
1992	May 4a	May 8b	May 11c
Clonal plants			
Skidegate a (54° N)	April 26a	April 29a	–
Skidegate b (54° N)	April 21a	April 25b	–
North Bend a (43° N)	April 29a	May 4b	–
North Bend b (43° N)	May 7a	May 10b	–
Nutrient-treated seedlings			
High	April 27a	April 28a	May 4b
Medium	April 25a	April 30b	May 2b
Low	April 29a	May 4b	May 12c

[1] Dates followed by the same letter within each row indicate that the fitted cumulative distribution functions were not significantly different (P = 0.05; using Chi-squared tests on the differences in deviance).

(Table 1). Plants growing outside the OTC in ambient CO_2 flushed seven days later than plants growing inside the OTC in ambient CO_2. In autumn, bud set was also affected by elevated CO_2 and chamber (Table 2). In 1992, plants receiving elevated CO_2 inside the OTC set bud 22 days earlier than those receiving ambient CO_2 inside the OTC, and 16 days later than those growing outside. The effect of elevated CO_2 on bud dormancy was to reduce the growing season on average by 24 days. In the autumn, there was no significant difference between bud dormancy of seedlings grown outside in ambient CO_2 and seedlings grown in the OTC in elevated CO_2.

Bud phenology of clonal plants

The timing of bud burst and bud set (Figure 3) was highly dependent on clone, with plants taken from the southerly provenance flushing and setting bud later than those from the northerly provenance. There was a significant effect of elevated CO_2 on the timing of bud burst and bud set for three of the four clones (Tables 1 and 2). The elevated CO_2 treatment did not significantly affect bud phenology in the Skidegate a clone, although bud burst was delayed and bud set was advanced by three and four days, respectively. In general, there was a larger difference in the timing of both bud burst and bud set between clones than between treatments.

Bud phenology of mineral-nutrient-treated seedlings

Plants growing in the OTC in elevated CO_2 and receiving low (10% of the potential rate) or medium (50% of the potential rate) nutrient supply rates, had significantly different dates of bud burst and bud set (Low = $P < 0.001$, Medium = $P < 0.01$) to

Table 2. The dates of 50% bud set in the autumn, for plants growing inside OTC's receiving 350 μmol mol^{-1} CO_2, (Ambient CO_2), or 700 μmol mol^{-1} CO_2, (Elevated CO_2) or outside receiving 350 μmol mol^{-1} CO_2, (Outside), in 3 experiments.

Treatment	CO_2 and chamber treatment		
	Ambient CO_2	Elevated CO_2	Outside
Potted seedlings			
1991	Sept 20a[1]	Sept 5b	–
1992	Sept 13a	Aug 22b	Aug 6b
Clonal plants			
Skidegate a (54° N)	Sept 11a	Sept 7a	–
Skidegate b (54° N)	Sept 19a	Sept 15b	–
North Bend a (43° N)	Sept 24a	Sept 15 b	–
North Bend b (43° N)	Oct 15a	Sept 28b	–
Nutrient-treated seedlings			
High	Oct 1a	Sept 29a	Sept 25a
Medium	Sept 27a	Sept 16b	Oct 2b
Low	Sept 18a	Sept 1b	July 22[2]

[1] Dates followed by the same letter within each row indicate that the fitted cumulative distribution functions were not significantly different (P = 0.05; using Chi-squared tests on the differences in deviance).

[2] The cumulative frequency function could not be fitted, the date of bud set was calculated from the raw data.

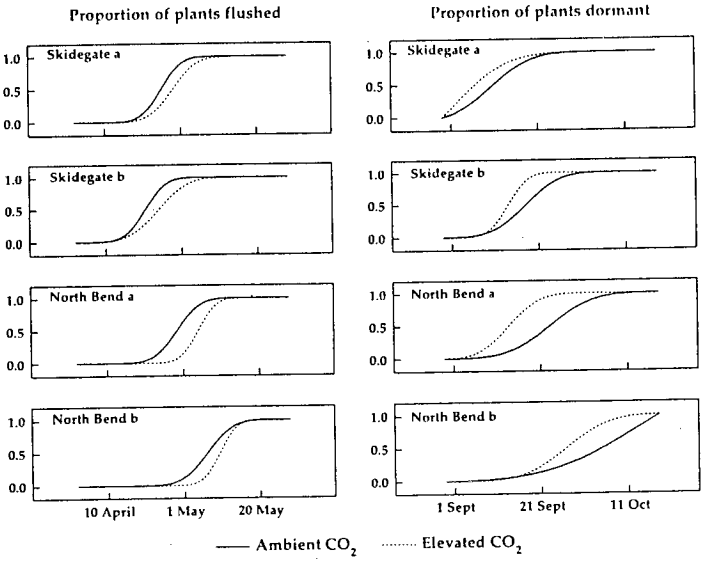

Figure 3. The fitted cumulative distribution function of bud burst and bud set for the Sitka spruce clonal plants, from North Bend (41.3° N) and Skidegate (53.2° N) provenances.

plants growing in the OTC in ambient CO_2 (Figure 4). The elevated CO_2 treatment delayed bud burst and advanced bud set. The effect of CO_2 on bud dormancy was ameliorated by high rates of nutrient supply: there was no significant difference in the timing of either bud burst or but set in plants receiving the high nutrient supply rate (200% of the potential rate). Plants receiving the low nutrient supply rate showed the biggest dormancy response to both elevated CO_2 and OTC. The elevated CO_2 treatment delayed bud burst by five days in the low and medium nutrient regimes and one day in the high nutrient regime (Table 1). Bud set was advanced in the elevated CO_2 treatment by 17, 11 and 2 days at low, medium, and high nutrient supply rates, respectively (Table 2). The effect of increasing the nutrient application rate from Low to High was to advance the timing of bud burst and delay bud set, resulting in an increased growing season of 15 days in ambient CO_2, 34 days in elevated CO_2 and 65 days outside.

Simulation model

A non-linear regression was produced using Equation 3 and values of $a = -56$, $b = 602$ and $\exp r = 0.991$ (Murray et al. 1989) for Sitka spruce seedlings growing outside in ambient CO_2 (Figure 5). The accumulated D_d required to bud burst for the plants growing in elevated CO_2 inside the OTC and in ambient CO_2 both inside and outside the OTC are shown in Figure 5. The number of chill days (C_d) received by the outside control plants, before bud burst in 1991, 1992 and 1993, was 87, 96 and 96, respectively. The parameterized model accurately predicted the thermal require-

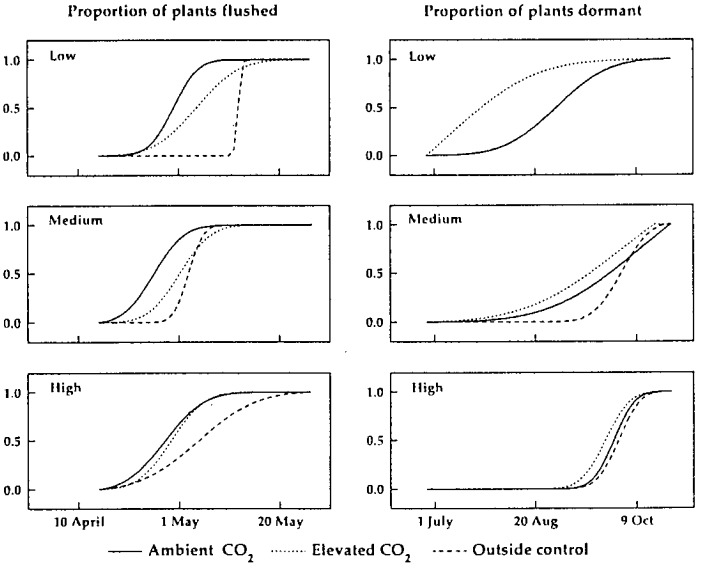

Figure 4. The fitted cumulative distribution function of bud burst and bud set for the Sitka spruce nutrient-treated seedlings. The optimum nutrient application rate was that required to sustain a 2% nitrogen concentration in the current-year foliage. The treatment application rates were, Low = 10%, Medium = 50%, and High = 200% of the optimum rate.

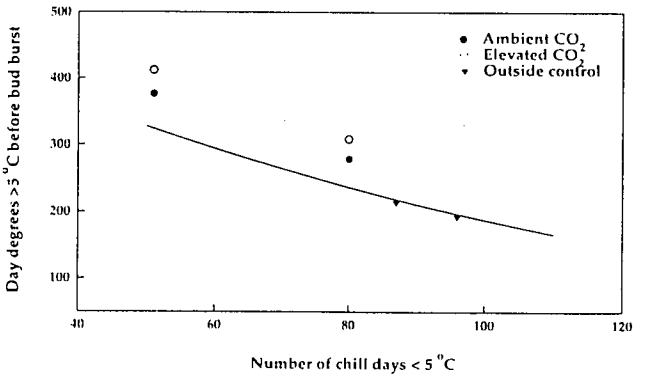

Figure 5. The relationship between day degrees to bud burst and number of chill days, given by the equation: $D_d = a + b \exp(rC_d)$, where D_d is the thermal time to bud burst, C_d is number of chill days and a, b and r are parameters obtained for Sitka spruce in ambient CO_2, (Murray et al. 1989). The symbols indicate where each of the treatments lie in relation to this line.

ments (D_d) to bud burst for the ambient CO_2 outside-grown control plants, given 87 and 96 chill days. As a result of the chamber warming effect, plants grown in the OTCs received fewer chill days before bud burst than the outside control plants (51 and 80 days), (Figure 1). The predicted values of D_d for both the ambient- and elevated-CO_2-treated plants growing in OTCs were lower than the observed values (Figure 5). The thermal requirement to bud burst was 50 and 80 day degrees higher than the predicted values at both levels of chilling, for the chamber-grown ambient- and elevated- CO_2-treated plants, respectively. Thus, the separate effects of chamber and elevated CO_2 on thermal time to bud burst were assumed to be a uniform increase of 50 D_d and 30 D_d across all chilling levels.

To simulate the effect of elevated CO_2 on spring bud phenology, the model parameter a estimated by Murray et al. (1989) was adjusted to account for the uniform increase in thermal requirement (30 D_d) of Sitka spruce growing in elevated CO_2. A model simulation was then run using both values of a (−56 and −26 for ambient and elevated CO_2 treatments, respectively) and the temperature records for Edinburgh, Braemar and Masset. Predictions of the date of bud burst and the minimum temperature on that date for 0, 2 and 4 °C uniform warming, were obtained for each site (Figure 6). The effect of elevated CO_2 alone (0 °C) was to delay bud burst at all three sites and to increase the minimum temperature on the date of bud burst at the cooler Braemar and Masset sites. The effect of climatic warming alone (ambient CO_2) was to advance the date of bud burst and to increase the temperature on that date at all three sites. The combined effect of elevated CO_2 and a 2 °C uniform warming was to bring forward the date of bud burst by 2 days at Edinburgh and 1 day at Braemar and delay flushing by 2 days at Masset; the mean minimum temperature on the date of bud burst was increased at all three sites. Elevated CO_2 and a 4 °C warming advanced bud burst by 14 days at Edinburgh, 10 days at Braemar and 12 days at Masset. The increase in the minimum temperature on each of those days was

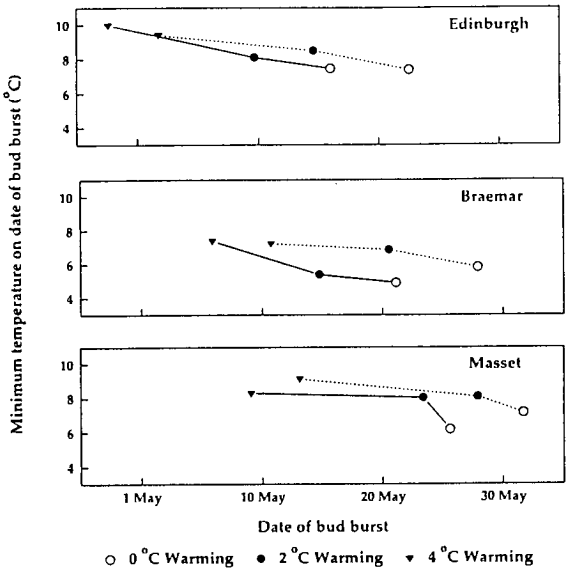

Figure 6. The predicted mean dates of bud burst, and the mean minimum daily temperature on that date, for Edinburgh, Braemar and Masset, for 0, 2 and 4 °C uniform warming, with (- - - -) and without (——) elevated CO_2.

1.6, 2.3 and 3.2 °C, respectively.

Discussion

We observed a pronounced effect of elevated CO_2 on bud dormancy. Sitka spruce grown in elevated CO_2 had a growing season that was, on average, 24 days shorter than that of Sitka spruce grown in ambient CO_2. This was a result of plants grown in elevated CO_2 flushing later in the spring and setting bud earlier in the autumn than plants grown in ambient CO_2.

Our results support the theory that a genetic factor is involved in the regulation of bud dormancy (Worrall and Mergen 1967, Perry 1971). The timing of bud burst and bud set was strongly influenced by provenance and clone, with the two more southerly clones from the North Bend provenance having longer growing seasons than either of the Skidegate clones. Maximization of the length of the growing season is expected to be more beneficial at more southerly locations, where there is a reduced risk of late spring and early autumn frosts, which would result in damage of non-dormant frost-sensitive tissue. This study also showed that there was the potential to select clones that would not be adversely affected by increases in atmospheric CO_2. Although only four clones were studied, the Skidegate a clone showed no significant effect of elevated CO_2 on bud dormancy, whereas the elevated CO_2 treatment significantly reduced the growing season of the other three clones.

There was a major interaction between CO_2 concentration and nutrient supply rate on the timing and duration of bud dormancy. Bud dormancy of plants receiving the

high nutrient supply rate was unaffected by CO_2 treatment, whereas plants receiving the low nutrient treatment had a significantly shorter growing season in elevated CO_2 (Tables 1 and 2). Therefore, Sitka spruce growing in enhanced atmospheric CO_2 concentrations (700 μmol mol^{-1}) may produce a larger phenological response to increasing nutrient supply rates, than Sitka spruce presently growing in ambient CO_2 concentrations. Silvola and Ahlholm (1993), who studied the effects of CO_2 concentration and nutrient status on *Salix phylicifolia* found that the length of the growing period varied by as much as 30% depending on the CO_2/nutrient ratio. In accordance with our findings, they found that CO_2 concentration and nutrient regime also affected bud dormancy. Therefore it is likely that increased atmospheric CO_2 concentrations in the future, will have a bigger effect on the timing of bud burst and bud set of Sitka spruce growing on nutrient-poor sites than on fertile sites. Sitka spruce on nutrient-poor sites will experience shorter growing seasons which may decrease its annual primary productivity, unless elevated CO_2 causes a comparative increase in relative growth rate. However, if trees growing on nutrient-poor sites are currently subject to frost damage, delayed bud burst and advanced bud set will decrease the risk of late spring and early autumn frosts. Elevated CO_2 would therefore have a net benefit on productivity. Increasing the nutrient supply rate lengthened the growing season, suggesting that the effect of elevated CO_2 on Sitka spruce bud dormancy at nutrient-poor sites would be reduced by the addition of fertilizer. Increasing the nutrient supply rate also ameliorates other plant responses to elevated CO_2, including reducing the effect of CO_2 on root–shoot partitioning (Eamus and Jarvis, 1989).

The large effect of the chamber on plant phenology was probably due to the increased temperatures within the chambers (Figure 1). Plants growing outside, at lower temperatures, had a significantly shorter growing season compared with chamber-grown plants. Figure 5 highlights a discrepancy between estimating thermal time to bud burst inside and outside OTCs. In this study, the thermal requirement to bud burst was calculated from daily mean air temperatures. The mean wind speed inside the OTC was 3 m s^{-1}, whereas it was 1 m s^{-1} outside in the control, resulting in a difference in the boundary layer resistance between the two environments (Monteith 1981). Thus as a result of heat convection, the difference between plant temperatures inside and outside the chambers will have been smaller than the difference between air temperatures. This could account for the apparent increase in thermal requirement (50 D_d) of the plants growing inside the OTCs. The chamber effect (50 D_d) on the thermal requirement to bud burst was larger than the CO_2 effect (30 D_d), in this study. Therefore, it is important to quantify the chamber effect when attempting to extrapolate results obtained from experiments in OTCs to the field.

Many factors control bud phenology and will have a major impact in determining the future survival and competitiveness of temperate tree species. To date, model simulations used to predict the effect of climatic warming on temperate tree species have not taken into account the direct impact of CO_2 on bud dormancy (Murray et al. 1989, Hänninen et al. 1990, Hänninen 1991), even though atmospheric CO_2 concentrations are rising at an increasing rate. We evaluated the direct impact of elevated CO_2 on bud dormancy and then simulated a 0, 2 and 4 °C uniform warming

using the model of Cannell and Smith (1983) parameterized for ambient and elevated CO_2. This bud burst model is one of a range that exist for woody perennials (Sarvas 1972, Sarvas 1974, Fuchigami et al. 1982, Cannell and Smith 1983*b*, Hänninen 1990, Thornley and Johnson 1990). Hunter and Lechowicz (1992) included this model in an evaluation of simulation models designed to predict the timing of spring bud burst in temperate trees. They concluded that the Cannell and Smith model was one of the models best suited to predict the date of bud burst in temperate trees. In addition, it has been used successfully to predict spring bud burst in a range of woody perennials, including Sitka spruce growing in Scotland (Murray et al. 1989). Our simulation results show that plants subjected to elevated CO_2 without any climatic warming will flush later in the spring at higher temperatures than at present (Figure 5). The extent of the effect depends on local site conditions, such as climate and soil nutrient status. Climatic warming alone, will advance the date of bud burst and increase the temperature on that date. However, when CO_2 and temperature change simultaneously the effect of elevated CO_2 on the timing of bud burst is reduced, or even reversed, depending on the local site climate. At Edinburgh, the mildest site in this study, the date of bud burst was predicted to advance, with temperatures warmer than at present; this would be beneficial to Sitka spruce, in terms of primary productivity and plant competitiveness. In contrast, at Masset, even with a 2 °C warming, spring bud burst was delayed by elevated CO_2.

Cannell and Smith (1983*b*) demonstrated that the likelihood of damaging spring frosts occurring around the time of bud burst is inversely proportional to the mean minimum temperature on the date of bud burst. Therefore, the warmer the temperature on the date of bud burst the lower the subsequent risk of frost damage. In each of the above cases the mean minimum temperature on the date of bud burst was predicted to increase, therefore the incidence of spring frost damage under elevated CO_2 and climatic warming will decrease at all three sites.

Previous model simulations, which have not accounted for the direct effect of elevated CO_2 on bud phenology, will overestimate the advance in the timing of bud burst, and underestimate the temperature on that date. Hänninen (1991) predicted that bud burst of trees growing in central Finland would occur in midwinter and that the trees would be subsequently exposed to temperatures between −27 and −10 °C. By including the ameliorating effect of elevated CO_2, these model predictions may have appeared less devastating. Our results demonstrate the importance of including the effect of elevated CO_2 when predicting phenological responses to climatic warming.

In conclusion, increasing atmospheric CO_2 concentrations in conjunction with climatic warming is likely to improve the survival of Sitka spruce in Britain, by reducing the risk of spring and autumn frost damage and lengthening the potential growing season.

Acknowledgments

This work was supported with funding from the CEC within the EPOC-CT90-0013 program and the UK NERC.

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APPENDIX C

Trees (1996) 10: 393–402

ORIGINAL ARTICLE

M.B. Murray · I.D. Leith · P.G. Jarvis

The effect of long term CO_2 enrichment on the growth, biomass partitioning and mineral nutrition of Sitka spruce (*Picea sitchensis* (Bong.) Carr.)

Received: 3 March 1995 / Accepted: 30 November 1995

Abstract Sitka spruce [*Picea sitchensis* (Bong.) Carr.] seedlings were grown for 3 years in an outside control plot or in ambient (~355 μmol mol^{-1}) or elevated (ambient + 350 μmol mol^{-1}) atmospheric CO_2 environments, within open top chambers (OTCs) at the Institute of Terrestrial Ecology, Edinburgh. Sequential harvests were carried out at the end of each growing season and throughout the 1991 growing season, five in all. Plants grown in elevated CO_2 had, (i) 35 and 10% larger root/shoot ratios at the end of the first and third season, respectively, (ii) significantly higher summer leader extension relative growth rates, which declined more rapidly in early autumn than ambient grown plants, (iii) after three growing seasons a significantly increased mean annual relative growth rate, (iv) consistently lower foliar nutrient concentrations, and (v) after two growing seasons smaller total projected needle areas. Plants grown inside OTCs were taller, heavier and had a smaller root/shoot ratio than those grown outside the chambers. There was no effect of CO_2 concentration on Sitka spruce leaf characteristics, although leaf area ratio, specific leaf area and leaf weight ratio all fell throughout the course of the 3 year experiment.

Key words Elevated CO_2 · Sitka spruce · Growth · Allocation · Nutrients

Introduction

Carbon dioxide is the most abundant greenhouse gas currently being released into the atmosphere through anthropogenic processes. It is widely accepted that the atmospheric level of CO_2 has risen from a pre-industrial value around 280 μmol mol^{-1} in 1900, to a present day level of around 355 μmol mol^{-1} and is increasing at a rate of about 1.6 μmol mol^{-1} per annum (Keeling 1993). Combustion of fossil fuels and the destruction of major terrestrial carbon pools, such as tropical rain forests, have been the major cause of this dramatic increase (Holdgate 1993). Such activities are unlikely to cease in the foreseeable future and indeed, are more likely to continue at an increasing rate well into the next century, despite recent attempts to introduce policies directed at reducing both CO_2 emissions and the destruction of tropical rain forests. The consequence of this is an estimated increase in the global-mean atmospheric CO_2 concentration of roughly 150 μmol mol^{-1} by the end of the twenty-first century (Gates et al. 1992). Although discrepancies exist between estimates of the rates at which CO_2 is predicted to increase, there is little doubt that overall global-mean atmospheric CO_2 concentrations will increase dramatically throughout the next century and beyond.

Because of direct effects of atmospheric CO_2 on plant photosynthesis and stomatal conductance, any rise in the atmospheric CO_2 concentration will directly affect the rate at which organic matter and plant nutrients are assimilated and internally cycled. Indirect effects of increased CO_2 concentrations will also affect plant competitiveness and survival, through altered photosynthate concentration, composition and translocation, growth rate, assimilate partitioning, growth form, reproduction, plant water status and plant tolerance to gaseous atmospheric pollutants (Acock and Allen 1985). This is especially true for plants with the C_3 photosynthetic pathway; a recent review by Poorter (1993) found that for C_3 species, plant growth was increased by 41%, compared to 22% for C_4 and 15% for CAM. In addition C_3 species constitute 95% of the earth's plant species, and will therefore be an important component in the earth's carbon cycle (Idso and Idso 1994).

The majority of published studies assessing the impact of rising CO_2 concentrations have been carried out on cereals, annual crop species or in the case of woody

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perennial species, over one or less than one growing season (that is on non-acclimated plants). Acclimation is especially important for a coniferous species such as Sitka spruce [*Picea sitchensis* (Bong.) Carr.], because its needle primordia are initiated the previous growing season. In the present study Sitka spruce seedlings were exposed to elevated CO_2 for 3 years. Luxmoore et al. (1993), published a detailed evaluation of experimental results of elevated CO_2 effects on forest tree species.

Tree crop species are an important group of C_3 plants to study, in terms of their economic value and the significant role they play in the global carbon balance (Ceulemans and Mousseau 1994). Another important aspect of trees is their longevity, this inevitably increases their susceptibility to changing CO_2 concentrations. In addition, trees will only experience a few breeding cycles within the time scale predicted for such change. This will reduce their ability to adapt genetically to the rapidly changing CO_2 environment.

Sitka spruce is the most widely planted timber producing species on upland sites in the UK, yielding on average approximately 15 m^3 ha^{-1} $year^{-1}$. Despite this, Sitka spruce has received little attention within the CO_2 scientific community. Consequently, the effect of future CO_2 concentrations on Sitka spruce is unknown.

Currently Sitka spruce tree breeders, selecting for wood yield, pick trees with a rapidly growing habit. This has generally established a lasting benefit on total yield per hectare and hence productivity of harvestable timber. However changes in allocation of biomass between plant components in elevated CO_2 is frequently reported and appears to be highly species specific (Brown and Higginbotham 1986; Norby and O'Neill 1991; Idso and Idso 1994; Rouhier et al. 1994). Therefore, in order to understand and predict the possible effect of future CO_2 concentrations on productivity, possible changes in selection criteria, and thus ultimate economic value of this important timber species, it is necessary to evaluate the long term impact of elevated CO_2 on Sitka spruce directly.

A long term study to evaluate the likely impact of a doubling of atmospheric CO_2 on the biomass and nutrient partitioning of Sitka spruce, over several growing seasons, was initiated in open top chambers (OTCs) at the Institute of Terrestrial Ecology, Edinburgh.

Materials and methods

Plant material

In June 1990, 2000 unflushed 1+1 bare-rooted Sitka spruce seedlings [Forestry Commission identity number 83(2015)S LOT2, provenance 20, origin Queen Charlotte Island] were taken from a cold store and potted into 2.0 dm^3 pots using a composite soil. The soil consisted of sphagnum peat, 5 mm quartz and sterilized loam in the ratio 13:4:3 (v/v). Vitax Q4 fertiliser (N:P:K, 5.3:7.5:10) was added at 4 g dm^3 of compost to the soil and thoroughly mixed.

The plants were then randomised, 250 selected per chamber, and evenly distributed between 10 randomised blocks within each of eight OTCs (2000 seedlings in total). In March 1991, to avoid the plants becoming pot bound, 30 from each of the eight chambers were repotted into 4.5 dm^3 pots using the above composite mix. An additional 30 plants were randomly selected from each of the CO_2 treatments, repotted and placed in two additional chambers, increasing the replicate number of chambers per treatment to five. At the start of the growing season the plants were top dressed with 5.5 g of a slow release fertiliser (Osmocote mini, 5–6 months formulation; composition: 18% N, 6% P_2O_5, 11% K_2O, 2% MgO, and trace elements; Grace-Sierra, Nottingham, UK). In March 1992, 15 plants from each of the 10 chambers were repotted into 18.0 dm^3 pots using the same composite soil as before, top dressed with 22 g of Osmocote mini, and returned to their respective chambers. The plants were watered by capillary matting during 1990 and 1991, and because of increased pot size and hence soil volume, by trickle irrigation in 1992.

OTCs and CO_2 treatment

Eight octagonal OTCs (Waytogrow Greenhouses Ltd, Essex, UK), were used in 1990. Four of the OTCs received ambient CO_2 (~355 µmol mol^{-1}) and four received elevated CO_2 (700 µmol mol^{-1}). In March 1991, the number of OTCs was increased to ten, giving five replicates per CO_2 treatment during 1991 and 1992. Each chamber was 2.7 m high with a floor area of 7.0 m^2, constructed from an octagonal aluminium frame with standard 3 mm horticultural glass side panels. For a more detailed description of the chambers and their properties see Fowler et al. (1989).

Ambient air was supplied to each chamber by individual fan units (EK31, Radial and Axial, Herts, UK). Prior to injection into all chambers, the ambient air was passed through a series of ten impregnated, activated charcoal filters to remove ozone, sulphur dioxide and nitrogen dioxide (Emcel filters, Machine control, Sussex, UK). The ambient CO_2 chambers then received this air directly via a polyethylene manifold (400 mm layflat tubing, McKinnon and Hay, Edinburgh, UK), 1.5 m above ground level. The CO_2 concentration in these chambers fluctuated diurnally around a mean daily value of 355 µmol mol^{-1}. The elevated CO_2 chambers received air which was supplemented with pure CO_2 to raise the ambient concentration by 355 µmol mol^{-1}, i.e. to double the average present day concentration. The pure, liquid CO_2 was stored on site in a 6 ton tank (Distillers MG, Lanarkshire, UK). The CO_2 was vaporised and passed through individual mass flow controllers [FC28, Tylan General (UK) Ltd, Wilts, UK] driven by an FC288 control box [Tylan General (UK) Ltd, Wilts, UK]. The vaporised CO_2 was fed directly into the ambient air stream within the chamber fan units at a pre-set flow rate, where it was mixed thoroughly before being released into the chambers. The CO_2 concentration inside the elevated chambers varied around 700 ± 80 µmol mol^{-1}, depending on the ambient concentration and external windspeeds which affected ambient air incursion through the open top.

CO_2 monitoring system

The PC controlled monitoring system consisted of an interface card (ADC42, Blue Chip Technology), a relay box, 2-way solenoid valves, infra-red gas analyser and control software. A diaphragm pump (B100-DE, Charles Austen Pumps, Surrey, UK) drew air continuously from all of the elevated CO_2 chambers and one of the ambient CO_2 chambers, through 4-mm-internal diameter nylon sample lines (Phase Separations, Clwyd, UK) to the monitoring cabin. Each sample line contained a 2-way solenoid valve which allowed the air stream to be vented to waste, or when activated, diverted to an infra-red gas analyser (IRGA; SB-300, The Analytical Development Co., Hoddesdon, UK). The air sample was drawn through the IRGA at a constant rate by an internal pump. The software program cycled through the air samples from each chamber in turn, allowing a 60 s period of purging through the IRGA followed by a 60 s period of recording; the average recorded CO_2 concentration over this period was then stored on hard disk.

Growth analysis

At the start of the experiment (June 1990), 15 plants were randomly selected and destructively harvested. The dry mass was determined

Table 1 Effects of CO_2 concentration and open top chamber on growth parameters of 5-year-old Sitka spruce [*Picea sitchensis* (Bong.) Carr.] after three growing seasons (June 1990 to February 1993). Values within each row followed by a different letter indicate a significant difference ($P \leq 0.05$)

Parameter	Initial harvest (June 1990)	Final harvest (February 1993)		
		OTC and ambient CO_2	OTC and elevated CO_2	Outside and ambient CO_2
Dry mass (g)	6.3 ± 1.1	792	828	718
Height (mm)	274 ± 11	1874a	1790a	1496b
Root collar diameter (mm)	4.0 ± 0.26	31.3	32.0	28.6
Root mass/shoot mass	0.40 ± 0.03	0.31	0.34	0.44

separately for roots, needles and shoots. In subsequent harvests the plant material was further subdivided into current and previous years stem and branch wood and needles. These harvests consisted of random samples of 30, 5, 5, 15, and 5 plants from each of the chambers in January 1991, June 1991, August 1991, February 1992 and February 1993, respectively. Outside control plants were included in the February 1992 and 1993 harvests. At each harvest, plant height, root collar diameter and leaf area were also measured. Projected needle area was determined using an image analysis system (IIR, Digithurst, Royston, UK). Needles were placed on a light box to increase edge definition, and black and white video images digitised at 512 × 512 pixel resolution. Threshold settings for binary imaging were determined prior to measurements, using calibration standards. Leaf area ratio (total projected needle area/total plant mass, $cm^2\ g^{-1}$), specific leaf area (projected needle area/needle mass, $cm^2\ g^{-1}$) and leaf mass ratio (leaf mass/total plant mass), were calculated for the June 1991, August 1991, February 1992 and February 1993 harvests.

Throughout the 1990 and 1992 growing seasons, measurements of weekly leader extension were made on all chamber grown plants, and in 1992 on the additional outside control plot. Weekly relative extension rates (R_w) were calculated from (Hunt 1978):

$$R_w = \frac{\ln l_2 - \ln l_1}{t_2 - t_1} \quad (1)$$

where l_1 and l_2 are leader length (mm) at times t_1 and t_2 (days), previous weeks and current weeks measurement, respectively. Since this form of sampling was not destructive, consecutive measurements were made on the same plants and pairing was not necessary. At the start of the experiment plants of similar size were paired in order to provide statistical estimates of error in mean annual relative growth rates of dry mass (R_m). R_m was calculated for the time intervals between each end of season harvest, using Eq. 1, where l_1 and l_2 was substituted with M_1 and M_2 (total plant dry mass) at times t_1 and t_2.

Nutrient analysis

In August 1991, February 1992 and February 1993, amounts of nitrogen (N), phosphorus (P) and potassium (K) were measured in the roots and both current and previous years needles and wood removed from the plant stem and branches (i.e. nine tissue classes). In January 1991 and June 1991 samples from stem tissue were not taken, so that there were only five tissue classes. Each plant tissue class was individually bulked by chamber, sub-sampled then ground using a mill (Wiley - DCFH48, Glen Creston, Stanmore, UK) to less than 0.8 mm, in preparation for the above analysis. An aliquot of the ground material was redried in an air-circulated oven at 105 °C for 3 h, and 350 mg of the oven-dry sample was digested by a modified Kjeldahl procedure in the presence of H_2O_2, with Li_2SO_4 to increase boiling point and Se as catalyst (Parkinson and Allen 1975). Concentrations of N and P were measured by continuous flow colorimetry (Skalar analytical) via indolephenol blue, and molybdenum blue respectively, and K was measured by flame emission spectrometry (Corning Flame photometer 430). Total carbon (C) was measured on all nine tissue types from the February 1993 harvest, using elemental analysis (Carlo Erba Strumentazione, Mod 1106, Fison Instruments, Sussex, UK). Samples were prepared for C analysis by initially grinding using a Wiley mill (type DCFH48, Glen Creston, Stanmore, UK) to less than 0.8 mm then ball milling to a fine particle size.

Statistical analyses

Differences in dry mass and nutrient content among treatments and harvests for each tissue class were tested by analysis of variance. A randomised split plot design was used with chamber as the main plot and plants the subplots. Fumigation with or without additional CO_2 was the treatment. The analysis of variance was performed using Genstat 5 software (Rothamsted Experimental Station, Harpendon, Herts, UK). Regression analysis was performed on root and shoot data using SigmaPlot (Jandel Scientific, Germany).

Results

Effects of elevated CO_2 on growth and biomass production

The initial (June 1990) and final (February 1993) values of growth parameters of Sitka spruce are given in Table 1. After 3 years there was no significant treatment difference between total dry mass, root collar diameter or root mass/shoot mass ratio. However, plants grown inside the OTCs were significantly taller than those grown outside.

There was no significant effect of elevated CO_2 on the total biomass of Sitka spruce plants raised in OTCs by the end of the 3 year period (Fig. 1a). Total biomass differences observed between the two CO_2 treatments in August 1991, may be attributed to variations in mean weekly relative growth rates (R_w), (Fig. 1b). During the first experimental season (1990), the R_w was similar in both treatments (Fig. 1b). This was probably because of the predetermined nature of Sitka's growth pattern and the early stage in the experiment. By mid-summer R_w peaked in both treatments, but was significantly ($P = 0.05$) higher in the elevated CO_2 treatment compared with the ambient CO_2 chamber treatment. Thereafter the R_w of elevated CO_2 treated plants declined more rapidly, becoming significantly lower during late summer and early autumn ($P = 0.01$). This accounts for the loss of growth enhancement found in the elevated CO_2 plants between August 1991 and February 1992. On 6 June 1992, after two full growing seasons in their respective treatments, the elevated CO_2 plants were growing significantly faster than either the ambient CO_2 chamber or outside control plants ($P = 0.01$ and 0.001 respectively),

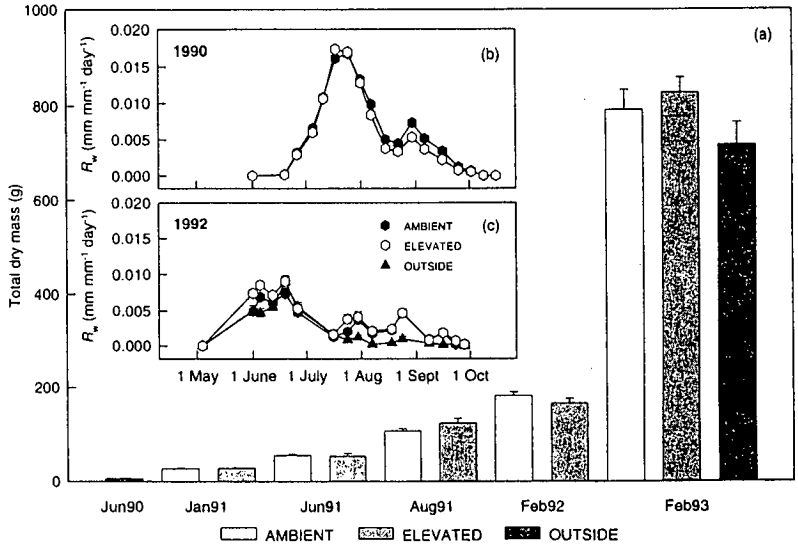

Fig. 1 Effects of OTC and CO_2 concentration on (a) total biomass produced over three growing seasons, (b) weekly relative growth rates of leader extension (R_w) in 1990 and (c) 1992. (For this and subsequent figures *Ambient* = chamber grown, ambient CO_2 (~350 µmol mol^{-1}) treated plants, *Elevated* = chamber grown, elevated CO_2 (~700 µmol mol^{-1}) treated plants, and *Outside* = plants growing outside chambers under ambient CO_2 (~350 µmol mol^{-1}). Values are means ± 1 SE)

Table 2 The mean annual relative growth rate of dry mass (R_m, % day^{-1}) for chamber grown ambient and elevated CO_2 treated plants in 1990, 1991 and 1992 ± 1 SE. Values within each column followed by a different letter indicate a significant difference ($P \leq 0.05$)

CO_2 treatment	1990	1991	1992
Ambient CO_2	0.47 ± 0.02	0.51 ± 0.02	0.42 ± 0.006 a
Elevated CO_2	0.47 ± 0.02	0.49 ± 0.009	0.45 ± 0.01 b

(Fig. 1c). There was a highly significant effect of chamber on mid- and late-summer leader extension. Plants growing outside the open top chambers had a significantly lower R_w than those inside the chambers irrespective of CO_2 treatment. The peak in R_w, although lower, occurred earlier and lasted longer in 1992 than 1990. This was because of the late starting date (1 June) for the 1990 field season.

Mean annual relative growth rates of woody biomass (R_m) did not differ between treatments during the first 2 years but were significantly higher (P = 0.05) in plants receiving elevated CO_2 compared to ambient CO_2 during the 3rd year, 1992 (Table 2). There was an initial increase in R_m from 1990 to 1991 followed by a decrease in 1992 for all treatments.

Partitioning of plant biomass

In January 1991, after one growing season in either elevated or ambient CO_2, Sitka spruce seedlings showed significant treatment effects on plant biomass distribution (Fig. 2a,b). Elevated CO_2 had no effect on shoot biomass (January 1991, 17.4 vs 18.1 g, P = 0.4), but significantly enhanced root biomass (January 1991, 11.2 vs 9.3 g, P = 0.01). Root dry mass was significantly larger in elevated CO_2 treated

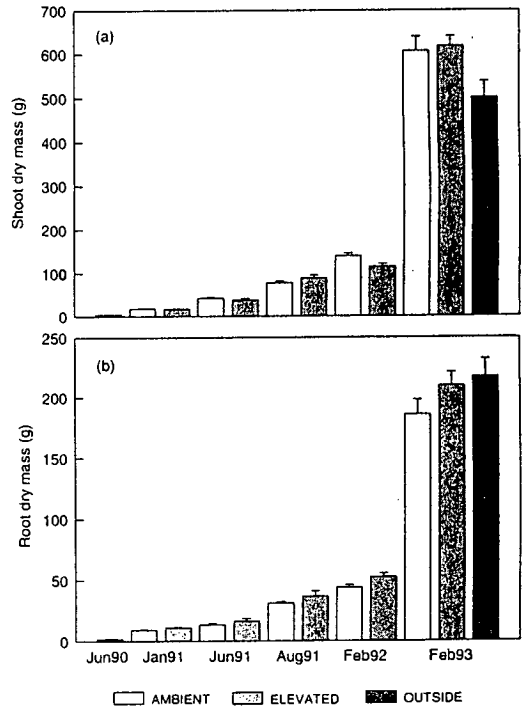

Fig. 2 Effects of OTC and CO_2 concentration on (a) shoot total dry mass and (b) root total dry mass at each harvest, from June 1990 to February 1993 inclusive. Values are means ± 1 SE

plants compared with ambient CO_2 treated plants, on all harvest dates. The effect of CO_2 on shoot partitioning was time dependent; the shoot dry mass of elevated CO_2 plants

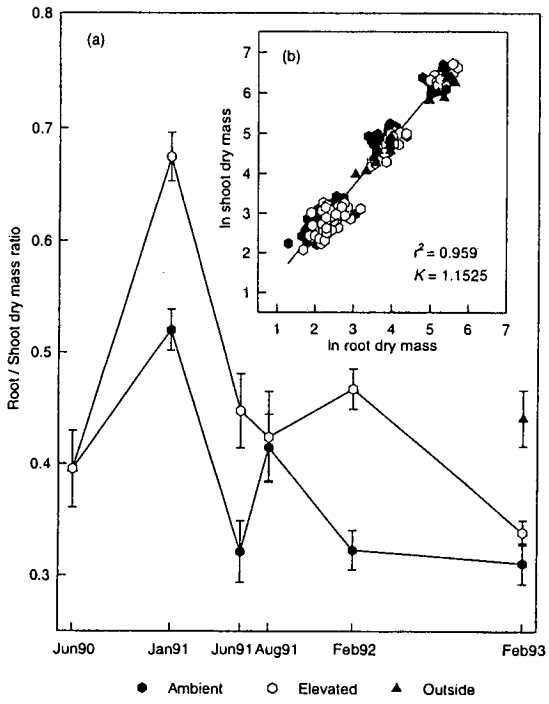

Fig. 3 a Changes in the root/shoot dry mass ratio from June 1990 to February 1993, under ambient and elevated CO_2: values are means ± 1 SE. Values for the outside control plot are included in 1993. b The linear relationship between ln root and ln shoot dry mass at each end-of-season harvest (January 1991, February 1992 and February 1993). (K = correlation coefficient)

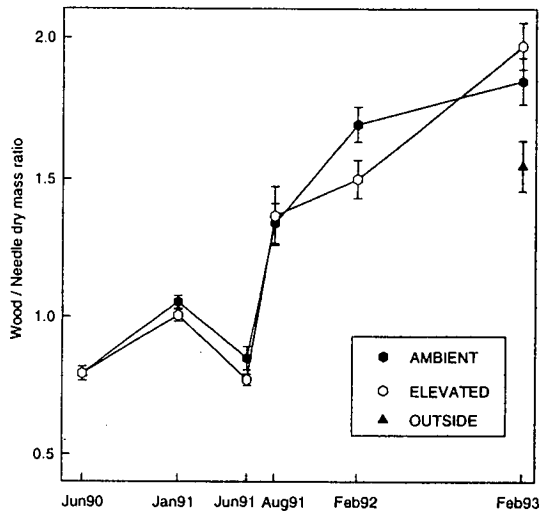

Fig. 4 Changes in the wood/needle dry mass ratio over three growing seasons in either ambient or elevated CO_2, or outside in control plot (last growing season only), (means ± 1 SE)

was smaller than that of ambient CO_2 plants in January 1991, June 1991 and February 1992, but larger in August 1991 and February 1993 (P = 0.4, 0.47, 0.011, 0.2 and 0.78, respectively).

Carbon allocated to the woody biomass was unaffected by elevated CO_2 in the 1st and 3rd years, but was significantly decreased in the 2nd year (P = 0.01; Table 3). There was little or no effect of elevated CO_2 on the allocation of wood between stem and branches. OTCs significantly increased the woody biomass of both stems and branches (P = 0.05).

The overall root mass/shoot mass ratio (R/S) of plants raised in both ambient and elevated CO_2 declined with time (Fig. 3a). Initially R/S ratios were significantly increased by elevated CO_2, but by the end of the third growing season, the effect of elevated CO_2 on R/S had disappeared. The R/S of plants growing outside was significantly higher than either of the two chamber treatments.

The allometric relationship between root and shoot dry mass was not significantly affected by elevated CO_2 throughout the 3 year experiment (Fig. 3b). There was nevertheless, an effect of chamber (though not significant) on root/shoot allometry; the slope of the relation between ln shoot mass and ln root mass (K) was increased for chamber grown plants (K = 1.16, r^2 = 0.96; K = 1.20, r^2 = 0.97 and K = 0.98, r^2 = 0.97, for ambient, elevated and outside treatments, respectively).

A steady increase in the wood mass/needle mass ratio occurred throughout the experiment in all treatments. There was no overall effect of CO_2 concentration on the wood/needle ratio, but outside control plants had a significantly lower ratio than chamber grown plants (P=0.01), (Fig. 4).

Table 3 Effects of OTC and CO_2 concentration on total wood biomass and partitioning between stem and branch dry mass at each harvest, from June 1990 to February 1993, inclusive. (A = ambient CO_2, E = elevated CO_2 and O = outside control. Values are means ± 1 SE. Values within each row followed by a different letter indicate a significant difference; ($P \leq 0.05$)

Harvest date	Jan 1991		Jun 1991		Aug 1991		Feb 1992		Feb 1993		
Treatment	A	E	A	E	A	E	A	E	A	E	O
Tissue Type (g)											
Wood dry mass	9.2±0.3	8.6±0.3	19.3±0.8	16.4±1.7	44±2.9	50±3.9	87±4.3 a	67±3.7 b	391±21 a	409±19 a	306±28 b
Stem dry mass	6.3±0.2	5.8±0.2	13.0±0.5	11.6±1.1	30±1.8	34±3.0	62±2.8 a	47±2.7 b	246±14 a	256±12 a	187±19 b
Branch dry mass	2.9±0.2	2.8±0.1	6.3±0.5	4.8±0.8	14±1.4	16±1.5	25±2.1 a	20±1.6 b	145±11 a	154±10 a	119±11 b

Table 4 Leaf properties of Sitka spruce grown inside OTCs after 2 (*FEB 1992*) and 3 (*FEB 1993*) years of fumigation with ambient or elevated CO_2, and grown outside in ambient air after two growing seasons. (*Current* = needles < 1 year old, *previous* = needles > 1 year old and *total* = current and previous years needles bulked. Values within each row followed by a different letter indicate a significant difference $P \leq 0.05$)

	FEB 1992			FEB 1993		
	Ambient	Elevated	Outside	Ambient	Elevated	Outside
Needle area (m^2)						
Total	24.8 ±1.1a	21.2 ±1.8b	–	84.8 ±4.7a	76.1 ±3.2b	70.7 ±5.2b
Current	18.9 ±1.0a	14.6 ±1.2b	–	66.9 ±3.7a	58.6 ±2.4b	54.9 ±4.8b
Previous	5.9 ±0.4	6.6 ±0.6	–	17.9 ±1.5	17.5 ±1.8	15.8 ±0.8
Specific leaf area ($cm^2\ g^{-1}$)						
Total	48.9 ±2.2a	46.0 ±2.3a	56.0 ±1.3b	39.8 ±1.0	36.4 ±0.7	36.1 ±1.7
Current	46.4 ±1.2a	41.3 ±1.0b	55.6 ±3.7c	40.9 ±1.3a	36.8 ±0.8b	37.3 ±2.0c
Previous	58.7 ±3.2	61.1 ±4.1	57.7 ±2.5	35.7 ±1.0	35.0 ±1.1	32.6 ±1.3
Leaf area ratio ($cm^2\ g^{-1}$)	13.8 ±0.6a	12.6 ±0.6a	17.7 ±0.7b	10.8 ±0.4	9.3 ±0.4	9.8 ±0.3
Leaf mass ratio	0.28±0.01a	0.28±0.01a	0.32±0.01b	0.27±0.01	0.25±0.01	0.28±0.01

Table 5 Effects of CO_2 concentration and OTC on tissue nitrogen concentrations (% of dry mass) of Sitka spruce [*Picea sitchensis* (Bong.) Carr.] for each harvest. (*A* = ambient CO_2, *E* = elevated CO_2 and *O* = outside control. Values are means with significant differences indicated by a different letter within each row for each harvest date. $P \leq 0.05$)

Harvest date	January 1991		June 1991		August 1991		February 1992			February 1993		
Treatment	A	E	A	E	A	E	A	E	O	A	E	O
Tissue type (g)												
Root	1.1a	0.9b	1.2a	1.0b	1.1	1.0	1.5	1.2	1.9	1.6	1.4	1.2
Current Year												
Stem												
Needles					1.9a	1.4b	1.8a	1.5b	2.1a	1.6	1.4	1.5
Wood					0.9	0.6	1.0	0.9	1.3	1.3	1.1	1.1
Branch												
Needles	1.8a	1.4b	1.5	1.4	1.7	1.4	1.9a	1.5b	2.1a	1.7	1.6	1.6
Wood	0.9	0.8	1.1	1.3	0.9a	0.7b	1.2	1.0	1.5	1.2	1.1	1.1
Previous Year												
Stem												
Needles					1.5a	1.2b	1.5a	1.3b	0.8c	1.2	1.1	1.1
Wood					0.5	0.5	0.5	0.5	0.4	0.6	0.5	0.6
Branch												
Needles	1.6a	1.4b	1.1	1.2	1.4	1.4	1.6	1.4	1.0	1.4	1.3	1.2
Wood	0.5	0.5	0.9	0.7	0.6	0.6	0.7	0.7	0.5	0.7	0.6	0.7

Effects of elevated CO_2 on leaf characteristics

By the end of the second and third growing seasons total projected needle area was significantly smaller in Sitka spruce plants treated with elevated CO_2 compared to plants grown in ambient CO_2 ($P = 0.05$, Table 4). This decrease in total needle area was attributed to a reduction in the needle area of current year foliage (< 1-year-old) in elevated CO_2 (Table 4). There was no significant effect of elevated CO_2 on the area of previous years needles (> 1-year-old), (Table 4). Again, the effect of elevated CO_2 on needle area was time dependent: samples taken in June 1991 showed no significant effect of treatment. There was also an effect of OTC on both current and previous year needles ($P = 0.05$ and n.s., respectively). Plants growing inside chambers had larger needle areas than those growing outside in the control plots.

Values for leaf area ratio (LAR), specific leaf area (SLA) and leaf mass ratio (LMR) fell significantly throughout the experiment (Table 4). Leaf area ratios and specific leaf areas of chamber grown plants subjected to elevated CO_2 were slightly lower than those receiving ambient CO_2, by the end of the 1991 and 1992 seasons, though not significantly so ($P > 0.05$; February 1992 and 1993 harvests, Table 4). Neither CO_2 concentration nor chamber affected the LMR of Sitka spruce (Table 4). By the end of the experiment no chamber effect was found on LAR, SLA or LMR. When the SLA was examined separately for current (< 1-year-old needles) and previous (> 1-year-old needles) year foliage, an effect of elevated CO_2 was found on current year needles in February 1992 and 1993 (Table 4). Plants receiving elevated CO_2 had lower SLA than ambient CO_2 treated plants ($P = 0.05$ and $P > 0.05$ for 1992 and 1993, respectively). There was no

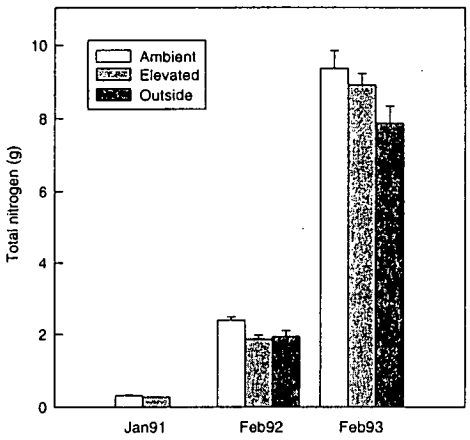

Fig. 5 Effects of OTC and CO_2 concentration on the mean total amount of nitrogen present per plant ± 1 SE, at the end of each growing season

treatment effect on the characteristics of the previous years foliage.

Effects of elevated CO_2 on plant nutrition

Foliar nitrogen concentration was consistently lower in the chamber grown, elevated CO_2 treated plants compared to chamber grown, ambient CO_2 treated plants (Table 5). Current year branch foliar N concentrations were significantly lower in the January 1991 and February 1992 end of season harvests. However, there was no significant difference in the N content of any plant component by the end of the experiment (February 1993), (Table 5). Foliar N concentration was highest in needles from current-year branches and lowest in needles from previous-year stem and branch wood. There was no statistically significant chamber effect on N concentration after plants had been growing outside for two full seasons.

Neither CO_2 concentration nor OTC had much effect on either root, wood or foliar concentrations of phosphorus or potassium throughout the duration of the 3 year experiment. After three growing seasons (February 1993 harvest), foliar nutrient concentrations (% oven dried mass) of phosphorus and potassium were 0.19 and 0.77, 0.18 and 0.74, and 0.17 and 0.74, for ambient CO_2, elevated CO_2 and ambient CO_2-outside plants, respectively. The P concentration in wood tissue from current-year branches, was significantly lower in elevated CO_2 plants than chamber grown ambient CO_2 plants, during August 1991 and February 1993 (0.2 and 0.22, respectively, $P = 0.05$). Despite this result there was no consistent pattern between CO_2 treatment and P values.

The total amount of nitrogen present in elevated CO_2 treated plants was consistently lower than chamber grown, ambient CO_2 treated plants ($P<0.05$, <0.05 and >0.05 for the January 1991, February 1992 and February 1993 harvests, respectively), (Fig. 5). Plants growing outside in the control plots also had a lower total nitrogen content compared with chamber grown, ambient CO_2 treated plants, though this was the result of the smaller amount of biomass present and not the nitrogen concentration per se. By the end of the third growing season, over 30% of total plant nitrogen was held in the foliage of branches less than 1 year old (Fig. 6). At the end of each growing season there was a significantly smaller amount of nitrogen held within needles on current year branches in elevated CO_2 compared with ambient CO_2 plants ($P<0.05$). The second largest sink for nitrogen was stem wood (including bark). There was a significant reduction in the total amount of nitrogen present in the stem wood (current + previous stem tissue) of elevated CO_2 treated plants compared with ambient CO_2 treated plants ($P<0.01$, Fig. 6).

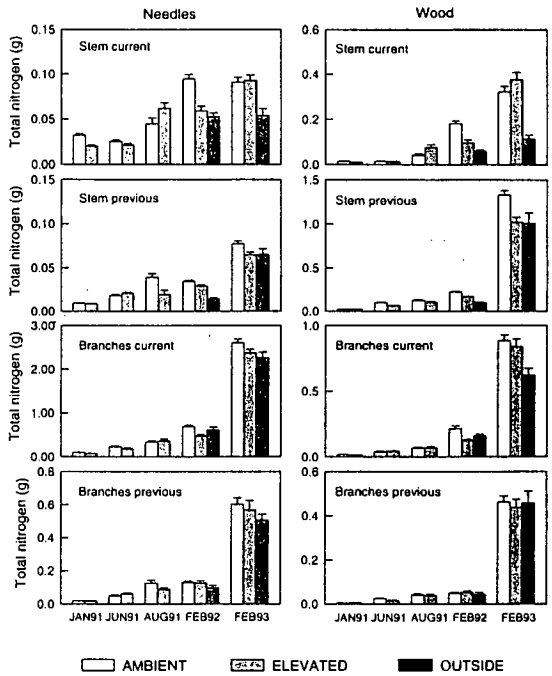

Fig. 6 Total amount of nitrogen partitioned between the needles (*left hand column of graphs*) and wood (*right hand column of graphs*), of current (< 1 year old) and previous year (> 1 year old) stem and branches for each harvest. Values are means ± 1 SE

Significant differences in the total amount of nitrogen held within tissue classes between chamber grown ambient CO_2 plants and plants growing in the outside control plot were largely attributable to differences in plant biomass rather than to tissue nitrogen concentration. There was no significant effect of elevated CO_2 or chamber on the partitioning of nitrogen between plant sinks.

The total carbon mass/nitrogen mass ratio at the end of the 3 year experiment was unaffected by elevated CO_2. It

was reduced in current year foliage and roots of plants growing in OTCs compared with those growing outside in the control plot.

Discussion

Changes in biomass accumulation and allocation are frequently reported for many C_3 species grown in elevated concentrations of atmospheric CO_2 (Tolley and Strain 1984; Johnson 1992; Pettersson et al. 1993; Silvola and Ahlholm 1993). It is therefore logical to expect an increase in net primary productivity (NPP) of Sitka spruce under conditions of enhanced atmospheric CO_2. However after 3 years fumigation with 700 μmol mol^{-1} CO_2, no increase in total plant height or biomass (g, dry mass) was observed. Although elevated CO_2 increased the root/shoot in this study by 35% in the first season, there was no significant change in the functional relationship between plant parts by the end of the third growing season. These results demonstrate that an enhanced level of CO_2 substrate may not necessarily result in an ultimate increase in NPP and hence greater timber yields for Sitka spruce.

The concentration of atmospheric CO_2 is just one of many environmental variables that control plant partitioning and productivity, e.g. local climate, soil and plant nutritional levels, competition and solar radiation (Bazzaz and Miao 1993; Rogers and Runion 1994). The balance between above-ground (harvestable timber) and below-ground plant components is as likely to be a reflection on the limitations of such environmental variables as elevated atmospheric CO_2 concentrations. Under conditions where photosynthesis is the limiting process, then carbon is preferentially partitioned to the shoots, but if either nitrogen or water are limiting growth then carbon is preferentially partitioned to the roots (Robinson 1986; Conroy 1992; Proe and Millard 1994).

Consequently in many cases, the experimental protocol and research techniques applied within the study, i.e. nutrient regimes, watering techniques and use of OTCs, directly affect plant growth and physiology. In fact, their impact may be as great if not greater than that of the CO_2 treatment itself. Our results demonstrate a strong influence of OTC on the growth pattern of Sitka spruce. Both shoot dry mass and plant height increased significantly in response to chamber, with the result that plants grown inside chambers had a lower root/shoot ratio compared with those grown outside. This response was probably because air temperatures inside OTCs were higher on days with high solar radiation, (see Murray et al. 1994 for a fuller explanation). Great care should therefore be taken when attempting to extrapolate elevated CO_2 results for purposes such as model vaildation or future scenario predictions.

Differences that exist amongst experimental studies reporting responses of plants to elevated CO_2 compared with those in ambient CO_2, most probably result from differences in nutrient and water availability, light quality (especially in experiments carried out in growth chambers) and pot size (Zak et al. 1993). Arp (1991) suggested that restricted available sinks for carbohydrates, such as are found with pot-bound plants which have a restricted rooting volume, may contribute to changes in photosynthetic capacity and thus affect ultimate productivity. This variable alone may account for many of the conflicting experimental results, a suggestion supported by Townsend (1993), who estimated that a rooting volume of 8 dm^3 was necessary for 3-year-old Sitka spruce seedlings, to ensure pot size was not confounding the experimental results. The plants in this study were repotted annually in order to avoid becoming pot bound. Though inadequate pot volumes can have a negative effect on growth stimulation of some tree species in elevated CO_2, this may not be true of all species. Kerstiens and Hawes (1994) concluded from their study on young cherry saplings, that there was no evidence of rooting volume reducing the stimulation of growth in elevated CO_2.

Enhanced root production and smaller projected needle areas in elevated CO_2 found in this and many other studies, may be the result of what is known as the "dilution or fertilisation" effect. Increased concentrations of atmospheric CO_2 may result in an increased demand by the trees for water and nutrients. Norby et al. (1992) found that increased photosynthesis was not accompanied by significant increases in leaf area or growth. Instead, the turnover of fine roots increased and leaf production decreased. Our findings are similar to these results and are typical responses of plants in which nutrition is limiting growth. Nutritional requirements are complex: increasing nutrient uptake via enhanced production and rapid turnover of fine roots may result in increased release and loss of nutrients from the soil rooting zone, a problem especially relevant for experiments carried out on potted plants. Zak et al. (1993) found a significantly larger pool of respired C in the rhizosphere of *Populus grandidentata* plants grown in elevated atmospheric CO_2. This suggests that respired C increases in response to greater root growth in elevated CO_2 and may result from enhanced root mortality, exudation, or cortical cell sloughing. The rate of nitrogen mineralisation was also shown to be higher in the elevated CO_2 soil, probably because of enhanced microbial populations and activity in the rhizosphere.

Our results show a decline in the concentration of nitrogen, on a unit dry mass of tissue basis, in elevated CO_2 plants compared with ambient CO_2 plants (1.6 vs 1.7%, after 3 years fumigation). This result is consistent with a number of other studies in which nutrient conditions were considered adequate although not luxuriant, for ambient CO_2. Sitka spruce is the most commonly planted tree species on nutrient-poor upland soils in the UK (Chandler and Dale 1990). We imposed a nutrient regime simulating likely field conditions to obtain results that could be extrapolated to the field. The importance of plant nutrition can be seen by comparing our results on Sitka spruce with those observed by Townend (1993). Where we found no significant increase in total biomass produced in elevated CO_2, Townend (1993) reported enhanced root and shoot and total growth in elevated CO_2. His plants were supplied

with un-limiting nutrients, resulting in foliar N concentrations of 2.9%, a concentration unlikely ever to be achieved in field conditions. Nitrogen concentrations reported here are by no means symptoms of acute deficiency. Optimum N foliar concentrations in plantations are generally in the range 1.2–2.0% (Everard 1973; Binns et al. 1980).

The results presented in this paper clearly demonstrate the importance of long term experimental studies, which allow plants to "acclimate" for several growing seasons. Had we presented our results after just 1 year of fumigation, elevated CO_2 would have been shown to significantly increase root/shoot ratios, decrease shoot biomass and have no effect on mean annual relative growth rates and needle areas. Similar results to these can be found in many recent publications (Brown and Higginbotham 1986; Campagna and Margolis 1989; El Kohen et al. 1992; Zak et al. 1993). However, after 3 years of fumigation the results are different. Root enhancement of elevated CO_2 treated plants was reduced to 10%. There was no significant effect of elevated CO_2 on the allometric relationship between roots and shoots. Also, plants growing in elevated CO_2 had significantly higher mean relative growth rates and significantly reduced current-year needle areas compared to the ambient CO_2 plants. These results are again consistent with the findings in a number of other studies (e.g. Eamus and Jarvis 1989; Norby and O'Neill 1991; Pettersson and McDonald 1992; Townend 1993).

In addition to the annual variation in our results, seasonal differences in biomass partitioning were observed. This was probably because of effects of elevated CO_2 on the timing of bud phenology (Murray et al. 1994). The late summer harvest of August 1991 showed that the plants in elevated CO_2 were at that time larger than those in ambient CO_2; however by the winter (February 1992) this result had been reversed. Murray et al. (1994) showed that Sitka spruce raised in elevated CO_2 set bud earlier in the autumn than Sitka spruce grown in ambient CO_2. Thus the shoot sink for fixed carbon is reduced earlier in the season in elevated CO_2, allowing the still photosynthetically active plants to allocate more carbon to the roots. This alters the root/shoot ratio and changes the carbon budget of plants raised in elevated CO_2 compared with plants growing in ambient CO_2 conditions.

In conclusion, elevated CO_2 may not necessarily enhance productivity. With a low nutrient supply, an increase in the root/shoot ratio may occur. If this is not accompanied by an overall increase in net carbon gain, above-ground harvestable timber production will be reduced. Despite such a possibility, valuable gains to the timber industry may be achieved on exposed nutrient poor sites, where increased root production may both enhance nutrient availability, and hence timber production, and increase wind stability. After 3 years of fumigation with nutritional conditions producing ~1.7% foliar nitrogen concentrations, we found no evidence of an increase in total plant biomass or shift in stem/branch ratio.

Acknowledgements This study was originally supported by funding from NERC and the EEC under the EPOCH programme. Funding has continued through the CEC Environment programme and NERC. It is part of the ECOCRAFT pan-European project co-ordinated by P. G. Jarvis. We thank EFG [Economic Forestry Group (Scotland), Forfar, Scotland] for supplying the plant material.

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