THE INFLUENCE OF CARBON DIOXIDE CONCENTRATION ON CARBON ASSIMILATION IN TROPICAL TREE SPECIES

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A thesis submitted for the degree of Doctor of Philosophy to the University of Edinburgh, 1998.

ABSTRACT

This thesis investigates the influence of carbon dioxide concentration on carbon assimilation in tropical tree species. To investigate the response of tropical tree species to elevated CO_2 concentration, seedlings of *Cedrela odorata* L. (Meliaceae) were grown in open-top chambers and exposed to atmospheric CO_2 at either ambient or twice-ambient concentrations. Nutrient supply rate was also altered to investigate its interaction with elevated CO_2 concentration. This experiment was repeated on different seedlings over two years, 1995 and 1996. The seedlings of *C. odorata* grown in 1996 showed an acclimation response to elevated CO_2 concentration, but those grown in 1995 did not. Plants grown in elevated CO_2 concentration were only larger than those grown in ambient CO_2 concentration in 1995 with a high rate of nutrient supply. It is hypothesised that high vapour pressure deficits restricted stomatal conductance and consequent photosynthesis in both years, but that this effect was particularly pronounced in 1996, when combined with a nutrient regime of excessively high concentration for the rate of growth. These effects are hypothesised to have triggered acclimation, or a reduction in CO_2 fixation capacity, as indicated by changes in derived values for the enzyme kinetic parameters of the carboxylation enzyme, ribulose 1, 5 bisphosphate carboxylase-oxygenase (Rubisco).

A biochemical model of photosynthesis (Farquhar *et al.* 1980, *Planta* 149, 78-90) was used to assess photosynthetic acclimation to elevated CO_2 concentration. The derived parameters for maximum rate of electron transport (J_{max}) and maximum velocity of the Rubisco enzyme (V_{cmax}) were compared between treatment CO_2 concentrations and both were significantly lower ($p < 0.05$) in plants that had been grown in elevated CO_2 concentration in 1996. Whole-plant gas exchange was monitored in 1996, where a decrease in net CO_2 uptake of plants grown in elevated CO_2 concentration was observed when compared with plants grown in ambient CO_2 concentration. Seedlings of *Schefflera macrostachya* Harms. (Araliaceae) were also included in the 1996 experiment and these showed no statistically significant response to elevated CO_2 concentration.

Field measurements of photosynthesis within-canopy were also made in an undisturbed rain forest in Brazil. The model described above was used to investigate differences in assimilation at five different strata of the canopy. Fitted values of J_{max} and V_{cmax} increased significantly ($p < 0.05$) with canopy height. Such results have not been previously published for a vertical profile of photosynthetic capacity in a tropical rain forest. Photosynthetic capacity was correlated with leaf nitrogen content. An increase in nitrogen concentration per unit of leaf area was also associated with an increase in leaf thickness. Both the increase in nitrogen concentration with increasing light and the increase in leaf thickness are mechanisms of optimising the distribution of photosynthetic capacity. Methods of combining data from these two types of investigation are discussed. Such integration is vital for prediction of the response of tropical forests to elevated CO_2 concentration.

Declaration

This thesis has been composed by myself from the results of my own work, except where stated otherwise, and has not been submitted in any other application for a degree.

ACKNOWLEDGEMENTS

I thank John Grace for his guidance and good humour throughout this project. I am particularly grateful for his careful reading of this thesis in its final stages. I also thank Paul Jarvis for the insight, time and patience that I received throughout my work, again, during writing in particular. I thank Andy Gray for sharpening my self-esteem, Diarmuid O'Neill for help with harvests, Craig Barton for generous technical assistance, Alex Harrower and Dave McKenzie for helping me to build things, and Bill and Bob for helping me to grow things. I also thank Tam and Joe, Mike Clearwater, Erica de Leau, John Findlay, Shiela Wilson, Ana Rey, Steve Scott and Ian Campbell for their help. I am particularly grateful to the "Brazil crew" (Patrick Meir, Yadvinder Malhi, Bart Kruijt, Jon Lloyd, Caroline Nichol) for their support in Manaus and for hosting me during the write-up. In addition, I acknowledge that the completion of my thesis was only possible with the friendship and support of other staff and students, specifically Jamesey Irvine, Belinda Medlyn, Vicky Temperton and Gail Jackson. I thank my many friends in Brazil who made my field work easier. In particular, I am grateful to Elisa Wandelli, Antonio Nobre, Graca, Eleneide, Mayra, Cassia and Silvano for their help with both scientific and logistic matters.

I acknowledge the financial support of the Commonwealth Scholarship Commission, who made this work possible, with assistance from Ede & Ravenscroft and the Davis Expedition Fund. In addition, I thank the Royal Botanic Garden of Edinburgh, particularly David Mitchell, for helping me to run my experiment with them, and also the two Brazilian institutes with whom I worked; INPA and EMBRAPA.

I acknowledge my friends, both here and in New Zealand, and my flatmates, particularly Bruce, Loeske, Cally & Beth who have kept me going at the times when I felt really low. My dear friend Maree has remained a constant source of love and encouragement throughout - thank you. My family in Scotland have also supported me tremendously in my crazy life here. Finally, I acknowledge the continued love and support of my parents who have been there for me when I most needed them.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Chapter One:
GENERAL INTRODUCTION

AN INTRODUCTION TO THE PROBLEM

With "millenium fever" upon us, we review the century and try to learn from the mistakes that have been made. The lessons are many. Among the greater awakenings of the twentieth century have been the realisation that the Earth's resources are finite, its resilience is not unlimited and that human societies evolve with dramatic consequences to the planet. Few people will be unaware that the global climate is changing and that we are likely to have exerted some influence on this phenomenon (IPCC 1995). Phrases such as "global warming" and "the greenhouse effect" have become common-place in every day conversation. Resolution of the likely scale of this climate change and the factors responsible for both its acceleration and amelioration are of utmost importance, both scientifically and socially.

In this thesis one aspect of the changing global climate has been selected for investigation, namely the rising concentration of CO_2 in the atmosphere. There exists less controversy about the continuing increase in the atmospheric concentration of this gas, than about the magnitude and direction of temperature change or the disruption of local and regional meteorological patterns (IPCC 1995). Keeling was probably the first to draw attention to the phenomenon of rising atmospheric CO_2 concentration, from measurements made since 1958 at Mauna Loa in the South Pacific ocean (Keeling *et al.* 1995). Atmospheric CO_2 concentration has increased by about 30 % since pre-industrial times and is expected to reach a concentration which is double that of the pre-industrial level by the year 2100 (IPCC 1995). Plants are the primary organisms of the terrestrial biosphere to respond directly, via photosynthetic enhancement, to an elevated concentration of CO_2. A concomitant increase in global temperature of about 2 °C by 2100 (IPCC 1995) is likely to further influence photosynthesis and respiration (Long 1991). In addition, nitrogen is being deposited on forests through air pollution, particularly in Europe and North America and this N-deposition is already affecting forests (Taylor, Johnson & Andersen 1994). We are still not sure of exactly how terrestrial ecosystems will respond to these increases, and we are particularly uncertain about the role that the tropical rain forest may play in the global carbon cycle (Brown *et al.* 1993).

The majority of studies of plant response to elevated CO_2 concentration have focused on glasshouse-grown crop species (Körner 1993). The responses of a range of temperate tree species have been well characterised but many questions still remain, particularly at the stand and ecosystem level (Luxmoore, Wullschleger & Hanson 1993). There are still relatively few studies of tropical plant response to elevated CO_2 concentration (Oberbauer, Strain & Fetcher 1985; Reekie & Bazzaz 1989; Hogan, Smith & Ziska 1991; Ziska *et al.* 1991; Körner & Arnone 1992; Berryman, Eamus & Duff 1993; Arnone & Körner 1995; Lovelock, Kyllo & Winter 1996)

which is surprising in view of the global importance of the tropical biomass. Tropical forest, including deciduous and montane forms, covers approximately 17.6 x 10^6 km^2 of the earth's surface and contains 59 % of global forest vegetation, or about 37 % of global carbon stored in either the vegetation or soil (Dixon *et al.* 1994). Although boreal forests contain a larger percentage of the global total (49 %), the amount of carbon stored in vegetation, as opposed to soil, is over twice as high in tropical forests. The remaining 14 % of the global carbon pool is stored in temperate forests. Global accounts of carbon flux indicate a discrepancy in the estimated amount of carbon being released to the atmosphere, in the form of CO_2, through the processes of fossil fuel burning and deforestation and observed atmospheric CO_2 concentration (Houghton 1995). This shortfall has been termed the "missing sink". The missing sink is likely to be apportioned between the ocean and the terrestrial biomass (Tans, Fung & Takahashi 1990). The determination of these proportions may be achieved using isotopic analyses. Using this method, the current estimates of terrestrial sink strength fluctuate between 0.5 and 1.9 Gt C yr^{-1} for the 1980s and 2.6 Gt C yr^{-1} given for 1992-93 (Melillo *et al.* 1996).

Some evidence suggests that carbon uptake in the temperate and boreal forests in the mid-latitudes of the northern hemisphere comprises the largest part of the terrestrial sink (Jarvis 1995). However, new evidence suggests that boreal forests may actually be a source of carbon when the large quantity of stored organic matter decomposes during the summer thaw (Goulden *et al.* 1998). The uncertainty associated with the estimation of tropical biomass and change in land-use make quantification of the exact contribution of tropical forest to global carbon balance difficult (Brown 1988; Houghton 1995). However, there are now two studies which suggest that Amazonian rain forests are carbon sinks (Grace *et al.* 1995a; Malhi *et al.* 1997).

This thesis provides quantitative data on the effects of an elevated concentration of atmospheric CO_2 on two tropical tree species, *Cedrela odorata* (L.) and *Schefflera macrostachya* (Harms.). In addition, the vertical profile of photosynthesis in an undisturbed tropical rain forest in Manaus, Brazil is characterised quantitatively and discussed in the broader context of a future elevated concentration of CO_2 in the atmosphere.

AIMS AND APPROACHES

Physiological responses to elevated CO_2 concentration have been studied intensively over the past fifteen years (Eamus & Jarvis 1989; Field *et al.* 1992; Luxmoore *et al.* 1993; Poorter 1993; Ceulemans & Mousseau 1994; Rogers, Runion & Krupa 1994) but the wide range in both methodology used and species' response have returned a highly variable data set. Experimenters have tackled the problem using a variety of techniques and with an eye for responses at different scales. The majority of experiments have used controlled environment and open-top chambers which can control natural environmental variation and therefore allow the elucidation of causal mechanisms (Payer *et al.* 1993). However, the results obtained from free air CO_2 enrichment experiments (Wall & Kimball 1993) and investigations of plant communities growing in naturally enriched CO_2 environments (Miglietta *et al.* 1993) are of greater ecological significance. Plant responses to elevated atmospheric CO_2 concentration have been studied from the scale of leaf physiology/anatomy through whole-plant carbon balance/gas exchange to canopy behaviour and interspecific dynamics, yet the integration of results from these scales is rare. This thesis combines measurements of the influence of CO_2 concentration on carbon assimilation from a range of scales and suggest how these results may be integrated.

Elevated atmospheric CO_2 concentration will increase plant photosynthesis (Sionit & Kramer 1986; Ceulemans & Mousseau 1994; Lloyd & Farquhar 1996) but the longevity of this "fertilisation effect", and the destination of the extra carbon fixed, remain uncertain. Because growth is not usually stimulated to the same extent as photosynthesis, it has been suggested that the increase in photosynthesis is not persistent and that acclimation in the form of either down-regulation of photosynthesis or end product inhibition occurs (Bowes 1993). In most medium-term studies some form of acclimation or "phenotypic adjustment to a short term change in environment" (Amthor 1995) has been observed (Ceulemans & Mousseau 1994; Gunderson & Wullschleger 1994). However, in many experiments the acclimation responses observed are thought to be experimental artefacts, whereby growth limitations imposed by poor light and nutrient regimes (Bowes 1993) and restricted rooting volumes (Arp 1991; Thomas & Strain 1991) inhibited the plant's ability to sustain a response to elevated CO_2 concentration. The acclimation of plants to elevated atmospheric CO_2 concentration is likely to occur but is unlikely to occur to the extent that there is no net benefit of an increased concentration of CO_2 to the plant (Gunderson & Wullschleger 1994). Plant response to elevated atmospheric CO_2 concentration will undoubtedly be moderated by the relative quality of other environmental variables but whether this interaction will necessarily reduce the effect of elevated CO_2 concentration requires further elucidation. Recent reviews have concluded that plants are likely to increase the efficiency of their resource use, particularly in the case of key limiting factors, such as nitrogen (Drake, Gonzàlez-Meler & Long 1997). Such an increase in resource-use efficiency has profound implications for an ecosystem as a whole (Drake *et al.* 1996b). In the present study, the interaction of elevated CO_2 concentration and nutrient stress is investigated for *C. odorata*, and allocation of extra assimilated carbon examined.

Given the relatively long life span of trees and the relatively short time span of this major environmental change, experimental manipulation of plants in a manner that mimics exactly the predicted global atmospheric change is impossible. The best way to deal with this situation is to integrate existing knowledge of plant physiological response to changes in atmospheric CO_2 concentration with forecast changes in the driving variables. The obvious way to achieve this integration is to combine global climate change models, usually general circulation models (GCMs), with plant response models. This thesis demonstrates the range of physiological responses of tropical tree species that must be investigated in order to predict plant growth in future and also highlights gaps in the existing database. Commentary on existing global climate change models and details of their mathematical integration with physiology-based models is beyond the scope of this thesis and good reviews of this subject are published elsewhere (Tans *et al.* 1990; Ågren *et al.* 1991; McMurtrie *et al.* 1992; Jarvis & Dewar 1993; Dixon *et al.* 1994; Schimel 1995).

Scaling-up plant responses to elevated CO_2 concentration from open-top chambers to future behaviour of whole ecosystems requires the use of plant photosynthesis models (Eamus & Jarvis 1989). The link between plant response and canopy response is best provided by the Farquhar *et al.* (1980) model of photosynthesis which can be applied to individual leaves and can also be used in quantitative models of whole-canopy photosynthesis. First, a verified model of photosynthesis for ambient conditions is derived from *in situ* measurements of leaf photosynthesis. The future behaviour of the plant photosynthetic parameters under elevated CO_2 concentration is then measured in plants exposed to elevated CO_2 concentration in open-top chambers. These changes in the parameters can then be incorporated into the whole-canopy model to give a prediction of ecosystem photosynthesis under future atmospheric CO_2 concentrations.

Specific aims of this thesis

This thesis aims:

1) To investigate the growth responses of seedlings of two tropical tree species, *C. odorata* and *S. macrostachya*, to elevated atmospheric CO_2 concentration in glasshouse conditions, and the interaction between rate of nutrient supply and CO_2 concentration using the Ingestad technique to control rate of nutrient supply. *C. odorata* is a fast-growing light-demanding species while *S. macrostachya* is a fast-growing canopy emergent which grows in full sunlight but is also shade-tolerant, suggesting a niche intermediate between pioneer and climax.

2) To investigate photosynthetic responses of *C. odorata* to elevated CO_2 concentrations in glasshouse conditions. Both short and medium-term photosynthetic responses to elevated CO_2 concentration were investigated.

3) To investigate whole-plant fluxes of CO_2 of *C. odorata* at ambient and elevated CO_2 concentration and to investigate both above and below-ground contributions to this flux.

4) To investigate photosynthetic capacity of leaves in a multi-layered, multi-species rain forest canopy in Brazil. The aim was to characterise vertical variation in photosynthetic capacity in order to improve canopy models of rain forest photosynthesis.

5) To suggest ways of integrating data from experiments on plants grown in elevated CO_2 concentration with a knowledge of leaf physiology *in situ* in a rain forest canopy. Data from this thesis could be used to calibrate a model of canopy

photosynthesis. Perturbations in external CO_2 concentration could be included in order to enable tentative predictions of the response of tropical forest trees to elevated atmospheric CO_2 concentration.

In the current chapter, an overview of existing research on the effects of elevated CO_2 concentration is given, with particular reference to tree species. Special attention is paid to work on tropical tree species and to studies which have attempted to "scale-up" from individual plants to ecosystem responses to elevated CO_2 concentration.

RESEARCH ON THE EFFECTS OF ELEVATED CO_2 CONCENTRATION ON TERRESTRIAL PLANTS

Methodology

The methodology adopted for the study of plant responses to elevated CO_2 concentration greatly affects observed results and consequent conclusions (Eamus & Jarvis 1989; Jarvis 1995). Research groups are only now beginning to standardise approaches for this purpose, thus allowing correlations between groups to be drawn on an international scale (Jäger & Weigel 1993). To compare oranges with apples is not necessarily fruitful, and results from existing studies should therefore be viewed from within the framework of selected methodology. In addition, extrapolation from individual plants in an experimental system to ecosystem scale predictions must be approached with caution.

Possibly the overriding difference between methods is the scale at which a system is viewed. Scale differs between experimenters on both a temporal and spatial level. Experiments on trees may be of short (weeks to months), medium (two to three growing seasons) or long (several years) term duration. For plants of short life span,

such as annual crops and flowers, it is possible to investigate response to increased atmospheric CO_2 concentration over the course of the entire lifetime within a few months (Norby, Wullschleger & Gunderson 1996). Whilst short-term experiments can offer valuable insights into physiological mechanisms involved in responses to elevated CO_2 concentration (Bowes 1991), they are of little predictive value for the long-term responses of long-lived plants such as trees (Eamus & Jarvis 1989; Mousseau & Saugier 1992; Luxmoore *et al.* 1993; Ceulemans & Mousseau 1994; Amthor 1995). On the other hand, long-term experiments are constrained by high financial cost (Eamus & Jarvis 1989; Mousseau & Saugier 1992) and by the fact that for most tree species the time taken for a seedling to reach maturity is likely to exceed the time predicted for a doubling in atmospheric CO_2 concentration (Eamus & Jarvis 1989; Lee & Jarvis 1995).

Responses to elevated CO_2 concentration, most notably the response of the CO_2-fixing enzyme, ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco), and other enzymes (Sage, Sharkey & Seemann 1988; Yelle *et al.* 1989; Stitt 1991), have been investigated at the sub-cellular scale. With increasing scale, changes in anatomy in individual leaves (Thomas & Harvey 1983; Conroy, Barlow & Bevege 1986; Woodward & Kelly 1995) plus phytochemistry (Wulff & Strain 1982; Norby *et al.* 1996) and gas exchange (Bunce 1992; Sage 1994), responses of individual branches on mature trees (Barton, Lee & Jarvis 1993) and whole-plant partitioning/allocation responses (Ackerson, Havelka & Boyle 1984; McConnaughay, Nicotra & Bazzaz 1996; Mousseau *et al.* 1996) have been investigated. Some investigations of ecosystem behaviour (Owensby *et al.* 1996) and tentative predictions of changes in stand dynamics have also been made (Bazzaz *et al.* 1989; Körner 1993). Integration of measurements taken over such a variety of temporal and spatial scales can be achieved through comprehensive mathematical modelling (Körner 1993; Jarvis 1995).

Growth Chambers

The use of controlled environment chambers allows control and manipulation of environmental variables but these differ appreciably from outside (Ceulemans & Mousseau 1994). Large growth units afford the possibility of investigation of ecosystem response (Körner & Arnone 1992) but their extrapolation to a natural environment is limited (Payer *et al.* 1993). It should also be noted that the environment within a growth chamber, contrary to general assumption, is not uniform and care should be taken to account for both systematic and random variation within as well as between chambers (Potvin 1993).

Open-Top Chambers

A large number of studies of plant responses to elevated CO_2 concentration have used open-top chambers (OTCs). Compared with growth chamber experiments these systems are relatively cheap to construct and require significantly less CO_2 to maintain an elevated concentration than free air CO_2 enrichment systems (Mousseau & Saugier 1992). They also allow substantial control of potential confounding environmental factors whilst still permitting exposure to natural, that is, outdoor conditions where desired (Hendrey, Lewin & Nagy 1993). As with growth chambers, OTCs are subject to substantial between and within chamber variation so replication and sampling strategies should allow for this (Fuhrer 1993). Low irradiation, unrealistic wind speeds and higher temperatures within chambers may also be a problem (Drake *et al.* 1989; Jäger & Weigel 1993), and general small volume makes the investigation of forest ecosystem and large tree response impossible (Schulze & Mooney 1993). However, the investigation of grassland ecosystems has been done successfully using this type of approach (Drake *et al.* 1996a).

Neither open-top nor growth chamber experiments can be used directly in predictions of a natural ecosystem situation (Long & Drake 1992; Jäger & Weigel 1993; Koch & Mooney 1996). Coupling of plants to atmosphere is reduced (Lee & Jarvis 1996), natural soil processes are difficult to mimic in growth chambers (Hendrey *et al.* 1993) and the majority of experiments in both types of chamber have not, until recently, used plants rooted naturally in soil (Eamus & Jarvis 1989; Arp 1991). It has now been conclusively shown that potted plants behave differently to plants rooted directly into the soil (Eamus & Jarvis 1989; Thomas & Strain 1991; Sage 1994; Mousseau *et al.* 1996). This is thought to be largely because of the correlated shortage of nutrient (Pettersson & McDonald 1994) but there is also much evidence to suggest that root restriction can inhibit the plant response to elevated CO_2 concentration both through a reduction in sink capacity (reviewed by Ceulemans & Mousseau 1994) and an altered ability to "mine" for nutrients (Sage 1994; Johnson *et al.* 1996).

Branch bags

For practical reasons mature trees have seldom been studied for their response to elevated CO_2 concentration. Branch bags have been proposed as a solution to this problem (Barton *et al.* 1993; Lee & Jarvis 1996). Their use depends on the assumption of branch independence (Sprugel, Hinckley & Schaap 1991) which clearly limits their applicability to whole tree gas exchange (Lee & Jarvis 1996). That is, interactions between branches cannot be inferred and no account is taken of differences in photosynthetic capacity between branches, so it is difficult to scale-up directly to the whole-plant level. However, information gathered in this way may be correlated with measurements obtained either from leaves or whole trees at other stages of the life cycle, through the use of models such as MAESTRO, in order to obtain a clear picture of overall species response (Lee & Jarvis 1996).

FACE experiments

Free air CO_2 enrichment (FACE) experiments approximate most closely to natural environmental conditions (Jäger & Weigel 1993). Experiments using this technology are large scale (up to 500 m^2), can be multispecific, and investigate plants in their natural environment (Pinter *et al.* 1996). This technology allows the possibility of combined investigation of elevated CO_2 concentration effects with responses to increases in temperature (Nijs *et al.* 1997) to improve predictions of global vegetation response. FACE experiments also create the opportunity to study soil sequestration of carbon in an elevated CO_2 concentration through the use of carbon isotopes (Nitschelm *et al.* 1997). The disadvantages are that this system is costly to maintain (Eamus & Jarvis 1989; Mousseau & Saugier 1992; Hendrey *et al.* 1993), the experiment may create an "island" effect whereby interactions from the surrounding ecosystem confound the experimental design (Koch & Mooney 1996) and is prone to the same problems of environmental confounding as classic ecological field experiments (Allen *et al.* 1992). As a response to the high cost, FACE units have now been produced in smaller sizes which are cheaper to run and are ideal for grass and pasture plants (Miglietta *et al.* 1997). Forests require a very large scale FACE unit, which consume large amounts of CO_2. However, experiments investigating the response of forests to elevated CO_2 concentration are currently in progress (*e.g.* Ellsworth *et al.* 1995).

A note on general methodological problems of existing research

The large number of investigations into the effects of elevated CO_2 concentration on terrestrial plants have mostly focused on Northern American or European species, the majority of which have been crop plants. There has been significantly little research on mixed species interactions (Zangerl & Bazzaz 1984; Bazzaz, Garbutt & Williams 1985; Bazzaz, Coleman & Morse 1990) and tropical systems have been largely ignored (Hogan *et al.* 1991). Also, most experiments involve either the

transplantation of seedlings or saplings growing in ambient conditions to a higher concentration of CO_2 or the germination and subsequent growth of plants in an elevated atmospheric CO_2 concentration. Both situations are problematic because in reality the rise in global atmospheric CO_2 concentration is a gradual one (Conway *et al.* 1988). The second scenario however, avoids the problem of sudden dramatic changes in the CO_2 concentration which are likely to produce physiological effects different to those seen in plants experiencing a gradual increase in atmospheric CO_2 concentration (Eamus & Jarvis 1989).

Photosynthesis

An increased concentration of atmospheric CO_2 increases the gradient in CO_2 concentration between the outside and inside of the leaf thus increasing flux of CO_2 into the leaf (Acock 1990). CO_2 is the primary substrate of photosynthesis (Harley *et al.* 1992), plus an activator of the ribulose 1, 5 bisphosphate carboxylase-oxygenase (Rubisco) enzyme and a modulator of its own fixation (Bowes 1991). It is a competitive substrate with oxygen (O_2) for Rubisco (Raven, Evert & Eichhorn 1986) so environmental perturbations which alter the balance of CO_2 and O_2 as substrates in favour of CO_2 improve carboxylation and suppress oxygenation (Farquhar, von Caemmerer & Berry 1980). This suppression of the competing photorespiratory pathway is reported to be the most important effect of an elevated CO_2 concentration, as this suppression requires no extra light, water or nitrogen so leaf efficiency is maximised for the same quantity of each of these resources (Drake *et al.* 1997). By contrast, an increase in plant growth with elevated CO_2 concentration, requires an increased consumption of these resources.

The process of photosynthesis can be effectively split into two major types of reaction: those requiring light energy and those which proceed without light and

effectively consume the products of the light reactions. Central to these "dark" reactions is the Rubisco enzyme which occurs in all photosynthetic organisms and comprises up to 50 % of the soluble protein of leaves (Lawlor 1993). A simplified diagram giving an overview of carbon metabolism in leaves is given below (Fig. 1.1). It should be noted that the rate of CO_2 assimilation, *ie.* conversion to a range of more complex organic molecules (Jones 1992), is limited not only by the dark reactions but also by the light reactions which provide nicotinamide adenine dinucleotide phosphate (NADPH) and adenosine tri-phosphate (ATP) to drive the photosynthetic carbon reduction (PCR) cycle (Farquhar *et al.* 1980).

The PCR cycle is common to all plants and the first product of the cycle, a three carbon compound (3 phosphoglycerate, 3PGA) defines the process as C_3 photosynthesis. Many higher plants have evolved additional mechanisms for concentrating CO_2 before the PCR cycle giving four-carbon carboxylic acids as their first products. This C_4 process may be split further, depending on the timing of the production of organic acids - plants that produce them immediately prior to their use in the PCR cycle are known as C_4 plants whereas crassulacean acid metabolism (CAM) plants produce the acids during the previous dark period (Lawlor 1993). 95% of terrestrial plants are C_3 species (Bowes 1993) but, those which are C_4 or CAM tend to inhabit hotter or more arid environments (Raven *et al.* 1986). Leaf anatomy varies according to photosynthetic pathway (Bolhàr-Nordenkampf & Draxler 1993). Most importantly, the C_4 and CAM pathways minimise losses in carbon fixation to the competing photorespiratory pathway (Bowes 1991). Therefore, suppression of photorespiration by increased atmospheric CO_2 concentration benefits C_3 plants proportionately more than C_4 or CAM plants. Humid tropical rain forest contains mainly C_3 trees with a small epiphytic CAM component. C_4 photosynthesis is only important in tropical graminaceous vegetation (Lüttge 1997).

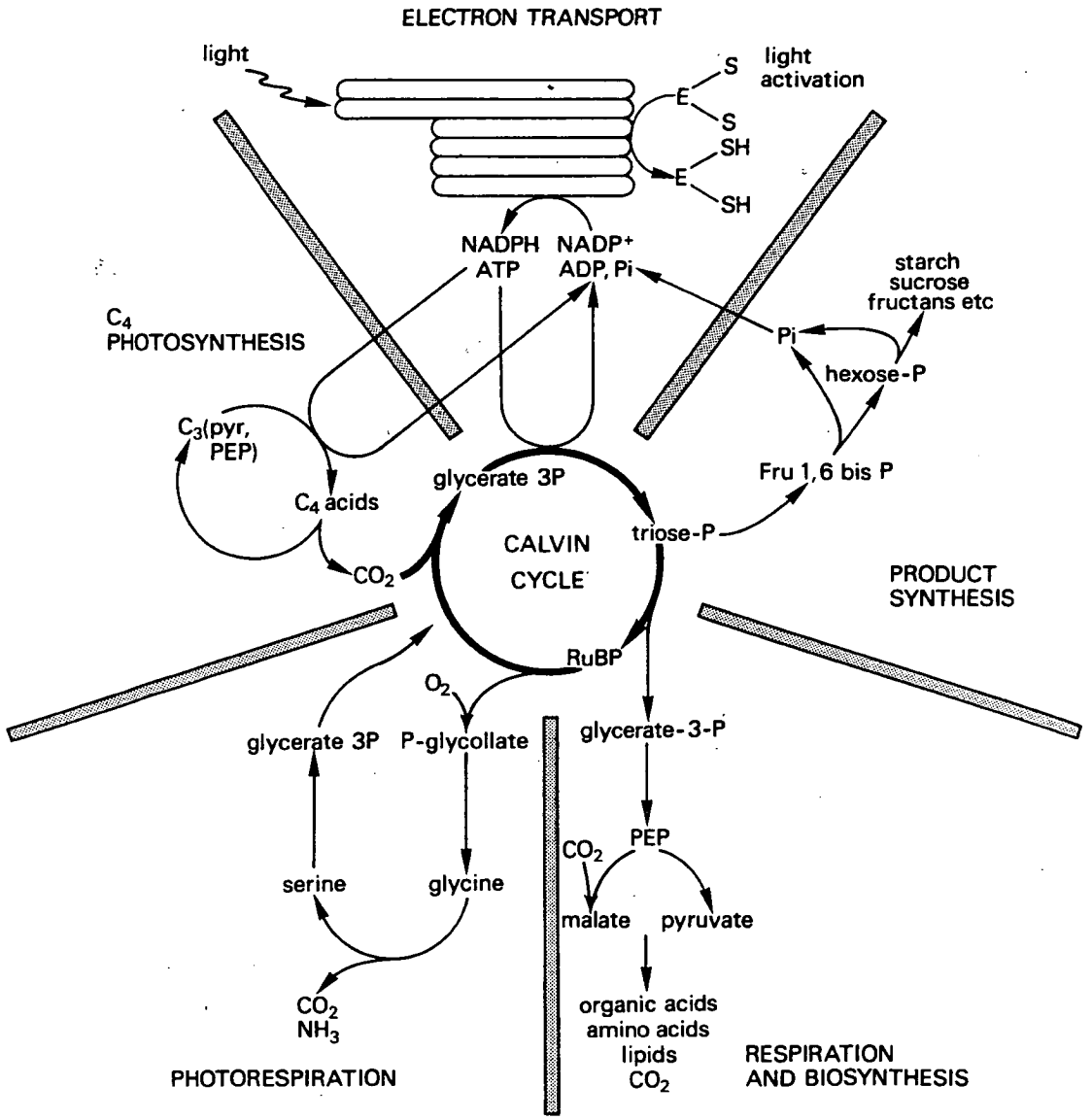


Figure 1.1 Carbon metabolism in leaves. The products from the light reactions in the thylakoids are ATP and NADPH and these are fed to the dark reactions of the Calvin cycle. This is also known as the photosynthetic carbon reduction cycle (PCR). CO_2 is fixed by the Rubisco enzyme in the PCR cycle to give glycerate-3-P. Rubisco also catalyses the competing reaction of oxygenation of RuBP or photorespiration. This is known as the photosynthetic carbon oxidation pathway (PCO). In C_3 plants the PCR cycle occurs in the mesophyll whereas in C_4 plants it occurs in the bundle sheath, the concentration of CO_2 into C_4 acids occurring in the mesophyll prior to entry into the PCR. Diagram reproduced from (Leegood 1993, Fig. 16.1, p248) with permission from Chapman &Hall.

The majority of studies have indeed shown an increase in photosynthesis in elevated CO_2 concentration, at least over the short term (Norby *et al.* 1992; Silvola & Ahlholm 1992; Gunderson, Norby & Wullschleger 1993). The average increase in net photosynthesis for woody tree species as a result of growth in double the present CO_2 concentration is about 50 % (Luxmoore *et al.* 1993). A few studies have shown no increase or even a decrease in photosynthesis with elevated CO_2 concentration (Gaudillère & Mousseau 1989; Kaushal, Guehl & Aussenac 1989; Bunce 1992), but these remain the exception rather than the rule. More commonly, an initial increase in photosynthesis is observed which slowly decreases or even reverses over time (Gaudillère & Mousseau 1989; Mousseau & Saugier 1992; Grulke, Hom & Roberts 1993). This latter response has been thought of as *acclimation* where photosynthesis is down-regulated over longer periods of time (Ceulemans & Mousseau 1994).

Acclimation

Both the existence and mechanism of acclimation are controversial. The term "acclimation" may be defined as "phenotypic adjustment to a short term change in environment" (Amthor 1995). Of course, in an experimental context the phenomenon may be observed over the "long term" duration of weeks to months (Ceulemans & Mousseau 1994), depending on the process being studied (Eamus & Jarvis 1989). Eamus and Jarvis (1989) proposed the term "acclimating" to describe plants which are still in the process of adjustment. This would apply to the majority of published studies.

Experimental evidence suggests that Rubisco activity and ribulose 1, 5 bisphosphate (RuBP) regeneration capacity can acclimate to the prevailing CO_2 concentration (Bowes 1991). It has been proposed that resources, such as nitrogen, may be reallocated away from Rubisco towards the more limiting components of the pathway such as light harvesting or carbohydrate synthesis. This may be determined by

indirect means such as the decrease in leaf nitrogen with a corresponding decrease in respiration (Wullschleger, Norby & Gunderson 1992). However, the exact pattern of reallocation is not consistent between species (Sage, Sharkey & Seemann 1989).

The tool most commonly used for the assessment of acclimation is the A/C_i curve (Gunderson & Wullschleger 1994). Net photosynthesis (A) is measured for values of intercellular CO_2 concentration (C_i) ranging from close to zero to *ca* 1000 μmol mol^{-1}. The resultant curve is interpreted as the result of the superimposition of two curves (Fig. 1.2, p. 19), one describing the dependence of assimilation on Rubisco activity (A_v) and the other the dependence on RuBP regeneration (A_j) which is in turn affected by the rate of electron transport from the light reactions (Farquhar *et al.* 1980). The net rate of leaf photosynthesis is therefore defined as:

$$A = \min\left(A_j, A_v\right) - R_d \qquad (1.1)$$

where R_d = the rate of dark respiration in the light (Farquhar *et al.* 1980).

An estimate of photosynthetic capacity can be obtained from the curves. This capacity is quantified using the parameters of J_{max} and V_{cmax}. J_{max} is the maximum rate of electron transport and V_{cmax} is the maximum velocity of the Rubisco enzyme.

The initial, near-linear part of the A/C_i curve shows the Rubisco limitation and may be described by the equation (Farquhar *et al.* 1980):

$$A_v = \frac{V_{cmax}(C_i - \Gamma^*)}{C_i + K'} \qquad (1.2)$$

The curvilinear and plateau sections of the curve indicate electron transport limitation and consequent limitation of RuBP regeneration and may be described by the equation (Farquhar *et al.* 1980):

$$A_j = \frac{J(C_i - \Gamma^*)}{4(C_i + 2\Gamma^*)} \qquad (1.3)$$

where J = rate of electron transport,

Γ^* = CO_2 compensation concentration in the absence of mitochondrial respiration (a value of 36.9 μmol mol^{-1} is used here (von Caemmerer *et al.* 1994)), and

K' = effective Michaelis-Menten constant of Rubisco.

Because Rubisco catalyses both carboxylation and oxygenation reactions its enzyme kinetics are complicated, specificity for each substrate altering with changes in temperature and concentration of each of the substrates present. The effective Michaelis-Menten constant, therefore, describes the interaction of the two reactions catalysed by Rubisco as dictated by O_2 concentration, *ie.* partial pressure, which dominates the reaction at low concentrations of CO_2.

The effective Michaelis-Menten constant may therefore be defined as (de Pury & Farquhar 1997):

$$K' = K_c\left(1 + \frac{O}{K_o}\right) \qquad (1.4)$$

where O = oxygen (O_2) partial pressure (205 x 10^3 μmol mol^{-1}),

K_o = Michaelis-Menten constant of Rubisco for O_2, a measure of the dependence of Rubisco velocity on O_2 concentration (a value of 248 x 10^3 μmol mol^{-1} is used here (von Caemmerer *et al.* 1994), and

K_c = Michaelis-Menten constant of Rubisco for CO_2 (a value of 404 μmol mol^{-1} is used here (von Caemmerer *et al.* 1994)).

K' = 738 μmol mol^{-1}

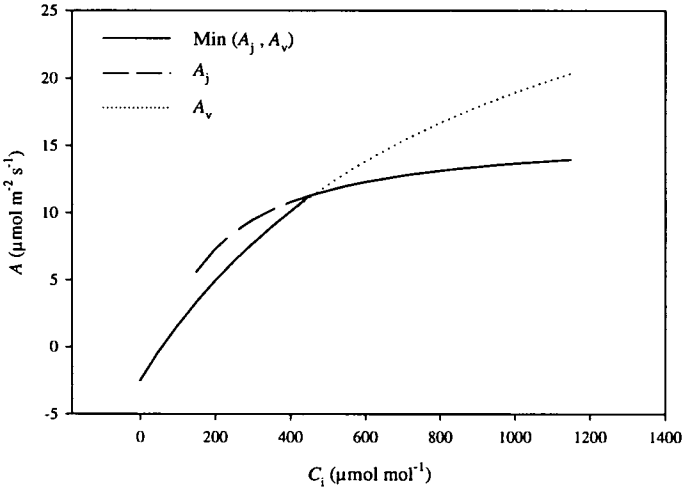


Figure 1.2 The relationship between the intercellular CO_2 concentration (C_i) and assimilation rate (A), known as the A/C_i curve (solid line). The graph illustrates A/C_i curve analysis showing A_j (photosynthesis limited by rate of light reactions) and A_v (photosynthesis limited by rate of carboxylation) and the combined minimum of these two curves, the A/C_i curve.

Limitation of carbohydrate synthesis by inorganic phosphate (P_i) also contributes to the plateau region of the A/C_i curve (Sharkey 1985; Harley *et al.* 1992) and may also be responsible for a part of the observed long-term acclimation of photosynthesis.

The shape of A/C_i curves may, therefore, be compared between CO_2 concentration treatments (Sage 1994) and provide quantitative assessment as to the extent of limitation by each of the three processes described above (von Caemmerer &

Farquhar 1981; Sharkey 1985). By analogy with the model for light acclimation (von Caemmerer & Farquhar 1981; Sharkey 1985; Harley & Sharkey 1991), it is inferred that acclimation to elevated CO_2 concentration will occur by a reduction in the investment of resources in Rubisco enzyme and an increase in investment in RuBP and P_i regeneration (Sage 1994). One recent review using A/C_i curves to estimate acclimation responses found that only a minority of species appear to have enhanced photosynthetic capacity in elevated CO_2 concentration and a consequent reduction of resource investment in excessive Rubisco capacity (Sage 1994). In a recent study of the response of five British species of contrasting functional type to elevated CO_2 concentration, only one species showed a significant difference in maximum carboxylation velocity (V_{cmax}), indicating acclimation (Stirling *et al.* 1997). However in another review of acclimation using A/C_i parameters to test acclimation it was concluded that most species did show an acclimation response (Gunderson & Wullschleger 1994). The authors conceded that acclimation was unlikely to be complete and that there would still be some net stimulation of photosynthesis in most species with exposure to elevated CO_2 concentration. Thus far, there are only a few published reports of A/C_i parameters in plants exposed to elevated concentrations of CO_2, but use of the technique is becoming more widespread so we expect to see more quantifiable acclimation investigations in the future.

Further evidence for acclimation of photosynthesis may be obtained from investigation of quantities of leaf chemicals. Most studies have reported increases in leaf starch (Overdieck 1990; Farrar & Williams 1991; Mousseau & Saugier 1992; Norby *et al.* 1996), decreases in leaf nutrient concentrations (Overdieck 1990; Mousseau & Saugier 1992; Norby *et al.* 1996) and often decreases in chlorophyll content (Wulff & Strain 1982; Oberbauer *et al.* 1985; Mousseau & Saugier 1992). The amount of N in a leaf correlates well with the amount of Rubisco, a decrease in which may indicate a shift in allocation of N towards enzymes in the RuBP

regeneration cycle (Evans 1989). Indeed, some studies using both A/C_i curves and measurements of quantities of photosynthetic enzymes have shown this to occur to some extent (Sage *et al.* 1989). An increase in foliar carbohydrate content relative to N has, in fact, been cited as the possible signal for feedback inhibition (Drake *et al.* 1997). It has been proposed that a build-up of a glucose-like substance could block the expression of the Rubisco protein (Sheen 1994; Koch 1996), effectively decreasing the investment in carboxylation.

Indirect evidence for acclimation may come from the study of plant respiration. In one study, in which respiration was partitioned between growth and maintenance components, it was found that the observed decrease in leaf respiration in elevated CO_2 concentration was largely a result of decreased maintenance respiration, corresponding with a decrease in leaf N (Wullschleger, Norby & Gunderson 1992). This presumably represents reduction in leaf Rubisco in response to the elevated CO_2 concentration.

Acclimation has also been reported for leaf anatomy and morphology (Thomas & Harvey 1983; Conroy *et al.* 1986; Radoglou & Jarvis 1990; Woodward & Kelly 1995), stomatal conductance (Tolley & Strain 1985; Hollinger 1987) and growth partitioning (Farrar & Williams 1991; Johnson *et al.* 1996; Norby *et al.* 1996) but these may be thought of as secondary effects in comparison with the fundamental effect of elevated atmospheric CO_2 concentration on photosynthesis.

Unfortunately, methodological limitations are correlated with the majority of observed acclimation responses (Ceulemans & Mousseau 1994; Gunderson & Wullschleger 1994; Sage 1994). Nutrient limitation and small pot size confound the establishment of true photosynthetic acclimation, the exact mechanism therefore remaining unclear.

Although less frequently studied than acclimation to elevated atmospheric CO_2 concentration, adaptation or "genotypic adjustment to prevailing environmental conditions" (Amthor 1995) will be at least as important in determining the behaviour of the forests of tomorrow.

Interaction of increased global temperature with elevated CO_2 concentration

Increases in photorespiration as a result of increased temperature are expected to be more than compensated for by increases in atmospheric CO_2 concentration at the individual plant scale (Long 1991). The combined effects of increasing temperature and increased CO_2 concentration in the atmosphere will have a proportionately larger influence on C_3 plants (Long 1991), increasing their competitive ability (Bazzaz *et al.* 1989). This is because C_4 and CAM plants already occupy hotter environments and minimise their photorespiratory losses so the suppression of the photosynthetic carbon oxidation pathway holds proportionally smaller advantage for these species. However, a reduction in the dark respiration of C_4 plants as a result of elevated CO_2 concentration may be of greater significance when a concomitant temperature increase occurs to the point of being limiting to growth in conjunction with low water availability (Drake *et al.* 1996b). In this case the elevated CO_2 may ameliorate the impact of the combined stress with significant impact on net ecosystem CO_2 exchange.

The exact effect of the interaction of elevated CO_2 concentration with increased temperature on whole ecosystems remains unresolved (Luxmoore *et al.* 1993). Whilst global warming may increase the length of growing seasons of ecosystems currently limited by temperature (Stirling *et al.* 1997) it may also give rise to substantial increases in the rates of microbial respiration and consequent soil CO_2

efflux, pushing the balance of carbon accumulation towards the atmosphere and away from the phytomass (Luxmoore *et al.* 1993). Beyond improving C_3 plants' competitive ability in mixed species situations, the combined effects of increased temperature and CO_2 concentration on ecosystem functioning remain unpredictable.

Growth

Increased photosynthesis in experiments using an elevated concentration of CO_2, leads to an average increase in tree growth of 32 % (Luxmoore *et al.* 1993; Wullschleger, Post & King 1995). The increase in growth is less than the average increase in C_3 photosynthesis for two main reasons. First, there are substantial respiratory costs associated with an increase in photosynthesis (Lloyd & Farquhar 1996). Second, the ability of a plant to translate increased photosynthesis into increased biomass appears to depend on its ability to increase its sink strength (Arp 1991; Farrar & Williams 1991; Mousseau *et al.* 1996). Plant sinks for photo-assimilate are those organs which either use a significant quantity of carbohydrate, such as rapidly growing shoot tips or flowers, or, store carbohydrate, such as roots. The best evidence for this comes from a comparison of soil-grown trees with plants kept in pots where the pot-restriction of root growth (roots being the strongest potential sink) has resulted in a correspondingly small increment in biomass despite increased photosynthesis (Mousseau *et al.* 1996). The soil-grown plants, however, showed a sustained increase in both photosynthesis and biomass increment.

There are numerous reports of changes in the partitioning of the biomass of herbaceous plants (reviewed by Farrar & Williams 1991) with elevated CO_2 concentration, but there is no consistent change in the direction of the root:shoot mass ratio, except for under conditions of nutrient limitation (Eamus & Jarvis 1989). It is now thought that it is both nutrient and sink growth limitation in pots that reduce

the potential increases in biomass of plants grown in elevated CO_2 concentrations. Other evidence suggests that the relationship is complex and factors such as pot shape may even play a role (McConnaughay, Berntson & Bazzaz 1993). Tree species have shown, on average, an equal increase in biomass of leaves, stems and roots where sink-growth was not limited (Wullschleger *et al.* 1995).

Plant nutrition

Sub-optimal nutrition is the most often cited reason for a reduced response to elevated CO_2 concentration (Brown & Higginbotham 1986; Conroy *et al.* 1986). This conclusion should be treated cautiously for three reasons.

First, nutrient deficiency is usually defined as a deficit in the total amount of nitrogen supplied to the plants. Ingestad (1981) has proposed that the rate, rather than total amount, of nutrient supply is a more important determinant of relative growth rate (RGR). RGR is independent of external concentrations of nutrient but linearly related to the relative addition rate.

Initial studies of plant responses to elevated CO_2 concentration used small pots and inadequate nutrient regimes. The interaction between nutrient limitation and increase in atmospheric CO_2 concentration has seldom been investigated under the more relevant Ingestad regime of nutrition where the rate rather than the total amount of nutrient supply is carefully controlled (Eamus & Jarvis 1989). RGR is mediated by the net uptake rate which is proportional to the addition rate. Therefore, nutrients should be added in exponentially increasing amounts to allow optimal growth, which is exponential in the case of seedlings (Ingestad 1982). The key to a successful nutrient regime is a constant concentration of nutrient within the plant so that the plant is then in a steady state with respect to nutrition (Ingestad & Agren 1992). It is important that the proportions of each nutrient match that of a plant in optimal

conditions because an over-abundance of one will affect the uptake of the others (Ingestad 1982). Thus, when the rate of nutrient supply is lower than that required for optimal growth, there is a lag phase which allows acclimation to this nutrient regime, where deficiency symptoms may show. After this lag, nutrient-deficiency symptoms disappear and the plant continues to follow an exponential growth curve, but at a lower rate than where there is a higher rate of supply (McDonald 1989). Where one nutrient only is limiting, the plant will again reallocate its carbon, water and other nutrient resources in order to acclimate to the deficiency and the optimal relative growth rate will be achieved after this lag period but deficiency symptoms will persist (McDonald, Ericsson & Ingestad 1991). When a traditional concentration-based nutrient supply regime has been used, there is an excess of available nutrients at the start of the growth period but nutrients become deficient during the exponential growth phase. Therefore, even high initial concentrations of nutrient are likely to be inadequate in maintaining optimal growth (Ingestad & Agren 1992). This may also be responsible for some of the observed increases in root:shoot ratio (Pettersson, McDonald & Stadenberg 1993).

Second, investigations have usually focused on absolute growth response at the expense of relative growth response. This neglects the possibility of plants which are "nutrient-limited" becoming more efficient in their use of resources (Drake *et al.* 1997). The focus on either absolute or relative effects of increased atmospheric CO_2 concentration can greatly affect the perception of a species response to elevated CO_2 (Körner 1993). When the rate of biomass accumulation (production ratio, a relative measure) between nutrient treatments is investigated, plants growing under low nutrient conditions may even be more responsive than well nourished plants (Lloyd & Farquhar 1996).

Third, the bulk of the early work on plant responses to elevated CO_2 concentration was done on crop species which are intensively managed to minimise their growth limitation by the environment (Koch & Mooney 1996). These studies are useful as crop species are very sensitive to changes in the environment but natural ecosystems are, by and large, limited by nutrients, light and, in some cases, water (Hogan *et al.* 1991; Körner & Arnone 1992; Koch & Mooney 1996). In particular, it has been proposed that temperate forests are nitrogen limited whilst tropical forests are phosphorus limited (Vitousek & Sanford 1986).

In some cases nitrogen deficiency has merely served to increase nitrogen use efficiency (van Kraalingen 1990) and in others it has been shown that the degree of "sub-optimal" nutrition is more important than whether or not the plants are at optimal nutrition (Johnson & Ball 1996). Clearly, the interaction of nutrition with response to elevated CO_2 concentration is not yet fully understood.

Stomatal responses

Stomatal responses to elevated CO_2 concentration can, like photosynthetic responses, be evaluated both on a short and a long time scale. In general, instantaneous measurements of stomatal conductance show a decrease with exposure to elevated CO_2 concentration (Tolley & Strain 1985; Gunderson *et al.* 1993; Ceulemans & Mousseau 1994). This leads to increased instantaneous transpiration efficiency (ITE) or increased water use efficiency (WUE) (Eamus 1991). Because WUE is defined as the ratio between the instantaneous rates of photosynthesis and transpiration (Ceulemans & Mousseau 1994), and photosynthesis is generally increased with exposure to elevated CO_2 concentration, it is possible to have an increased WUE even if the transpiration of an individual plant is decreased (Acock 1990).

There is little evidence at present to support a longer-term acclimation response of stomata to the increase in atmospheric CO_2 concentration (Eamus & Jarvis 1989; Eamus 1991) although stomatal densities are often decreased. In 74 % of cases reviewed by Woodward & Kelly (1995), stomatal density was decreased in elevated CO_2 concentration. The average decrease in density was 14.3 %. In addition, the decrease in stomatal conductance seems to persist long-term although the exact mechanism of CO_2 control of stomata remains unresolved (Mott 1990). The mechanism is likely to involve the C_i/C_a (intercellular/atmospheric CO_2 concentration) balance, a shift in this ratio potentially indicating long term acclimation of stomata to elevated CO_2 concentration (Sage 1994). So far there has been no consistent pattern of C_i/C_a alteration except for in the case of water or humidity stress where the ratio declined in plants grown in elevated CO_2 concentration, further supporting the idea of increased impact in droughted conditions (Sage 1994). Further investigation of transpirational water loss, sap flow and cavitation in whole trees is advisable to improve estimations of water use efficiency at the stand scale (Ceulemans & Mousseau 1994).

Respiration

Reports of the effects of elevated CO_2 concentration on plant respiration are varied. The most important effect of an increase in elevated CO_2 concentration is, arguably, its suppression of the photosynthetic carbon oxidation pathway (PCO, Drake *et al.* 1997). Photorespiration, the Rubisco-catalysed oxygenation reaction which produces CO_2 in competition to carbon assimilation, is decreased by 10 to 30 % on average (Amthor 1995).

Catabolic respiration, however, or the process of carbohydrate breakdown to provide energy for growth and maintenance, has been reported both to increase (Poorter, Pot

& Lambers 1988; Nijs, Impens & Behaeghe 1989) (roots only in the latter study) and to decrease (Bunce 1992; Wullschleger *et al.* 1992). It is to be expected that at least transient increases in growth respiration must occur where there is additional new growth in the plant (Farrar & Williams 1991; Amthor 1995). The majority of studies, however, report an overall decrease in respiration (Ceulemans & Mousseau 1994) even if this decrease is not maintained over the entire growing season (El Kohen, Pontailler & Mousseau 1991). The average instantaneous decrease in dark respiration is about 20 % for a doubling of the atmospheric CO_2 concentration (Drake *et al.* 1997). The long-term effect is less easy to predict and the mechanism for a potential acclimation of respiration remains unclear (Mousseau & Saugier 1992).

Short-term (reversible) and long-term (acclimation) effects of elevated CO_2 concentration on dark respiration have been ascribed as direct and indirect effects respectively. Direct effects on respiration include instantaneous changes in respiratory enzyme functioning with elevated CO_2 concentration whereas indirect effects are observed at a later stage as a result of other processes such as plant growth (Amthor 1991). A mechanism for the direct effect of elevated CO_2 concentration on respiration has recently been proposed (Drake *et al.* 1997). Two of the mitochondrial electron transport enzymes, cytochrome *c* oxidase and succinate dehydrogenase, are directly inhibited by elevated concentrations of CO_2 therefore inhibiting the oxidation of the substrates used by these enzymes (Gonzàlez-Meler *et al.* 1996).

Because the amount of foliar respiration is proportional to the amount of energy-consuming proteins such as Rubisco and other enzymes present (Bouma *et al.* 1994), studies showing a longer-term decrease in the amount of leaf Rubisco (Sage *et al.* 1989; Wullschleger *et al.* 1992) suggest that leaf respiration should also decline. However, when respiration is expressed as a proportion of shoot or leaf mass there is

no long-term difference between ambient and elevated CO_2 concentrations (Drake *et al.* 1997).

Foliar anatomical and morphological responses

Some changes in leaf anatomy and morphology have been noted with increased atmospheric CO_2 concentration. An increase in dry mass to leaf area ratio is commonly observed (Gunderson & Wullschleger 1994) but this may be the result of at least two entirely different processes. First, starch accumulation will usually result in an increase in specific leaf mass (Farrar & Williams 1991). Second, leaf thickness, usually inferred from measurements of specific leaf area (SLA), is commonly increased as a result of increases in either the mesophyll cell area and intercellular space (Radoglou & Jarvis 1990) or an additional cell layer (Thomas & Harvey 1983; Mousseau & Enoch 1989). Increases in leaf number and area have also been reported (Tolley & Strain 1985; Brown & Higginbotham 1986; Koch *et al.* 1986) but these are possibly merely a consequence of accelerated ontogeny rather than a specific increased CO_2 concentration response (Berryman, *et al.* 1993).

Whole-plant morphological and phenological responses

Some authors suggest that it is the growth strategy of an individual species which drives and constrains the response to elevated CO_2 concentration (Bazzaz 1990; Bazzaz & Miao 1993; Körner 1993; Mousseau *et al.* 1996). Indeed it has been shown that indeterminate species, such as beech, have a larger capacity to respond to changes in CO_2 concentration because their growth in a particular season is not predetermined by that of the previous growing season (Stitt & Schulze 1994; Lee & Jarvis 1995; Pinter *et al.* 1996). In addition, degree of maturity affects responsiveness, with seedlings showing the largest response capacity (Lee & Jarvis

1995). Changes in growth allocations (Mortensen & Sandvik 1987; Farrar & Williams 1991; Mousseau *et al.* 1996) and branching patterns (Sionit *et al.* 1984; Sionit & Kramer 1986) have been reported and these have a large potential to affect the plant's ongoing capacity to respond. For example, in an experiment which compared the growth responses of beech (*Fagus sylvatica*) and sweet chestnut (*Castanea sativa*), it was concluded that the larger capacity of beech to maintain new sinks led to the absence of down-regulation of photosynthetic/growth response in comparison with chestnut (Mousseau *et al.* 1996).

Phenological changes have also been reported for a number of plants. It seems that a hastening of ontogeny, particularly in seedlings, is common (Berryman *et al.* 1993), suggesting a potentially earlier arrival at maximum photosynthetic efficiency. However, delays in bud burst (Lee *et al.* 1993) and hastened onset of leaf senescence (Mousseau & Enoch 1989) may lead to a reduction in the growing season of temperate tree species (Murray *et al.* 1994). Sink development in general, such as the production of fruit and flowers, seems to be improved in elevated CO_2 concentration (Ceulemans & Mousseau 1994; Idso & Kimball 1997), an earlier onset of which has serious implications for mixed species communities (Bazzaz 1990). This will be especially important in communities where pollination is dependent on animals or when the growing season is short.

Below-ground processes

Increases in root:shoot mass ratios have been observed in a wide variety of herbaceous species with exposure to elevated CO_2 concentration (Farrar & Williams 1991), but a more detailed examination of below-ground response is required (Rogers *et al.* 1994). An increase in fine root production has often been reported in tree species (Norby *et al.* 1992; Johnson *et al.* 1996) and this could be translated into

more rhizodeposition (Rogers *et al.* 1994), which may already account for as much as 40 % of photo-assimilate (Paterson *et al.* 1997). These effects significantly increase ability to "mine" soil nitrogen in times of high nitrogen demand and are therefore vital in plant response, particularly at the ecosystem scale (Johnson & Ball 1996).

It is thought that the biggest impact of elevated atmospheric CO_2 concentration on below-ground processes will be mediated by the change in litter quality (Paterson *et al.* 1997). For example, in birch it is estimated that an increased C:N ratio of leaf litter would decrease soil mineralisation but increase relative uptake of microbial N (Berntson & Bazzaz 1997). This would change soil organic matter and possibly also the microbial succession in the soil (Paterson *et al.* 1997).

There has been a great deal of speculation on the possible increase in carbon sequestration in tropical and subtropical soils as a foil to an increasing atmospheric CO_2 concentration (Batjes & Sombroek 1997). However, unless a substantial change in management policy occurs to increase the total area of forested surface it seems unlikely that the amount of carbon stored in soils will increase solely as a result of increased root growth. Another proposed mechanism for counter-balancing the elevated CO_2 concentration is an increase in resource-use efficiency of natural systems. This would effectively increase the amount of energy input to an ecosystem stimulating soil microbial processes, increasing nutrient turnover and ultimately leading to accumulation of soil carbon (Drake *et al.* 1996b).

Species/stand interactions

Obviously an increase in competitive ability of C_3 plants has the potential to alter greatly species balance in some ecosystems (Bazzaz *et al.* 1989). However, inherent differences in species' response to elevated CO_2 concentration within C_3 plants may

be equally dramatic (Körner 1993). It is possibly the occurrence of increased WUE that will have the biggest effect on the relative dominance of species (Strain & Cure 1985; Acock 1990; Hogan *et al.* 1991). In addition, weedy species will probably prosper as they are potentially highly plastic in their response (Patterson & Flint 1990).

Some studies have shown that plants of different successional status differ in their capacity to respond to elevated CO_2 concentration, particularly with other environmental limitations. For example, Bazzaz and Miao (1993) studied six deciduous forest trees of varying shade tolerance and found that the response of early successional species to elevated CO_2 concentration was strongly constrained by nutrient supply, whereas the late successional species were constrained more strongly by light.

Natural systems will change in species composition, the dominant factor being morphotype (Körner 1993) but as yet there is no clear way to "pick winners" (Bazzaz *et al.* 1985). Increased usage of FACE experiments will increase the potential for investigation of ecosystem interactions *in situ*, such as the experiments in grasslands (Blum 1993; Miglietta *et al.* 1997) and the current pine forest stand experiment (Ellsworth *et al.* 1995).

Interactions with other environmental variables

As discussed earlier, the interaction of atmospheric CO_2 concentration with soil nutrient status is thought to be one of the key determinants of plant response to elevated CO_2 concentration. What of other constraints such as light and water?

There is some evidence to suggest that the stimulatory effect of elevated CO_2 concentration is larger at low photosynthetic photon flux density (PPFD) thus having a larger relative effect on the growth enhancement of shade leaves (Idso *et al.* 1994). This is possibly because of a shift in the balance of nitrogen partitioning with relatively more N being partitioned to thylakoid proteins in low PPFD to improve the processes of electron transport and RuBP regeneration (Evans 1989). In addition, it is thought that the response of tree seedlings to light is a species-dependent phenomenon, as mentioned above (Bazzaz & Miao 1993). Indeed one study found a greater stimulation of photosynthesis by increased CO_2 concentration in conditions of low PPFD in one species but the opposite result in another (Tolley & Strain 1984).

Because light-use efficiency generally increases with CO_2 concentration, and forest species near to the forest floor operate close to the light compensation point, a small increase in light-use efficiency from a reduction in the competing photorespiration reaction has the potential to greatly increase photosynthesis in these species (Drake *et al.* 1997). Even taller sub-canopy species are light limited for part of the day and an increase will probably occur here also.

Decreased stomatal conductance conserves soil water and may therefore have a larger response on plants growing in water-limited environments than those in environments where water is not limiting (Tolley & Strain 1984; Strain & Cure 1985; Conroy *et al.* 1986). One study investigated the response of plants to the atmospheric CO_2 concentration range from the last glacial maximum to the present time and concluded that water use efficiency has increased and has altered species abundance (Polley *et al.* 1993). The effect of elevated CO_2 concentration is potentially larger where an increase in WUE is sufficient to offset the commonly observed increase in leaf area index (LAI) with exposure to elevated CO_2 concentration (Eamus & Jarvis 1989). A recent survey concluded that LAI, a measure of leaf area per unit of ground

area, is not significantly increased with exposure to elevated CO_2 concentration (Drake *et al.* 1997). If there is a concomitant increase in global temperature WUE may become even more important, depending on how soil moisture is affected (Eamus 1991). An increase in temperature is expected to increase soil microbial respiration and consequent root growth as a result of increased decomposition and nutrient availability (Luxmoore *et al.* 1993). Therefore it should also be considered that an increased area of rooting system increases water consumption making even plant scale predictions about WUE very difficult (Strain & Cure 1985; Eamus 1991). In addition, increased temperature increases leaf to air vapour pressure deficit, another potential method of decreasing water use efficiency (Nijs *et al.* 1997).

Historical information

In an attempt to get a better perspective on ecosystem changes with time we can use some information on plant response to existing increases in atmospheric CO_2 concentration. Tree ring data are conflicting (Wullschleger *et al.* 1995) but do seem to indicate an overall increase in plant growth since industrialisation (Luxmoore *et al.* 1993). Although allowances have already been made for variation in temperature and precipitation (LaMarche *et al.* 1984), the coincidence of post-industrialisation N-deposition with elevated CO_2 concentration increase make positive responses in tree ring width difficult to interpret (Körner 1993). There is, however, supplementary evidence from ecosystem flux measurements for an increased sink capacity in re-growing northern hemisphere forests (IPCC 1995). Even after accounting for the effects of N-deposition there appears to be an effect of increased CO_2 concentration as well. Annual growth rings are not formed in tropical rain forest species (Esau 1965), eliminating the use of this technique to investigate past responses of the tropical rain forest to the increase in atmospheric CO_2 concentration.

Results from natural CO_2 sources provide an almost model system for the study of long term responses. When trees grown beside CO_2 - emitting springs for 30 years in Italy were compared with nearby control trees an overall 12 % increase in tree radial width was observed (Hättenschwiler *et al.* 1997). Upon examination of the tree ring chronologies from this site it was observed that growth stimulation could be largely attributed to responses when the trees were young. There was no difference in annual ring width by 25 to 30 years of age. Contamination with H_2S was considered to be minimal at the sites.

Information may also be gained from the study of autotroph response to changes in CO_2 concentration over a geological time span. In general, adaptations have involved either a change in Rubisco kinetics or an adoption of methods to concentrate CO_2 internally. Elevated CO_2 concentration environments are associated with an increased specific activity of Rubisco which, in turn, is decreased in its concentration and its affinity for CO_2 (Bowes 1991). This information correlates well with current investigations of Rubisco kinetics in plants grown in elevated CO_2 concentration conditions (Stitt 1991; Sage 1994).

THE SPECIAL CASE OF TROPICAL TREES

Tropical rain forest is renowned for its structural complexity and great diversity. It is characterised by constantly high temperatures and rainfall and has the largest number of co-existing plant and animal species in any terrestrial ecosystem (Whitmore 1990). There are still many gaps in our knowledge of the structure and function of tropical forest (Mooney *et al.* 1980), particularly in the area of root function and acquisition of soil nutrients (Tans *et al.* 1990).

The Amazon basin contains the largest continuous area of tropical rain forest in the world, most of this lying in Brazil. The forest is heterogeneous and may be further subdivided into several life zones on the basis of climate and geography (Raven *et al.* 1986). Field work for this thesis was completed in an area of mixed-species lowland *terra firme* rain forest. This type of forest covers 60 - 70 % of the Amazon (Pires 1978) and grows on deeply weathered, nutrient-poor soils, usually oxisols or ultisols (Herrera 1985).

Above-ground processes

Tropical forests differ from temperate forests in community structure and nutrient cycling dynamics (Uhl & Jordan 1984; Vitousek & Sanford 1986; Lugo & Brown 1990). Canopy structure is more complex, a major difference being the presence of tropical lianas (Croat 1978) whose physiology differs substantially from that of tropical trees (Zotz & Winter 1996). Above-ground processes are clearly fundamental to our understanding of tropical ecosystem carbon balance. Gas exchanges of forest trees are well coupled to the atmosphere (Jarvis 1985), although evidence suggests that coupling is poorer in tropical rain forests when compared with temperate forests (Kruijt *et al.* 1996). In addition, it appears that plants growing at 1 m or less from the ground are subjected to naturally elevated concentrations of CO_2 for most of the day (Medina *et al.* 1986).

Tropical forests have a higher density of foliage which has a greater uniformity in its vertical distribution than in temperate forests (Shuttleworth 1989). Therefore, a lower percentage of incoming radiation is thought to penetrate the canopy to the ground level. The albedo, or reflection coefficient for solar radiation (Jones 1992), is reported to be similar to that for temperate evergreen, rather than deciduous, forests (Shuttleworth 1989).

The effects of seasonal changes in rainfall differ according to species and forest type. In some species of evergreen tropical trees it has been shown that the water status is little affected by seasonal variation in rainfall (Zotz & Winter 1994) whereas in dry tropical forests water shortage during the dry season may greatly affect daily carbon gain (Lugo *et al.* 1978). Leaf scale studies have shown mid-day reductions in stomatal conductance and assimilated carbon on days when leaf temperatures reach 35 °C or higher and leaf to air vapour pressure differences increase above 40 mPa Pa^{-1} (Zotz & Winter 1996). This has been corroborated by studies of net carbon exchange in tropical *terra firme* forest which show a decrease in ecosystem productivity during the dry season (Malhi *et al.* 1997; Williams *et al.* 1997).

If we are to improve our understanding of carbon physiology in tropical forest we will need to increase both our collection of empirical data and its integration into models of ecosystem process. The new technology of eddy covariance allows measurement of large scale carbon flux (Grace *et al.* 1995b) but the component parts of this flux still require further examination. Much information can be gained from leaf scale studies. One such study has shown a very consistent relationship between the maximum rate of photosynthesis, A_{max}, and 24 h C gain, $A_{24\ h}$, of tropical tree leaves *in situ* (Zotz & Winter 1993), the relationship being described by the equation:

$$A_{24\ h} = 21.2A_{max} - 2.1\ (r^2 = 0.92)$$

These authors suggest that the tightness of this relationship is the result of a low photosynthetic photon flux density (PPFD) saturation point of most canopy leaves allowing light saturation at 50 % of maximum PPFD and consequently reducing the effect of variation in PPFD. They also report that the average length of the photoperiod affects the slope of the $A_{max}/A_{24\ h}$ relationship, therefore resulting in a

different relationship in temperate trees, where the day length may be significantly longer during the growing season.

Much attention has been focused on the close relationship between carbon and nitrogen content of tissues (Tateno & Chapin 1997) as a potential shortcut to predicting photosynthetic capacity of individual species or canopy layers in forest systems. It has been shown that strong vertical gradients of stomatal conductance and maximum photosynthetic rate exist in canopies of Amazonian forest (Roberts, Cabral & Ferreira de Aguiar 1990; McWilliam *et al.* 1996). This is most commonly linked to the concentration of foliar nitrogen (Field & Mooney 1986; Hirose & Werger 1987) as it is thought that tree species have the ability to reallocate nitrogen resources within a vertical profile in order to maximise photosynthetic capacity at the sites of maximum PPFD, that is, near the top of the canopy (Field 1983; Pons & Bergkotte 1996). This "optimisation" however is rarely perfect (Hirose & Werger 1987; Terashima & Hikosaka 1995) suggesting that the mechanism of photosynthetic acclimation to light environment is not yet fully understood. In fact, in one *in situ* study of Amazonian tree species, spread across a wide variety of light environments, there was considerable variation in the relationship between nitrogen content and A_{max} between species (Reich *et al.* 1994, Reich & Walters 1994). Clearly, there is a need for further leaf scale measurements to enhance our descriptions of tropical forest ecosystem functioning.

There is a paucity of data on responses of tropical species to elevated CO_2 concentration. From the small amount of existing literature it would appear that, as with plants from other terrestrial systems, tropical tree species have the potential to increase photosynthesis, growth and water use efficiency as a result of increased atmospheric CO_2 concentration (Hogan *et al.* 1991; Berryman *et al.* 1994). Oberbauer *et al.* (1985) investigated the responses of two tropical species each from a

different ecological niche. Both showed increases in biomass, particularly the pioneer species *Ochroma lagopus*. A reduced photosynthetic rate was also reported for both species in elevated CO_2 concentration. This paper has been criticised on the basis of small pot size and consequent sink restriction (Ziska *et al.* 1991). Presumably the observed reduction in photosynthetic rates is an "acclimation" response as photosynthesis must have been higher in plants grown in elevated CO_2 concentration at the start of the season or else there would be no difference in overall growth.

Reekie and Bazzaz (1989) also reported an absence of increased photosynthesis in seedlings of five tropical tree species grown in elevated CO_2 concentration. Again small pots (0.67 dm^3) were used and no attempts were made to ensure that the nutrient regime was sufficient. Ziska *et al.* (1991) experimented on nine tropical species, including representatives of each of the C_3/C_4/CAM photosynthetic pathways in both ambient tropical conditions and pots of large size (12.5 dm^3). In this scenario, photosynthesis was increased in all five C_3 species and growth was increased in four out of the five grown in elevated CO_2 concentration.

Below-ground processes

Our understanding of the below-ground processes of tropical forest remains inadequate (Sedjo 1988) despite their key importance in the construction of a global C balance sheet (Mulkey, Chazdon & Smith 1996). Nutrient cycling is particularly strongly coupled to forest dynamics in tropical ecosystems, with biomass partitioning reflecting the need to conserve nutrients (Jordan 1989). For example, the majority of roots lie in the top 0.1 - 0.3 m of the soil, coincident with the maximum concentrations of mineral nutrients (Whitmore 1990). Amazonian *terra firme* forest has a root mat of an average thickness of 20 cm on the surface of the mineral soil

(Sanford 1987) with about 50 % of total fine root mass lying in the uppermost 10 cm of mineral soil (Sanford & Cuevas 1996). Nutrients are reported to be fairly evenly distributed between above- and below-ground parts of the ecosystem for most tropical forests, with nutrient addition, both in solution and as an aerosol, occurring during rain (Whitmore 1990).

A key feature of the debate on probable forest response to elevated CO_2 concentration is the effect of nutrient limitation on responses to elevated CO_2 concentration. It is crucial that nutrient dynamics of tropical forests are assessed in their own right, as there is much evidence to suggest that, unlike temperate forests where nitrogen is the key limiting nutrient, phosphorus is more important in its effect on growth limitation of tropical ecosystems (Vitousek & Sanford 1986). This may have major impacts on the system's capacity to respond to increases in atmospheric CO_2 concentration because deficiency of phosphorus may impose a more crucial limit to Rubisco activation and RuBP regeneration than nitrogen. Because the allocation pattern of nitrogen appears more flexible, the emphasis of control would be shifted from nitrogen to phosphorus availability (Bowes 1991) in tropical systems.

Ecosystem processes

The introduction of eddy covariance techniques for the measurement of tropical canopy/atmosphere interaction has advanced the scope of ecophysiological research at the ecosystem scale significantly (Shuttleworth 1988; Fan *et al.* 1990; Grace *et al.* 1995a; Grace *et al.* 1995b, Lloyd *et al.* 1995b). Measurement of vertical profiles of CO_2 flux have characterised the diurnal cycle of CO_2 uptake in the Amazon forest (Wofsy, Harriss & Kaplan 1988). This study showed CO_2 uptake during the day and build up during the night. The CO_2 which builds up overnight is then flushed out into the atmosphere early in the morning when the air becomes more turbulent.

Further investigations of carbon flux in an undisturbed rain forest in Rondônia, Brazil have yielded net uptake of CO_2 over a 44-day period (Grace *et al.* 1995a). When combined with leaf scale data it was possible to produce a simple model of rain forest productivity which explained canopy CO_2 uptake in terms of basic physiological processes and therefore allowed some prediction of forest response to increases in atmospheric CO_2 concentration (Lloyd *et al.* 1995b). Flux measurements are being repeated in another region of Amazonian rain forest (Malhi *et al.* 1997) and integrated with canopy models to show evidence of seasonality in CO_2 uptake (Williams *et al.* 1997). The forest was shown still to be a net sink for carbon but this capacity was reduced towards the end of the dry season, probably as a result of reduced stomatal conductance and consequent photosynthetic limitation (Malhi *et al.* 1997; Williams *et al.* 1997).

Measurements of net ecosystem flux may be coupled with carbon isotope analyses to give a dynamic view of carbon uptake and assimilation by the forest as a whole. Analysis of carbon isotope contents of leaf samples through a vertical profile of the canopy provides a method for integrating flux measurements with physiological measurements and this approach has been used successfully to identify sources and sinks of carbon within Amazonian forest (Kruijt *et al.* 1996). These data showed the importance of soil respiration as a source of CO_2 which is also re-used by leaves in the lower levels of the canopy.

Isotope studies have also been used in the investigation of the relative importance of evaporation and transpiration in water recycling in Amazonia (Moreira *et al.* 1997). This is particularly important within the context of deforestation as these authors found that the forest ambient water vapour was almost entirely derived from transpiration of the forest, a change in which would dramatically alter the hydrological cycle in the region.

Körner and Arnone did the first study of direct relevance to tropical ecosystem response to elevated CO_2 concentration (Körner & Arnone 1992). They created a small artificial tropical ecosystem and discovered no significant increases in stand biomass, leaf area index or stomatal behaviour in elevated CO_2 concentration. Although this work does not exactly "mimic a natural situation" it is still the only published research on tropical ecosystem response to elevated CO_2 concentration. Further work of a similar nature is in progress at the Arizona Biosphere 2 site (Lin 1997). Other authors have suggested that the response of tropical ecosystems may be smaller than that of temperate and boreal forest, with a mean tree biomass increase of only 18 % compared with an overall mean tree response of 32 % when temperate and boreal forest trees were included (Wullschleger *et al.* 1995). Some bias will have occurred in this calculated estimate, however, if the figures were taken from the studies detailed above where a reduced sink capacity is likely to have induced acclimation.

Because of expected increases in water use efficiency it has been suggested that some tropical plants in particular may be able to extend their ranges into previously unfavourable sites putting the onus of selection back on interspecific competition (Hogan *et al.* 1991). However, a corresponding increase in temperature may well exacerbate existing nutrient deficiencies in tropical forest resulting in an overall decrease in tropical biomass (Goudriaan & Unsworth 1990). It seems likely that, in any event, there will be a significant increase in carbon passed to the soil (Körner & Arnone 1992; Koch & Mooney 1996). Clearly there is an urgent need for further research into tropical plant responses at all scales but particularly at the ecosystem scale (Hogan *et al.* 1991; Koch & Mooney 1996).

GLOBAL MODELS OF PLANT GROWTH IN RELATION TO CO_2 CONCENTRATION

Because of the relatively small time span of the predicted changes in atmospheric CO_2 concentrations, the longevity of trees and the complexity of plant responses, models which integrate the information of different experimenters are imperative (Eamus & Jarvis 1989; Körner 1993; Jarvis 1995; Reynolds *et al.* 1996). Scaling up from individual ecophysiological studies to ecosystem scale can in itself be an invaluable tool in the testing of hypotheses and identifying missing components of interpretations (Field & Ehleringer 1993; Reynolds *et al.* 1996). With respect to the interaction of rising CO_2 concentration and terrestrial ecosystems, models have been used both at the plant scale, to predict stand responses to increased CO_2 concentration (McMurtrie & Wang 1993), and at the global scale in investigation of planet carbon balance (Tans *et al.* 1990).

Different models emphasise different aspects of the problem (Reynolds *et al.* 1996). Several models such as MAESTRO, BIOMASS or G'DAY have been used to simulate canopy response to an instantaneous doubling in atmospheric CO_2 concentration (C_a). MAESTRO (Wang & Jarvis 1990) and BIOMASS (McMurtrie, Rook & Kelliher 1990) are physiology-based models of canopy processes whereas G'DAY is a soil-plant model (McMurtrie *et al.* 1992). A model such as MAESTRO which is sufficiently complex to use physiological information provided on a number of scales is a valuable scaling tool for integrating responses of different sized and aged trees to elevated CO_2 concentration (Lee & Jarvis 1996).

Information from models predicting stand response to climate change may then be validated with measurements of CO_2 and H_2O exchange above existing forest canopies using state-of-the-art eddy covariance techniques (Lee & Jarvis 1996). The

information obtained from these *in situ* ecosystem measurements has already been applied to estimates of Amazon basin productivity (Lloyd *et al.* 1995b) as discussed earlier. The future of ecosystem prediction will look bright when we are confident with an estimate of current Amazon basin carbon sink capacity, for example, of 8.5 ($\pm$ 2) mol m^{-2} yr^{-1} (Grace *et al.* 1995a), and can combine this with a physiology-obtained estimate for plant response to elevated CO_2 concentration in which we are equally confident. For example, one model is predicting a 27 % initial increase in the productivity of a *Pinus radiata* stand, reducing to an 8 % stimulation as a sustained response (McMurtrie *et al.* 1992). If we had a similar estimate of the response of tropical forest to elevated CO_2 concentration, we could put a figure on potential increase in sink capacity in this region of the world.

Meanwhile, lack of data on both positive and negative feedbacks from the biosphere on elevated atmospheric CO_2 concentration is limiting the application of models to future predictions (Schimel 1995). Schimel's review concluded that those responses observed in the laboratory are likely to be a lot larger than those actually occurring in forest ecosystems. With respect to global carbon balance, there still exists considerable uncertainty in estimation of the exact value of global sinks and sources (Jarvis & Dewar 1993; Dixon *et al.* 1994; IPCC 1995; Schimel 1995), making a good estimate of future global carbon balance unlikely in the near future. However, model predictions are improving all the time and there is much need to integrate results from experimental manipulation of the CO_2 levels with *in situ* canopy physiology response of tree species, particularly in the tropical rain forest.

NOTES ON THE SPECIES USED

Seedlings of tropical trees from two different ecological niches were selected for investigation. *C. odorata* is a shade-intolerant pioneer and *S. macrostachya* is a fast-

growing canopy emergent which thrives both in shade and full sunlight. *C. odorata* is a feature of central and South American rain forests whereas *S. macrostachya* originates from Australian monsoon vine thickets.

Cedrela odorata

The genus *Cedrela* was first named by Linnaeus in 1759 (Keay 1996). It is a member of the Meliaceae family which is found throughout the tropics (Pennington 1981). *C. odorata* is an economically important timber crop in the neotropics (Longman & Jeník 1974; Newton *et al.* 1993) and is considered a fast growing pioneer species (Pennington & Styles 1975). The trees are deciduous, producing new flushes about once a year (Grijpma 1974 in Newton *et al.* 1993). In natural conditions *C. odorata* performs best on freely draining soils or ridge tops and is intolerant of poor drainage and compacted soils (Newton *et al.* 1993). There is a considerable variation in height growth with provenance.

Schefflera macrostachya

Schefflera is a large genus (200 species) within the Araliaceae which consists mainly of trees and shrubs from tropical regions (Mabberley 1987). Araliaceae contains ivy (*Hedera helix*) and ginseng and many of the species have been known to cause allergic contact dermatitis (Mitchell 1981). *S. macrostachya* is a commonly cultivated houseplant, with palmately compound leaves, which originated in Australia and is commonly known as the Queensland umbrella tree (Mabberley 1987). It is a fast-growing species, often multi-stemmed, which can grow both in the shade and in full sunlight.

In situ study

Little has been published about the ecology of specific Amazonian tree species. However, those measured in the canopy in Brazil were largely canopy emergent species. This includes *Jacaranda copaia*, *Goupia glabra*, *Helicostilis sp.* and *Inga sp.* *Memora sp.* is a liana and *Oenocarpus sp.* is an arborescent palm.

OUTLINE OF THIS THESIS

In **Chapter Two**, data are presented for changes in *C. odorata* and *S. macrostachya* biomass and allocation of biomass after exposure to elevated CO_2 concentration. In addition, morphology of *C. odorata* is examined for any change in ontogeny. Partitioning of nutrients (N, P, K, Ca, Mg) is examined to investigate changes in foliar biochemistry.

In *C. odorata*, leaves are investigated at both the anatomical and phytochemical scales for further indication of changes in leaf biochemistry. Both cross sections of leaves and stomatal densities are investigated using scanning electron microscopy and light microscopy respectively. An investigation of leaf chlorophyll and carbohydrate concentrations are presented.

In **Chapter Three**, instantaneous measurements of photosynthesis are presented along with longer term responses. Foliar responses to changes in internal CO_2 concentration are investigated and presented in the form of A/C_i curves. For this analysis the Farquhar *et al.* (1980) model is fitted and values are presented for mathematical parameters describing the leaf response to elevated CO_2 concentration. These are compared between elevated and ambient CO_2 concentration treatments to

test for any long-term modification in photosynthetic efficiency (acclimation). Changes in stomatal conductance are examined briefly.

Measurements made in a whole-plant gas exchange chamber are presented for individual plants contained for 24 hour periods. These are compared between elevated and ambient CO_2 concentration treatments to provide carbon flux budgets for each treatment. Light responses at each growth concentration are also investigated.

In **Chapter Four**, changes in photosynthesis over a 24 hour period are presented for individual canopy levels of a Brazilian rain forest. These are analysed in terms of the variables driving ecosystem processes such as light and humidity. Vertical profiles of photosynthetic capacity are presented using the same technique for A/C_i curve fitting as in the glasshouse-grown *C. odorata*. In addition, vertical profiles of foliar nitrogen concentration are presented to examine the correlation of photosynthetic capacity with Rubisco content, indicated by leaf nitrogen concentration. These are discussed in the context of there being one driving variable, namely light, for which photosynthetic activity is maximised in its distribution through the canopy.

Finally, in **Chapter Five**, suggestions are given for the integration of experimental results from studies of plant responses to elevated CO_2 concentration with *in situ* investigations of leaf physiology. Steps are suggested for how to scale-up predictions of tropical tree response to elevated CO_2 concentration to the ecosystem level. These are presented together with recommendations for future research.

Chapter Two:
Effects of an elevated concentration of atmospheric CO_2 on the growth of two species of tropical trees in their seedling stage: *Cedrela odorata* L. and *Schefflera macrostachya* Harms.

INTRODUCTION

The expected increase in atmospheric carbon dioxide to 500 μmol mol^{-1} by the year 2100 (IPCC 1995) has prompted intensive investigation of possible plant responses to the proposed "CO_2 fertilisation effect". Increased atmospheric CO_2 concentration will likely enhance photosynthesis and predictions of consequent average increases in biomass accumulation range from 32 % for tree species (Luxmoore *et al.* 1993; Wullschleger *et al.* 1995) to 58 % for herbaceous crop species (Poorter 1993). Tropical forest comprises 59 % of global forest vegetation (Dixon *et al.* 1994), yet relatively few authors have studied the responses of tropical tree species to elevated CO_2 concentration (Oberbauer *et al.* 1985; Reekie & Bazzaz 1989; Hogan *et al.* 1991; Ziska *et al.* 1991; Körner & Arnone 1992).

In this chapter I investigate the response of two tropical tree species, *Cedrela odorata* L. and *Schefflera machrostachya* Harms. to elevated atmospheric CO_2 concentration. As there is little existing information on tropical species' responses to elevated CO_2 concentration, this study was designed to fill some of the gaps in the existing data base and also to resolve some of the conflicts reported by other studies. In addition, it is proposed that such detail as reported here is required for modelling forest response to elevated concentration (McMurtrie *et al.* 1992). It is envisaged that the information from the current study will eventually be used in such an investigation.

Data from studies of tropical tree species are conflicting as some authors report little or no increase in photosynthesis with elevated CO_2 concentration (Oberbauer *et al.* 1985; Reekie & Bazzaz 1989) whereas Ziska *et al.* (1991) found sizeable increases both in photosynthesis and growth of all four tropical tree species measured. It seems likely that root restriction resulting from the use of small pots constrained the growth response in the first two experiments (Ziska *et al.* 1991) and that tropical tree species are just as likely as other C_3 plants to increase photosynthesis, growth and water use efficiency as a result of increased atmospheric CO_2 concentration (Hogan *et al.* 1991; Berryman *et al.* 1994). It was hypothesised that the growth of the two tropical tree species investigated in this study, *C. odorata* and *S. macrostachya*, would be stimulated by an increase in elevated CO_2 concentration. This chapter provides data on the growth response of these species to elevated atmospheric CO_2 concentration, in an experiment with pots of a size assumed to ensure that root restriction did not occur.

Sub-optimal nutrition is thought to limit the extent of plant response to elevated CO_2 concentration although plants grown in these conditions do still show a response to the increase in CO_2 concentration (Pettersson & McDonald 1992; Radoglou & Jarvis 1992; Pettersson *et al.* 1993). Allocation between sources and sinks of carbon

compounds may be important (Farrar & Williams 1991; Stitt 1991; Long & Drake 1992). Experiments done with small pot volumes have been criticised because where roots are restricted, plant response to elevated CO_2 concentration may be inhibited through both a reduction in sink capacity (Thomas & Strain 1991) and a reduced ability to "mine" for nutrients (Sage 1994; Johnson *et al.* 1996). Provided that pot volume is adequate for "normal" rooting behaviour, the effects of variable sink strength may be overcome by ensuring stable plant nitrogen concentration (Pettersson & McDonald 1994). Where the Ingestad technique (Ingestad 1982) is employed it is possible to control the rate of nutrient addition such that plants are said to be in a "steady state" of nutrition even though this rate might be below that required for maximum growth (Ingestad & Agren 1992). It was hypothesised that the growth response of *C. odorata* would be reduced when nutrient supply was limiting. In the present experiment, nutrient addition rate was carefully controlled to allow elucidation of the response of *C. odorata* to the interaction of elevated atmospheric CO_2 concentration with nutrient limitation.

Reported plant responses to elevated CO_2 concentration vary widely, the extent of this variation probably depending on the range of research methodology. Differences between plants grown in ambient and elevated CO_2 concentrations have been observed within leaves as well as at the scale of whole-plant changes in biomass and allocation. Decreases in stomatal density (Woodward & Kelly 1995), foliar chlorophyll (DeLucia, Sasek & Strain 1985; Mousseau & Saugier 1992), nitrogen (Mousseau & Saugier 1992) and sucrose concentrations (Norby *et al.* 1996) have been recorded in open-top chamber (OTC) experiments along with increases in foliar starch concentration (DeLucia *et al.* 1985; Socias, Medrano & Sharkey 1993; Norby *et al.* 1996) and leaf thickness (Thomas & Harvey 1983; Mousseau & Enoch 1989; Radoglou & Jarvis 1990). These observations are common but not universal. It was hypothesised that there may be anatomical and biochemical leaf responses to elevated

atmospheric CO_2 concentration. Data are presented on foliar chlorophyll, starch, monosaccharide and oligosaccharide concentrations along with whole-plant analyses of nutrient concentrations.

In some species the stimulation of growth with elevated CO_2 concentration has been attributed to accelerated ontogeny (Berryman *et al.* 1993; Tissue, Thomas & Strain 1997). Where an ontogenetic shift involves a change in the timing of the development of one organ relative to another, this change may be reflected in the mathematical index relating the size of one organ, or part thereof, to another (Niklas 1994). It was hypothesised that a change in the ontogeny of leaves may be reflected in the allometric relationship between rachis length and total leaf area. This study investigated specifically whether there were any morphological or developmental changes in leaves of *C. odorata* in response to growth in elevated CO_2 concentration. In addition, it was hypothesised that there may be gross morphological or developmental changes in other organs, including shifts in the relative allocation between organs (Ackerson *et al.* 1984; McConnaughay *et al.* 1996; Mousseau *et al.* 1996). Whole-plant growth response of *C. odorata* and *S. macrostachya* was investigated accordingly.

METHODS

C. odorata seedlings grown during the Summer of 1995

Plant material and growth conditions

Seed from the Oxford Forestry Institute in Costa Rica of *C. odorata* provenances San Antonio and Teupasente was supplied by the Institute of Terrestrial Ecology (ITE). This was sown in sand, and seedlings were transferred to a "Perlite" (Silvaperl ™) growing medium in 1 dm^3 containers. The plants were of an average height of 21 mm at the time of planting in individual containers. 88 of these were then transferred to eight open-top chambers (OTCs) inside a glasshouse at the Royal Botanic Garden of Edinburgh (RBGE, 55 °57′ N, 3 °12 ′ W, elevation 40 m) where they were distributed evenly throughout all eight chambers using a split-plot randomised block design. The plants were carefully transferred, minimising root disturbance, to bigger pots (4 dm^3) after six weeks when at an average height of 70 mm. Each plant stood in its own saucer in which water and nutrient feed could accumulate. All plants were irrigated daily with tap water. Plants were rotated within a chamber every two weeks to minimise the effects of within-chamber gradients in temperature, light or CO_2 concentration.

Open-top chambers

Eight OTCs, four for exposure to ambient CO_2 concentration and four for exposure to elevated CO_2 concentration, were used. These were made from transparent polypropylene and each occupied a ground area of 1.3 m^2. CO_2 treatment was blocked so that the chambers in a line were alternately supplied with either an ambient or an elevated concentration of CO_2. This minimised the effect of any glasshouse gradient in either temperature or light between treatments. The glasshouse at RBGE was maintained at tropical temperatures (15 °C minimum night

temperature and maximum day temperature of 45 °C) and humidity (65 - 85 % relative humidity). Heat was supplied to the glasshouse in ducts at floor level from a central boiler in the RBGE grounds. Heat was usually only required at night. Ventilation during the day provided the only means of cooling. Floors and free-standing plants in the glasshouse were watered regularly to maintain a tropical humidity in the glasshouse. Air at tropical temperature and humidity was blown into the OTCs from inside the glasshouse. It was then extracted from the chambers using a centrifugal fan (Turbo SDX 4S, Roof Units Group, Dudley, UK) which pulled air from all chambers via an overhead duct at a system rate of 0.27 $m^3 s^{-1}$, giving a flow of 2.0 $m^3 min^{-1}$ per OTC or one air change per chamber per minute.

Records were taken of air temperature, photosynthetic photon flux density (PPFD), and relative humidity (RH) on a datalogger (DL2, Delta-T Devices, Cambridge, UK). Air temperature was measured in all eight OTCs using University of Edinburgh (EU) thermistor probes (RS components, Corby, UK), shielded from solar radiation with tin foil. Measurements were made every five seconds and average measurements were stored every ten minutes. PPFD was measured using EU sensors (Sinclair 1995) in the first, fourth and eighth OTCs every five seconds and average values were stored every ten minutes. RH was measured using a temperature/humidity probe (HMP 35A, Vaisala, Helsinki, Finland) in the third OTC every five minutes and values were averaged every ten. Further data on sunshine hours during the months of the experiment, for Edinburgh in general, were extracted from Meteorological Office records provided by the weather station at the RBGE.

For the CO_2-enriched OTCs, incoming air was supplemented with pure CO_2 from cylinders (Distillers plc, Glasgow, UK). Ethylene contamination was previously tested for in CO_2 from this source and could not be detected (Radoglou & Jarvis 1990). Ethylene was therefore assumed to be absent from this experiment. The CO_2

concentration of ambient OTC's ranged between 325 and 475 μmol mol^{-1} whilst elevated CO_2 OTCs were maintained between 600 and 800 μmol mol^{-1}. There was some increase in ambient glasshouse CO_2 concentration despite attempts to collect CO_2-rich air and vent it outside the glasshouse. The CO_2 concentration in each chamber was continually measured using an infrared gas analyser (WMA1, PP systems, Herts, UK) which was calibrated regularly using a known concentration of CO_2. Sampling and adjustment of CO_2 supply was fully automated with the use of a personal computer fitted with interface cards (AOP6, Blue Chip Technology, Clwyd, UK) and mass flow controllers (Barton 1997).

Nutrient regime

In each OTC, half of the plants were assigned to a high nutrient supply rate and half to a low rate, by a fully randomised design. One leaf sample from each of four healthy *C. odorata* seedlings growing in a glasshouse in tropical conditions was taken and its nutrient concentration determined. The nutrient solution (Appendix 3) described by Ingestad and Lund (1986), which was originally devised for birch, was modified to suit the nutrient concentrations observed in *C. odorata* leaves. In particular, extra calcium was added to the solution. Allowances were made for nutrient concentrations in the tap water supply.

Nutrient solution was applied to the rooting medium three times a week. Conductivity and pH of the nutrient solution were monitored to ensure that its application was at a concentration and pH appropriate for physiological activity. The same volume of solution was fed to each plant and this was contained within each plant saucer at the time of feeding. For rate of nutrient supply to match that of growth, dilution of stock solution before addition to plants was decreased exponentially as it was assumed that while the plants were in this seedling stage their rapid growth rate would be exponential (Ingestad & Agren 1992). There were two

rates of supply of nutrient solution - high and low. Previous work suggested that the maximum growth rate of *C. odorata* in glasshouse conditions was 3.24 % per day (Ramos & Grace 1990) so the "high nutrient" plants were supplied at a rate to allow growth of 5 % per day and the "low nutrient" plants at a rate of 1 % per day.

At the start of the experiment there were 22 plants in each of the four treatments: elevated CO_2 concentration and high rate of nutrient application (HCHN), elevated CO_2 concentration and low rate of nutrient application (HCLN), ambient CO_2 concentration and high rate of nutrient application (LCHN), ambient CO_2 concentration and low rate of nutrient application (LCLN).

Growth measurements

Non-destructive measurements were made weekly of plant height, number of leaves and length of the longest rachis per plant.

Intermediate harvests enabled measurement of leaf area (LiCor 3100 area meter, Lincoln, Nebraska, USA), root and shoot dry mass and subsequent calculation of relative growth rate (RGR). For dry mass determination root samples were carefully washed by hand to minimise fine root loss. These and the shoot samples were oven-dried at 80 °C to constant mass.

Nine plants were harvested before the start of the experiment to determine initial mass. Two further small harvests, each of 16 randomly-selected plants (one plant from each nutrient treatment per OTC) were made at 41 and 80 days, respectively. The final harvest of the 56 remaining plants (14 per treatment) was made 119 days from the start of the experiment. Nutrient analyses were performed on leaf samples after the first intermediate harvest to check the nutrient regime.

At the final harvest leaf areas were measured and the dry mass for all plants determined as above. Shoot samples were further separated into stem and leaf components. Mean % increases in dry mass of each component as a result of exposure to elevated CO_2 concentration were calculated as:

$$\Delta = \frac{(m_e - m_a)}{m_a} \times 100 \qquad (2.1)$$

where

m_e = mass of tissue grown at elevated CO_2 concentration, and

m_a = mass of tissue grown at ambient CO_2 concentration.

In addition to mean relative growth rate (RGR, R) for the entire growing season, calculations were made of mean net assimilation rate (NAR, E), final leaf area ratio (LAR, L), and final specific leaf area (SLA, S) and tested for statistical differences. These are defined as follows:

$$R = \frac{(\ln m_2 - \ln m_1)}{t_2 - t_1} \times 100 \qquad (2.2)$$

$$E = \frac{m_2 - m_1}{A_{L2} - A_{L1}} \times \frac{\ln A_{L2} - \ln A_{L1}}{t_2 - t_1} \qquad (2.3)$$

$$L = \frac{A_{L2}}{m_2} \qquad (2.4)$$

$$S = \frac{A_{L2}}{m_L} \qquad (2.5)$$

where

t_1 = time 1,

t_2 = time 2,

m_1 = tree dry mass at t_1,

m_2 = tree dry mass at t_2,

m_L = mass of leaves at t_2,

A_{L1} = leaf area at t_1, and

A_{L2} = leaf area at t_2.

It was assumed that mass increased without discontinuity between t_1 and t_2 in the calculation of RGR. Assumptions made during the calculation of NAR were that A and m varied with time with the same linear allometric relationship (Kvet *et al.* 1971), for the period between the start and finish of the experiment. Values for m_1 were obtained from the initial harvest, and values for A_{L1} were obtained from the allometric relationship between plant mass and leaf area which was established using all simultaneous measurements of plant mass and leaf area (Fig. 2.1). An average of these was used as an estimate of initial mass and leaf area of plants that were harvested at t_2. Both LAR and SLA are given for one moment in time, namely the final harvest. Plants were so small at preceding harvests that dry mass was not calculated for stems and leaves individually. RGR, LAR, NAR and SLA were calculated for each individual plant and averages used for statistical comparisons between treatments.

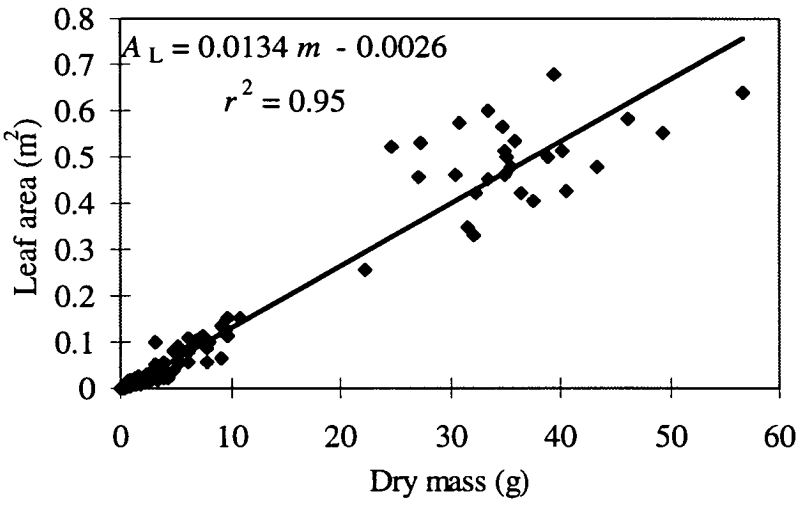


Figure 2.1 The allometric relationship between plant dry mass and leaf area of *C. odorata*. Data are combined from all harvests of both 1995 & 1996 at which leaf area was measured. A_L = leaf area. m = plant dry mass. n = 195.

Leaf anatomy

At the final harvest, impressions of eight abaxial leaflet surfaces per treatment, each from a different plant, were made using a dental impression material (Kerr Extrude, Cottrels and Co., Edinburgh, UK). Prior investigation had revealed few or no stomata on adaxial surfaces. Imprints were then taken with clear nail varnish and these were viewed under a light microscope (Ortholux II, Leitz, Wetzlar, Germany) and stomatal densities determined for four fields of view per impression.

Hand sections were made of four fully illuminated leaflets on a consistently sunny day. These were stained with a KI/I_2 stain to check for starch in the mesophyll chloroplasts as opposed to the bundle sheath cells to confirm *C. odorata* as a plant which uses only the C_3 photosynthetic pathway (Bolhàr-Nordenkampf & Draxler 1993).

Tissue nutrient concentration

At the final harvest one sample of each of stem, leaf and root tissue of all 56 plants was analysed for nutrient (N, P, K, Ca, Mg) concentration. The samples were oven-dried at 80 °C to constant mass and then all tissues were finely ground on a centrifugal grinder (Retsch, Glen Creston, Stanmore, UK). All dried and ground samples (0.1 g of each) were acid/peroxide-digested (adapted from Grimshaw, Allen & Baker 1989), diluted with water and analysed for nutrient concentration using one of two methods. N and P were analysed colorimetrically on a flow injection analyser (Flow Solution 3000, Perstorp Analytical, Wilsonville, USA). Ca, Mg and K were analysed by atomic absorption spectroscopy using an atomic absorption spectrophotometer (Unicam 919, Unicam, Cambridge, UK). See Appendix 4 for full details of the method.

Monosaccharide, oligosaccharide and starch concentration

Monosaccharide, oligosaccharide and starch concentrations were determined for a sub-sample of leaves at final harvest. These samples were removed from the plants between 11 am and 1 pm and stored in a freezer at -20 °C before freeze drying to constant mass. The 54 plants with the largest leaf area were analysed for starch concentration. Monosaccharide and oligosaccharide concentration was determined for as many plants as there was sufficient remaining sample (52 samples).

Mono- and oligosaccharides from freeze-dried leaf samples (0.05 g) were extracted in de-ionised water, centrifuged and the supernatant collected and diluted (modified from Grimshaw *et al.* (1989)). This was analysed for mono- and oligosaccharides using a carbopac (PA1) column on a high performance liquid chromatograph (Dionex DX500, Dionex, Camberley, UK). See Appendix 4 for further details.

For analysis of starch 0.05 g samples of freeze-dried leaves were dissolved in perchloric acid and an iodine colorimetric analysis performed (adapted from Grimshaw *et al.* 1989) to determine concentration. See Appendix 4 for full details.

Chlorophyll concentration

For chlorophyll determination, three leaf discs were removed from three different healthy leaflets of all plants at the time of the final harvest. These samples were stored in a freezer at -20 °C until required.

The method of Porra, Thompson & Kriedemann (1989) was used to determine leaf chlorophyll content per unit leaf area. Three leaf discs per plant were placed in sealable glass vials. N,N-dimethylformamide (DMF, 3 cm^3) was added to the vials and they were then sealed and incubated in the dark at room temperature until the leaf discs were white (two to three days). Absorbances of the solutions were measured at 647, 664 and 750 nm on a spectrophotometer (Series 2, Cecil Grating, Cambridge, UK) and the equations of Porra *et al.* (1989) used to convert the absorbances to concentrations of chlorophyll *a* and *b* in solution. These concentrations were then converted to mass of chlorophyll per unit leaf area.

Statistical analysis

Treatment differences were analysed using an hierarchical two way analysis of variance (ANOVA) contained in SAS for Windows version 6.12 (SAS Institute Inc. 1990). This test avoids "pseudo-replication" (Hurlbert 1984) by nesting the CO_2 treatment within chambers and testing the CO_2 effect using the error associated with variations between chambers rather than between individuals with the same CO_2 regime (Potvin 1993). It should be noted therefore that standard errors and *n.*'s listed for all results are neither the between nutrient treatment errors nor the between CO_2 treatment errors. They are merely an indication of the variation within each group as

it is not feasible to show all errors according to each level of nesting within a treatment. Assumptions of normality and equal variance were tested using normal plots and Hartley's test for homogeneity of variance (Milliken & Johnson 1992). Data were transformed using either log or square root transformations where appropriate. Paired comparisons were made *a posteriori*, using Bonferroni correction intervals to protect against type one errors (Williams 1994). These differences were also investigated using a Tukey's honestly significant difference test (Fowler & Cohen 1990).

The fit of the allometric relationship between rachis length and leaf area or plant height and mass was assessed using the coefficient of determination, r^2. Differences in the allometric relationship of rachis length with leaf area between nutrient and CO_2 treatments were investigated using an analysis of covariance also contained in SAS (SAS Institute Inc. 1990).

Differences between OTCs and provenances (San Antonio and Teupasente) were also tested. Although provenances of *C. odorata* were mixed the differences between them were found not to be statistically significant ($p > 0.05$) and all subsequent tests were performed on a pooled data set.

C. odorata and *S. macrostachya* seedlings grown during the Summer of 1996

Plant material and growth conditions

C. odorata San Antonio was again sown in sand but 108 seedlings were then transferred to individual pots (1 dm^3) containing expanded clay (Hydroleca™) and moved to OTCs when the plants were at an average height of 30 mm. The plants were distributed evenly through the six OTCs using the same experimental design as in 1995 (three OTCs per CO_2 treatment). The OTCs were housed within a

glasshouse at the University of Edinburgh (EU, 55 °55 ′ N, 3 °11 ′ W, elevation 80 m). All plants were transferred to bigger (5 dm^3) pots at five weeks of growth in the OTCs when at an average height of 58 mm.

Differences in inherent growth potential of *C. odorata* plants grown in Hydroleca™ as opposed to Perlite were tested for in the Summer of 1997. It was hypothesised that the media might differ in their nutrient adsorption capacity and therefore interfere with plant growth through adsorption of nutrients on the media surface. Ten *C. odorata* seedlings were grown in separate pots of each growth medium for 18 weeks. Plants were liquid-fed with Vitax™ "high N foliar feed" (N:P:K 35:5:10) at regular intervals. Initial and final plant heights were measured and compared between treatments. Final dry mass was also measured and % increases in dry mass from start to finish were calculated using the allometric relationship between plant height and dry mass (Fig. 2.2) to infer initial masses.

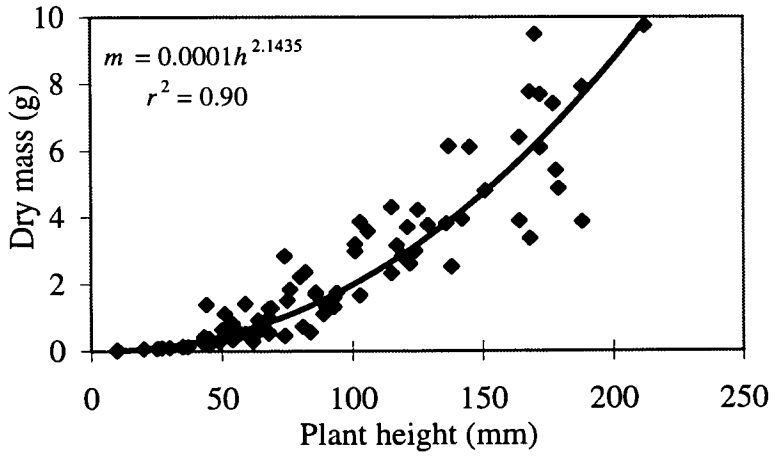


Figure 2.2 The allometric relationship between height and dry mass of *C. odorata* in 1995. m = plant mass. h = plant height. Data from all plants of all CO_2 concentration and nutrient treatments have been combined. All plants from all harvests have been included. n = 88.

Also included in the OTCs in 1996 were 24 plants of *S. macrostachya* (Araliaceae), germinated in the previous year. Known as Queensland Umbrella Tree, this popular ornamental plant has been cultivated for use as an indoor and outdoor plant in many countries around the world. The plants were grown for 102 days in OTCs in a potting mix (peat: fine sand: coarse (10 mm) sand 6:1:1 with 1 kg of ground lime and 1.65 kg of Vitax™ Q_4HN (N:P:K 5.3:7.5:10) fertiliser for every 400 dm^3 of mix) in pots of volume 2 dm^3, 5 dm^3 or 10 dm^3, depending on the initial plant size. Plants were divided into size classes at the start of the growing season and distributed evenly between high and low CO_2 concentration treatments, 12 to each, four per OTC. All plants were irrigated as necessary. An example of *C. odorata and S. macrostachya* being grown within the same OTC can be seen in Appendix 2.

Open-top chambers

Air from outside the glasshouse at EU was blown in from intakes near the ground. It was heated whenever its temperature dropped below 20 °C. Holes for glasshouse fan heaters (Autoheat MP3, Findlay Irvine, Penicuik, UK) were made in the side of the OTCs, air for which was supplied from the OTC via a loop of flexi-ducting. The thermostat on the heaters could therefore be used to maintain an OTC temperature of 20 °C, particularly at night. Records were taken of air temperature, PPFD, and RH with a datalogger (DL2, Delta-T Devices, Cambridge, UK). Temperature and PPFD were measured in all six OTCs and RH was again measured in only the third OTC. The same sensors and logger were used as in 1995 and values were averaged for ten minute periods as in 1995. Again, further meteorological data of sunshine hours in Edinburgh during experimental months were taken from Meteorological Office records from the weather station at the RBGE.

Ambient OTCs ranged in their CO_2 concentration between 250 and 450 $\mu mol\ mol^{-1}$ whilst the elevated OTCs were maintained at a CO_2 concentration between 600 and

800 μmol mol^{-1}. CO_2 concentration was monitored and adjusted as in 1995 for the majority of the 1996 growing season. For part of 1996, however, the CO_2 supply rate was controlled by the use of manually-set needle valves. See Appendices 1 and 2 for full details of the systems.

Nutrient regime

The growth data obtained for *C. odorata* in 1995 confirmed that a maximum relative growth rate (RGR) of 5 % per day was a good estimate. High nutrient plants in 1996 were therefore fed with the same recipe and rate of supply as in 1995. However, the low nutrient plants were supplied with sufficient nutrient to allow a RGR of 3 % per day to reduce the magnitude of the difference between treatments. It was calculated that a 3 % RGR should result in an average final plant mass 25 % that of the plants growing with the high nutrient supply rate. De-ionised water was used so it was not necessary to correct the nutrient solution for contamination from the town water supply. Special care was taken with watering to ensure no loss of supplied nutrient as a result of overflow in irrigation.

The recipe was altered 71 days into the 1996 experiment when calcium appeared to be present in leaf tissue in excessively high concentrations. The original Ingestad solution (Ingestad & Lund 1986) was used for the remainder of the growing season.

The rate of nutrient application was also modified once, immediately after harvest two (40 days). Because plants had grown at less than the expected RGR, the rate of nutrient supply was altered to suit the measured rather than calculated mass for that time. In effect the exponential rate of nutrient application was reset to an earlier point on the nutrient addition curve. There were 15 plants at the final harvest in each of the four CO_2 concentration and nutrient application rate treatments.

No attempts were made to control the rate of nutrient supply to *S. macrostachya* and plants were fed only once with Osmocote ™ slow release fertiliser after five weeks of the experiment. Therefore there was only one nutrient treatment.

Growth measurements

Weekly changes in *C. odorata* plant height and leaf number were measured as for 1995. Rate of rachis elongation of a single tagged leaf per plant was followed. The tagged leaf was that uppermost on the main stem at the start of the experiment and weekly measurements were also taken of the length of the internode immediately below.

Starting mass of the seedlings (m_1) was inferred from the allometric relationship between height and mass of the *C. odorata* plants grown in 1995 (Fig. 2.2, p.62). The first sub-harvest of 16 randomly-selected plants (four per treatment) was 23 days after the start of the experiment. Two subsequent sub-harvests, each of 16 randomly-selected plants (four per treatment), were made 17 and 47 days after the first harvest. It was observed that the high nutrient, elevated CO_2 concentration treated plants from harvest three were particularly stunted in comparison with those from the other treatments. These seedlings appeared to have failed to establish upon transplantation after germination. RGR calculations therefore omit the results from harvest three and only results from the other three harvests are shown. The remaining 60 plants (15 per treatment) were harvested 101 days after the start of the experiment (harvest four). Calculations of RGR, NAR, LAR and SLA were made as in 1995 with A_{LI} inferred from the allometric relationship of leaf area with plant mass (Fig. 2.1, p. 58).

All rachis lengths of all leaves remaining on *C. odorata* plants at the time of harvest were measured at harvests three and four. These data were used to compute the allometric relationship between rachis length and total leaf area. The relationships

were compared between treatments to test for any change in the relative timing of events during leaf ontogeny. Also during the final harvest, all internode lengths of plants were measured and the organ type (branch, leaf or abscised leaf) noted to investigate any change in morphology or gross morphological development.

Samples of the growth medium of four high nutrient plants of each of elevated and ambient CO_2 concentration levels were analysed by the Scottish Agricultural College (SAC) for total organic carbon content. In addition, control samples of Hydroleca™ only (*i.e.* Hydroleca with nutrient additions but no plants) were analysed from two OTCs of each of the elevated and ambient CO_2 treatments.

Weekly measurements of height and leaf number were made for all *S. macrostachya* plants. All *S. macrostachya* plants were harvested at the conclusion of the 102 day experiment. Leaf areas and numbers, dry masses of roots, stem and leaves and plant heights were measured at this time.

Leaf anatomy

Stomatal densities were measured in both *C. odorata* and *S. macrostachya* at the final harvest as in 1995.

In addition, three *C. odorata* leaflet samples of each of the high and low CO_2 concentration plants, all supplied with high nutrient, were stored at -20 °C. These were later mounted in a support/mounting media for cryo-microtomy (Tissue Tek, Agar Scientific Limited, Stansted, UK) and sectioned transversely on a cryostat (Reicher, Leica UK Ltd, Milton Keynes, UK) to produce a flat face. Samples were then transferred to a tissue drier (Speedivac Pearse, Edwards High Vacuum, Crawley, UK) which used a Peltier cooled stage under vacuum to freeze-dry the tissue in a constant manner. Dry samples were mounted on 12.5 mm stubs and carbon dagged

to improve conductivity. They were then gold coated for three minutes at 20 mA and 0.08 Torr using a sputter coater (Emscope SC500 A, Agar Scientific Limited, Stansted, UK). Samples were viewed on a scanning electron microscope (Stereoscan 250, Cambridge, UK), and pictures taken on Kodak TMAX 100 film, to be examined for gross anatomical differences in numbers of cell layers.

Tissue nutrient concentration

At the final harvest root, stem and leaf tissues of all 60 *C. odorata* plants were analysed for nutrient (N, P, K, Ca, Mg) concentrations.

In *S. macrostachya* samples of root and shoot (stem and leaf combined) dry matter were analysed for nutrient content for all 24 plants (12 per treatment).

Single sugar and starch concentration

The 44 *C. odorata* plants of largest leaf area were sampled for mono- and oligosaccharide concentration and starch analyses were performed on all samples present in sufficient quantity after monosaccharide analysis ($n = 40$).

Mono- and oligosaccharide, starch and chlorophyll concentrations were not determined for *S. macrostachya.*

Chlorophyll concentration

Three leaf discs from all *C. odorata* plants were removed and stored in a freezer at -20 °C as in 1995. These were analysed for chlorophyll concentration as in the previous year. On the day prior to final harvest, *in-situ* measurements were also made of leaf chlorophyll content on one uppermost fully expanded but not apparently senescing leaf of the 13 most healthy plants per CO_2 concentration and nutrient

treatment using a portable chlorophyll meter (SPAD-502, Minolta Camera Co., Osaka, Japan).

Statistical analysis

The same procedures were used to analyse growth differences between treatments of *C. odorata* as in 1995. Differences were tested between ambient and elevated CO_2 concentration treatments in *S. macrostachya* using a one way analysis of variance, with nesting, contained in SAS for Windows version 6.12 (SAS Institute Inc. 1990).

RESULTS

Growth conditions

In both years the mean temperature and PPFD was very similar between chambers. In 1995 chambers four and six (both elevated CO_2 concentration chambers) showed a smaller range in temperature for most of the time (Fig. 2.3). Chamber eight (elevated CO_2 concentration) had a larger range of recorded PPFD (Fig. 2.4). In 1996 there was more variation between the mean temperature (Fig. 2.5) of each chamber although the chambers experienced a similar range of temperatures. PPFD was almost constant between chambers with the exception of chamber six (elevated CO_2 concentration) which was consistently darker (Fig. 2.6).

There was, however, some difference in the average growing conditions between years. The mean temperature for the season was 1.6 °C warmer at RBGE than at EU. Average temperature in 1995 was 24.1 $\pm$ a standard error of 0.04 °C as opposed to 22.52 $\pm$ 0.02 °C in 1996. Daytime PPFD (hours when PPFD was greater than 0) was also higher on average in 1995. The mean PPFD was 285.8 $\pm$ 4.2 μmol m^{-2} s^{-1} compared with 211.4 $\pm$ 1.0 μmol m^{-2} s^{-1} in 1996. Data from Meteorological Office records also showed a difference between the two years. 1995 had an average of 258.1 total hours of sunshine per month for the months of the experiment whereas 1996 had an average of 157.5 total hours per month.

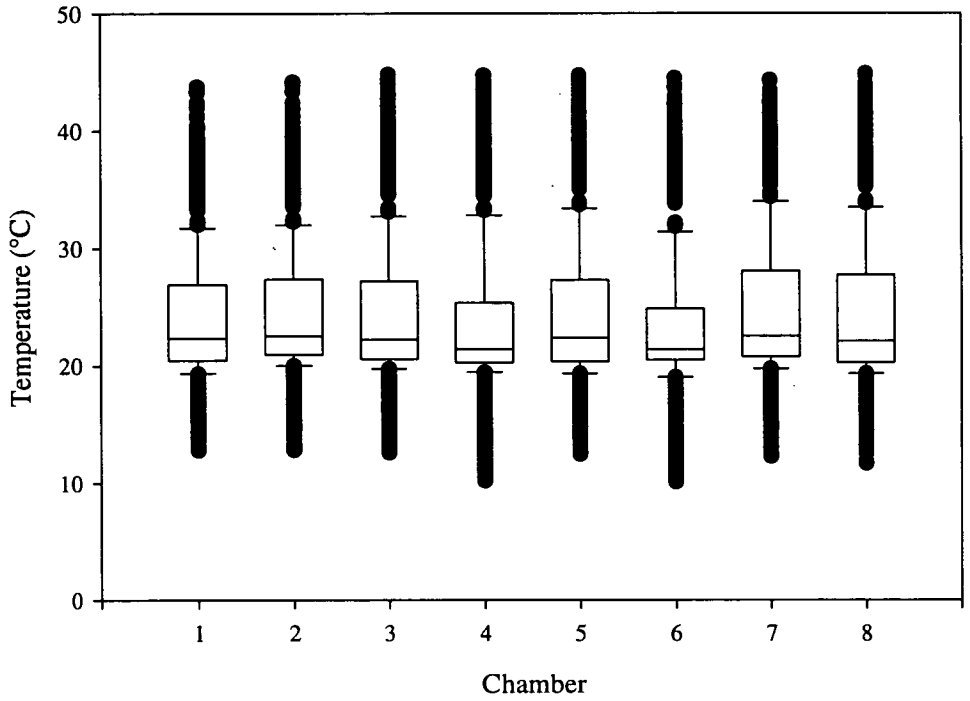


Figure 2.3 Box plot of range of temperatures observed in chambers at Royal Botanical Gardens of Edinburgh site 1995. The mean of each data set is shown as the central bar of the box with the delimiters of the box representing the 25th and 75th percentiles. Capped bars represent the 10th and 90th percentiles with values lying outside of these percentiles shown as •. Temperature was measured in each chamber every five seconds but average values per ten minutes are shown here.

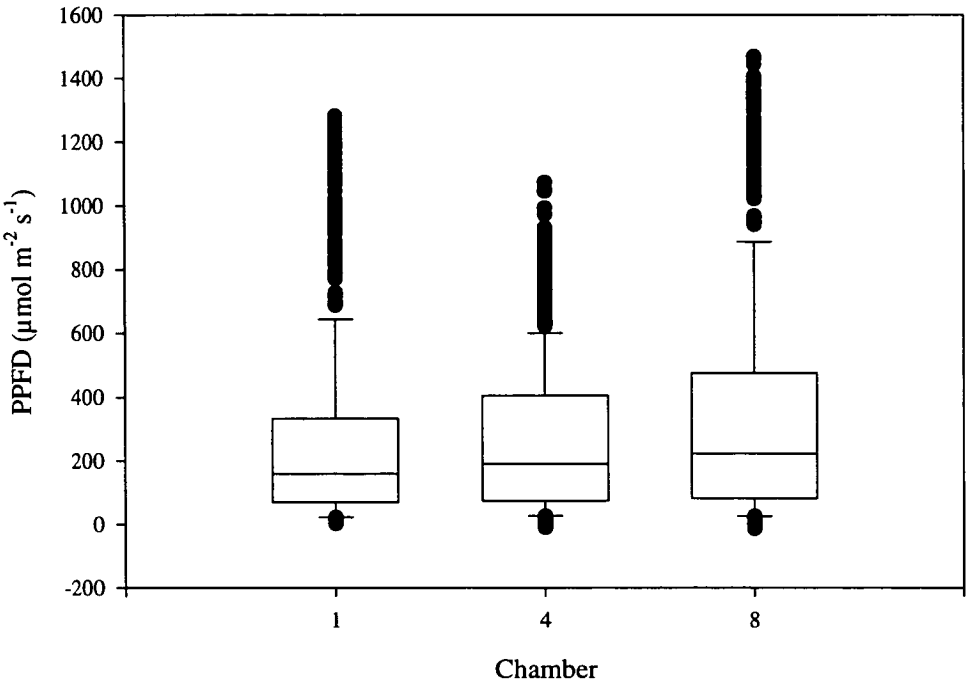


Figure 2.4 Box plot of range of day PPFD values observed in each of three chambers measured at Royal Botanic Gardens of Edinburgh site in 1995. The mean of each data set is shown as the central bar of the box with the delimiters of the box representing the 25th and 75th percentiles. Capped bars represent the 10th and 90th percentiles with values lying outside of these percentiles shown as •. PPFD was measured every five seconds but average values for every ten minutes are shown here.

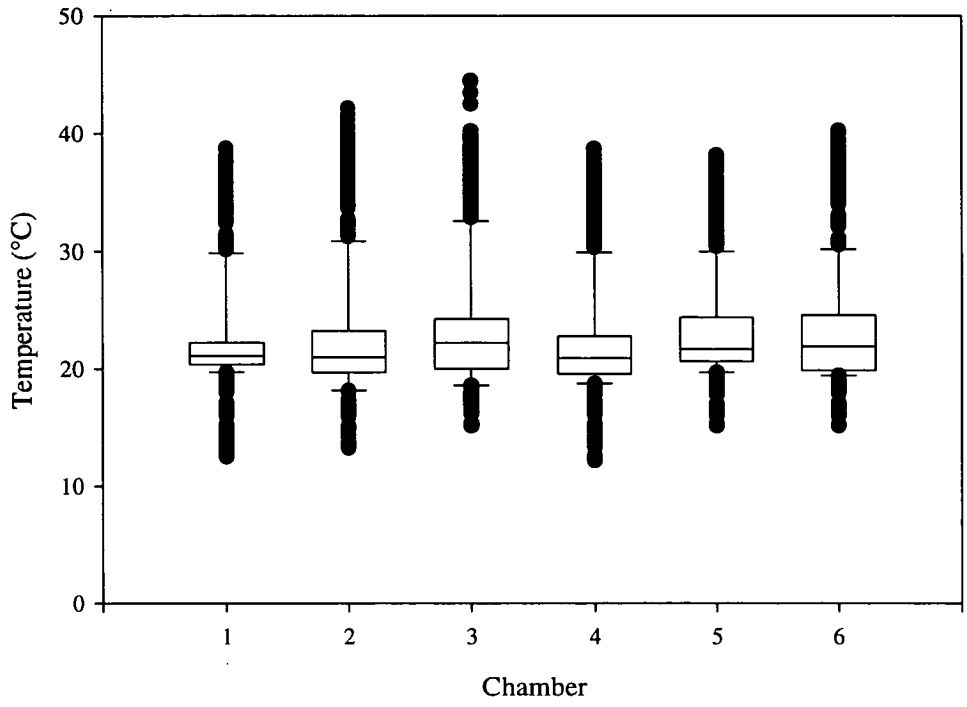


Figure 2.5 Box plot of range of temperatures observed in chambers at University of Edinburgh site in 1996. The mean of each data set is shown as the central bar of the box with the delimiters of the box representing the 25th and 75th percentiles. Capped bars represent the 10th and 90th percentiles with values lying outside of these percentiles shown as •. Temperature was measured in each chamber every five seconds but average values per ten minutes are shown here.

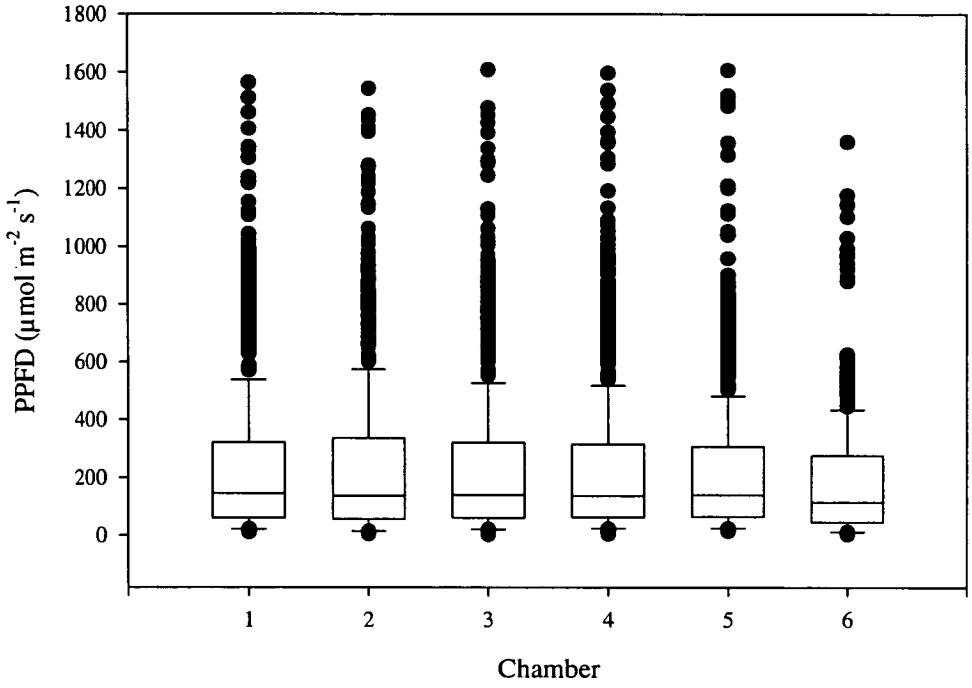


Figure 2.6 Box plot showing range of day PPFD values observed in each of the six chambers measured at the University of Edinburgh site in 1996. The mean of each data set is shown as the central bar of the box with the delimiters of the box representing the 25th and 75th percentiles. Capped bars represent the 10th and 90th percentiles with values lying outside of these percentiles shown as •. PPFD was measured every five seconds but average values for every ten minutes are shown here.

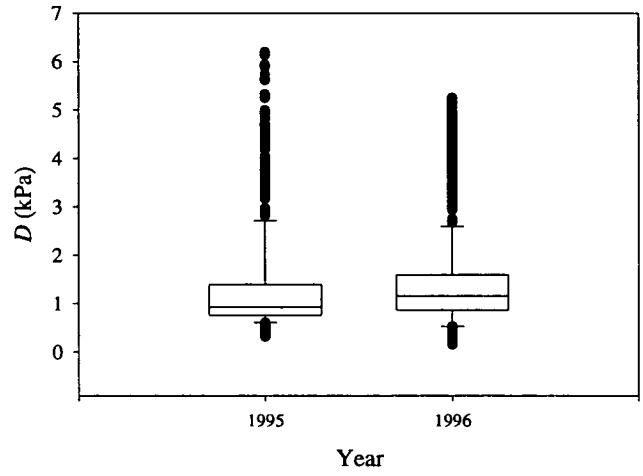


Figure 2.7 Box plot of D in one measured chamber from each of 1995 and 1996. The mean of each data set is shown as the central bar of the box with the delimiters of the box representing the 25th and 75th percentiles. Capped bars represent the 10th and 90th percentiles with values lying outside of these percentiles shown as •.

The range of vapour pressure deficit (D) was greater in 1995 but the mean was smaller with the 1995 average being 1.30 $\pm$ 0.03 kPa and the 1996 average being 1.37 $\pm$ 0.01 kPa (Fig. 2.7). D was significantly higher in 1996 ($p < 0.05$).

Growth of *C. odorata*

1995 growth

At the final harvest, mass varied both with CO_2 concentration and with nutrient supply rate (Fig. 2.8, p. 76). The average increase in root biomass (Δ) in elevated CO_2 concentration with a high rate of nutrient supply was 23 % ($p < 0.05$). Stem dry mass and total dry mass both showed a trend towards an increased biomass with a high rate of nutrient supply. There was no significant difference between CO_2 treatments for plants at a low rate of nutrient supply ($p > 0.05$). The biomass was

significantly reduced with a low supply of nutrient in both CO_2 concentrations ($p < 0.05$, Table 2.1).

Table 2.1 Probability values associated with significant differences between plants grown in elevated and ambient CO_2 concentrations with two different nutrient regimes (high and low) in 1995. DM = dry mass, RGR = relative growth rate. Probability values are given for the overall significance of each factor of the two way hierarchical ANOVA. Also given are the significant differences between pairs using Tukey's HSD tests where $p < 0.05$. These are denoted by the following letters: a = statistically significant difference between LCHN and LCLN, b = statistically significant difference between LCHN and HCLN, c = statistically significant difference between HCHN and HCLN, d = statistically significant difference between HCHN and LCLN, e = statistically significant difference between HCHN and LCHN, f = statistically significant difference between HCLN and LCLN. Treatments were as follows: HCHN (elevated CO_2 concentrations, high rate of nutrient supply), HCLN (elevated CO_2 concentrations, low rate of nutrient supply), LCHN (ambient CO_2 concentrations, high rate of nutrient supply), LCLN (ambient CO_2 concentrations, low rate of nutrient supply). Note that although the CO_2 effect may be significant in the ANOVA, the Tukey difference may only lie between plants of differing nutrient treatment.

Variable	p value CO_2	p value nutrient	p value interaction	Tukey difference in CO_2 treatment
Stem DM	0.0284	0.0001	0.2194	a, b, c, d
Root DM	0.0080	0.0001	0.1697	a, b, c, d, e
Total DM	0.0118	0.0001	0.2939	a, b, c, d
RGR	0.037	0.0001	0.1630	a, b, c, d, f
SLA	0.0548	0.0141	0.2062	
NAR	0.0957	0.0001	0.9967	

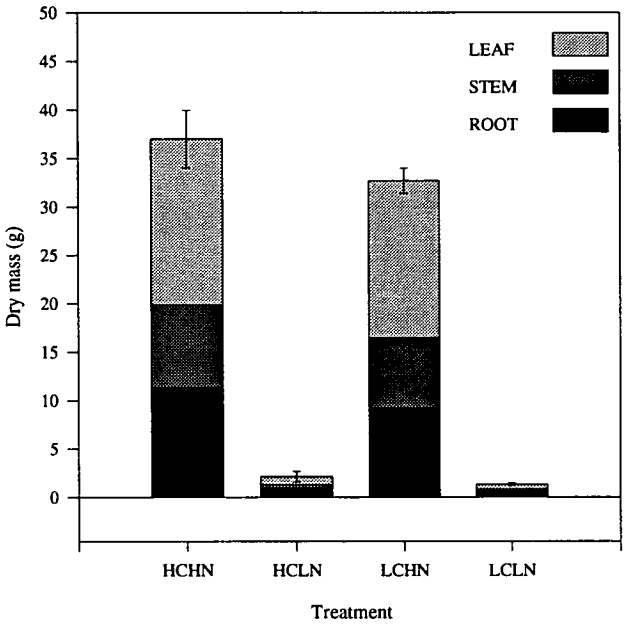


Figure 2.8 Mean values of dry mass at final harvest in 1995 of *C. odorata* for each of root, stem and leaf components. Plant treatments were as follows: HCHN (elevated CO_2, high rate of nutrient application), HCLN (elevated CO_2, low rate of nutrient application), LCHN (ambient CO_2, low rate of nutrient application), LCLN (ambient CO_2, low rate of nutrient application). One standard error is shown for each mean value of total plant dry mass. $n = 14$ for all treatments and components.

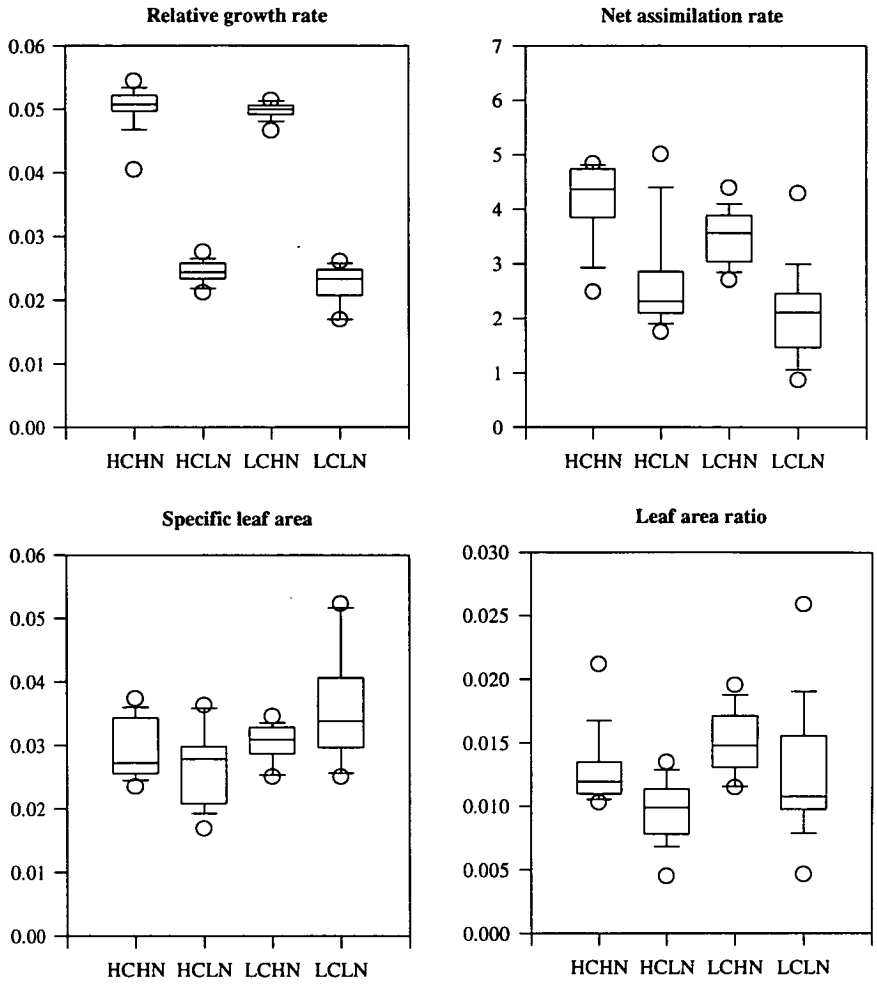


Figure 2.9 Box plots of the growth ratios of RGR (d^{-1}), NAR ($g\ m^{-2}\ d^{-1}$), SLA ($m^2\ g^{-1}$) and LAR ($m^2\ g^{-1}$) for *C. odorata*, 1995. The mean of each data set is shown as the central bar of the box with the delimiters of the box representing the 25th and 75th percentiles. Capped bars represent the 10th and 90th percentiles with values lying outside of these percentiles shown as O. Each graph shows all four treatments which were as follows: HCHN (elevated CO_2 concentration, high rate of nutrient application), HCLN (elevated CO_2 concentration, low rate of nutrient application), LCHN (ambient CO_2 concentration, high rate of nutrient application) and LCLN (ambient CO_2 concentration, low rate of nutrient application). $n = 14$ per treatment.

NAR was not significantly increased in elevated CO_2 concentration although this difference was also approaching significance ($p = 0.1$, Fig. 2.9). RGR calculated for the entire duration of the experiment was significantly higher in HCHN plants only

when compared with LCLN plants ($p < 0.05$, Fig. 2.9). There was no difference between HCHN and LCHN plants ($p > 0.05$). However, HCLN plants had a significantly higher RGR than LCLN plants ($p < 0.05$, Fig. 2.10). Despite this difference, HCLN plants were not significantly larger than LCLN plants at the end of the growing season, as a result of the large variation in biomass within these two treatments ($p > 0.05$). There was no significant decrease in SLA with elevated CO_2 concentration although the probability of a difference also approached statistical significance ($p = 0.05$, Table 2.1, p. 75).

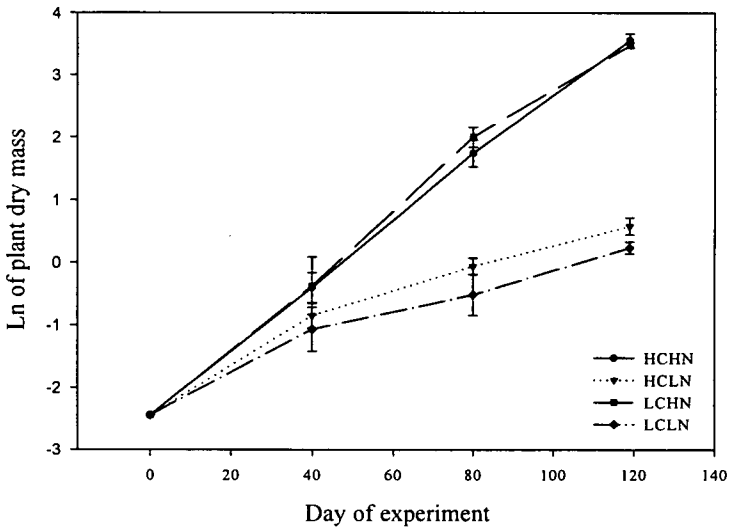


Figure 2.10 Natural logarithms of plant dry mass over time of each of the four CO_2 concentration and nutrient treatments for *C. odorata* in 1995. Relative growth rates are represented by the slopes of the lines. Data shown are from days 0-119 after the start of the experiment. Data were obtained from three consecutive harvests. Mean values for each treatment at each harvest are shown with one standard error. $n = 4$ per treatment for harvests at 41 and 80 days and $n = 14$ per treatment for the final harvest at 119 days. CO_2 concentration and nutrient treatments were as follows: HCHN (elevated CO_2 concentration, high rate of nutrient application), HCLN (elevated CO_2 concentration, low rate of nutrient application), LCHN (ambient CO_2 concentration, high rate of nutrient application) and LCLN (ambient CO_2 concentration, low rate of nutrient application).

There were no differences detected in morphology between plants grown in elevated and ambient CO_2 concentration conditions. The average height, number and area of

leaves, and length of the longest rachis per plant did not differ between CO_2 concentration treatment ($p > 0.05$ in all cases), but all were significantly larger in the high nutrient supply treatments than in the low nutrient supply treatments ($p < 0.05$, Table 2.2, Appendix 6). Similarly, there was little change in the relative heights of the treatments with time (Fig. 2.11).

Table 2.2 Morphometric measurements of *C. odorata* at final harvest, 1995. All are shown as averages plus or minus one standard error. Rachis length is given as the longest rachis on the plant at the time of measurement. n = 14 plants for all treatments and variables, except for stomatal density where n = 32 fields of view per treatment. Treatments were as follows: HCHN (elevated CO_2 concentration, high rate of nutrient application), HCLN (elevated CO_2 concentration, low rate of nutrient application), LCHN (ambient CO_2 concentration, high rate of nutrient application) and LCLN (ambient CO_2 concentration, low rate of nutrient application). Means with different superscripts within the same row are significantly different ($p < 0.05$).

	HCHN	HCLN	LCHN	LCLN
Leaf area (cm^2)	4616 ± 323^a	190 ± 36^b	4932 ± 254^a	148 ± 7^b
Final height (mm)	610 ± 35^a	98 ± 14^b	594 ± 28^a	76 ± 5^b
R:S ratio	0.45 ± 0.02^a	0.97 ± 0.05^b	0.40 ± 0.02^a	0.88 ± 0.05^b
Rachis length (mm)	538 ± 19^a	127 ± 15^b	529 ± 17^a	95 ± 4^b
Number of leaves	19 ± 0.7^a	11 ± 0.4^b	19 ± 0.5^a	10 ± 0.5^b
Stomatal density (no. mm^{-2})	234 ± 9^a	219 ± 7^a	244 ± 8^a	208 ± 11^a

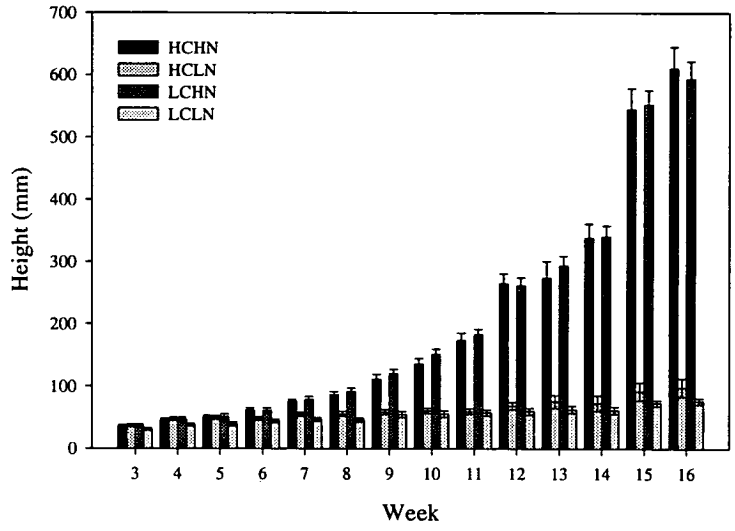


Figure 2.11 Growth of *C. odorata* in 1995. Mean values of plant height are shown for each CO_2 and nutrient treatment and week of experiment. Plant treatments were as follows: HCHN = elevated CO_2 concentration, high rate of nutrient application, HCLN = elevated CO_2 concentration, low rate of nutrient application, LCHN = ambient CO_2 concentration, high rate of nutrient application, LCLN = ambient CO_2 concentration, low rate of nutrient application. Mean values are shown for each treatment $\pm$ one standard error of the mean. $n = 22$ per treatment for weeks 3 - 7, $n = 18$ for weeks 8 - 12, $n = 14$ for weeks 13 - 16.

1996 growth

In 1996, elevated CO_2 concentration had no significant effect on *C. odorata* biomass ($p > 0.05$ in all cases). Mass varied significantly only with rate of nutrient application ($p < 0.05$, Fig. 2.12). At a high rate of nutrient addition, plants grown in elevated CO_2 concentration appeared smaller than their ambient CO_2 counterparts although this difference was not significant. Elevated CO_2 concentration appeared to have a stimulatory effect on plants grown at a low rate of nutrient addition although this effect was not significant.

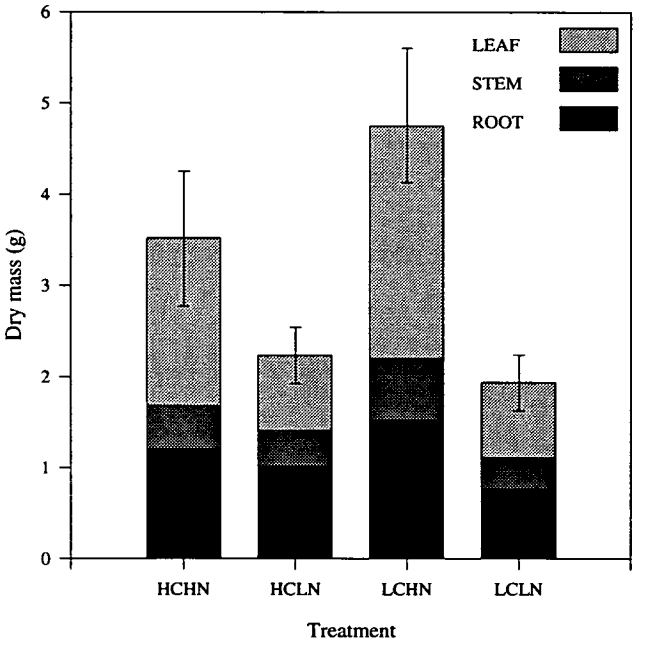


Figure 2.12 Mean values of dry mass at final harvest in 1996 of *C. odorata* for each of root, stem and leaf components. Plant treatments were as follows: HCHN (elevated CO_2, high rate of nutrient application), HCLN (elevated CO_2, low rate of nutrient application), LCHN (ambient CO_2, low rate of nutrient application), LCLN (ambient CO_2, low rate of nutrient application). One standard error is shown for the mean values of total plant dry mass. $n = 15$ for all treatments and components.

SLA appeared to decrease with elevated CO_2 concentration (Fig. 2.13), but again this difference was not statistically significant ($p > 0.05$). There were no significant differences in RGR between treatments ($p > 0.05$, Figures 2.13 and 2.14).

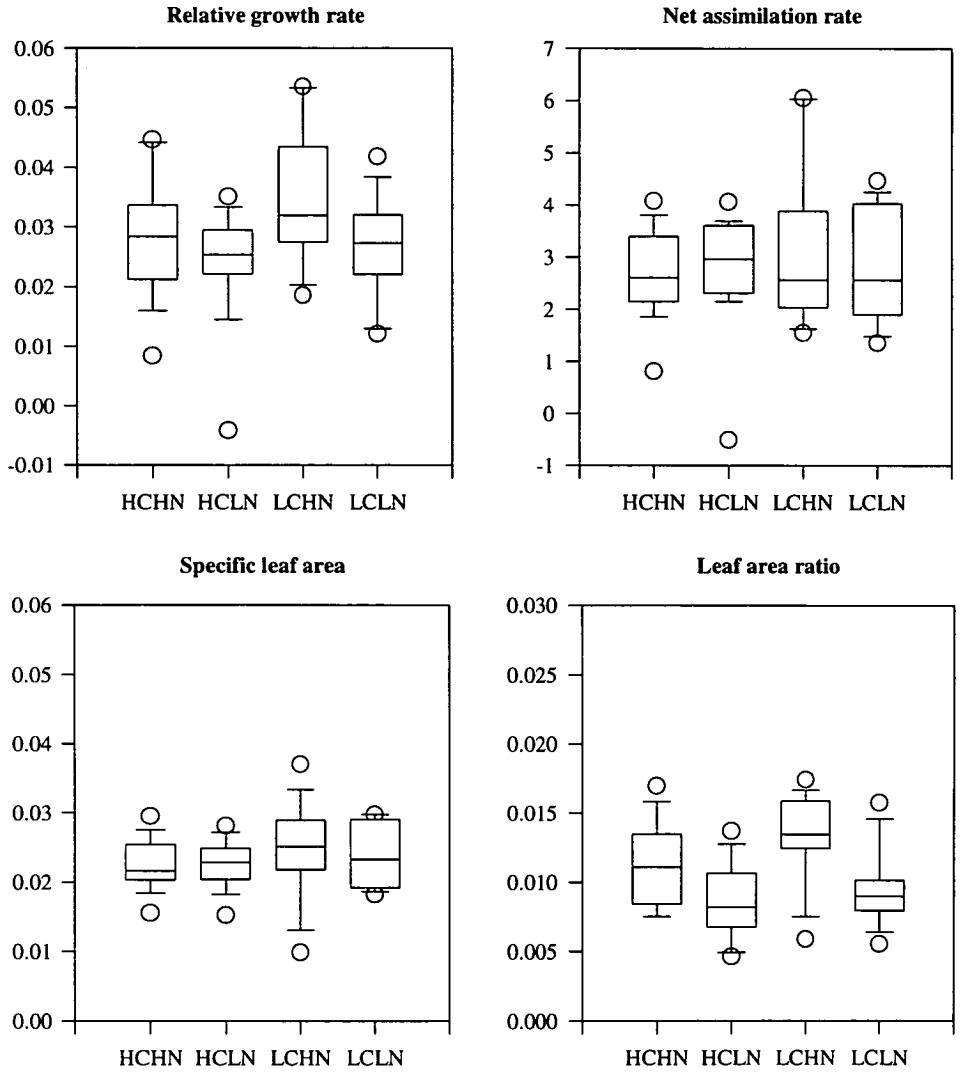


Figure 2.13 Box plots of the growth ratios of RGR (d^{-1}), NAR (g m^{-2} d^{-1}), SLA (m^2 g^{-1}) and LAR (m^2 g^{-1}) for *C. odorata*, 1996. The mean of each data set is shown as the central bar of the box with the delimiters of the box representing the 25th and 75th percentiles. Capped bars represent the 10th and 90th percentiles with values lying outside of these percentiles shown as O. Each graph shows all four treatments which were as follows: HCHN (elevated CO_2 concentration, high rate of nutrient application), HCLN (elevated CO_2 concentration, low rate of nutrient application), LCHN (ambient CO_2 concentration, high rate of nutrient application) and LCLN (ambient CO_2 concentration, low rate of nutrient application). n = 15 per treatment.

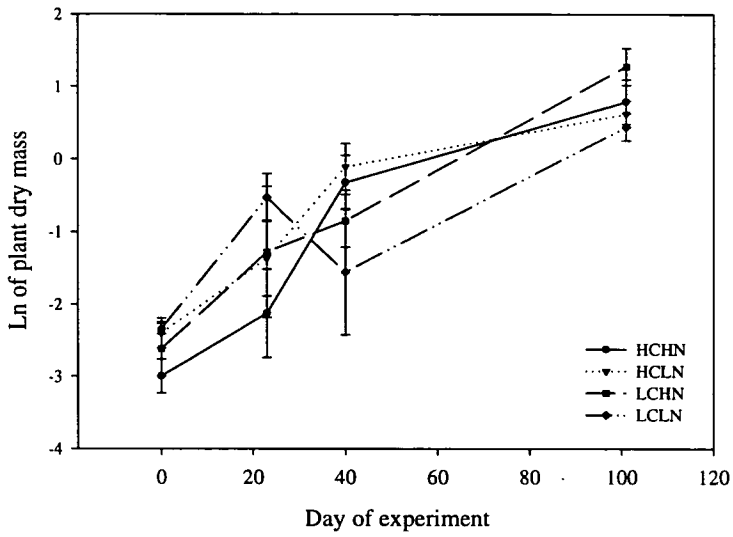


Figure 2.14 Natural logarithms of plant dry mass over time of each of the four CO_2 concentration and nutrient treatments of *C. odorata* in 1996. Data are shown from days 0 to 101 after the start of the experiment. Data for time 0 were estimated from the allometric relationship between plant height and dry mass. Data for subsequent times were obtained from three consecutive harvests. Mean values for each treatment are shown per harvest plus or minus one standard error of the mean. n = 4 per treatment for harvests at 23 and 40 days and n = 15 per treatment for the final harvest at 101 days. HCHN = elevated CO_2 concentration, high rate of nutrient application, HCLN = elevated CO_2 concentration, low rate of nutrient application, LCHN = ambient CO_2 concentration, high rate of nutrient application and LCLN = ambient CO_2 concentration, low rate of nutrient application.

There were no detectable differences ($p > 0.05$) in stem growth patterns or allocation according to CO_2 concentration treatment, although the low nutrient plants appeared to be smaller, because of shorter internode lengths ($p < 0.05$) rather than a smaller number of internodes (Table 2.3). Mean leaf number did not vary significantly with either nutrient or CO_2 concentration treatment ($p > 0.05$). A full list of all variables measured and their significant differences is given in Appendix 6, p. 241.

Table 2.3 Morphometric measurements of *C. odorata* at final harvest, 1996. All are shown as averages plus or minus one standard error. Rachis length is given as the final length of the rachis of the leaf tagged at the beginning of the season. Internode length and number are derived from the measurement of all internodes at final harvest. n = 15 plants for all treatments and variables, except for stomatal density where n = 32 fields of view per treatment. Treatments were as follows: HCHN (elevated CO_2 concentration, high rate of nutrient application), HCLN (elevated CO_2 concentration, low rate of nutrient application), LCHN (ambient CO_2 concentration, high rate of nutrient application) and LCLN (ambient CO_2 concentration, low rate of nutrient application). Means with different superscripts within the same row are significantly different ($p < 0.05$).

	HCHN	HCLN	LCHN	LCLN
Leaf area (cm^2)	411 ± 103^a	179 ± 25^b	629 ± 103^a	199 ± 38^b
Final height (mm)	137 ± 19^a	89 ± 8^b	140 ± 15^a	94 ± 9^b
R:S ratio	0.59 ± 0.05^a	0.86 ± 0.05^b	0.52 ± 0.04^a	0.75 ± 0.05^b
Rachis length (mm)	37 ± 4^a $n = 6$	38 ± 4^a $n = 12$	45 ± 3^a $n = 7$	44 ± 3^a $n = 11$
% increase in tagged internode length	169 ± 30^a	118 ± 17^a	185 ± 49^a	136 ± 36^a
Internode length (mm)	6.0 ± 0.4^a	4.6 ± 0.4^b	6.1 ± 0.5^a	4.8 ± 0.4^b
Number of internodes	20 ± 1^a	20 ± 1^a	23 ± 1^a	19 ± 1^a
Number of leaves	10 ± 0.7^a	11 ± 0.7^a	13 ± 0.7^a	10 ± 0.7^a
Stomatal density (no. mm^{-2})	313 ± 11^a	388 ± 12^b	324 ± 14^a	413 ± 15^b

Mean plant heights appeared to diverge between treatments after about five weeks of growth (Fig. 2.15). HCHN plants appeared shorter than their LCHN counterparts, just as HCLN plants appeared taller than their LCLN counterparts from this time on. Despite this trend, the CO_2 treatment differences were not significant ($p > 0.05$).

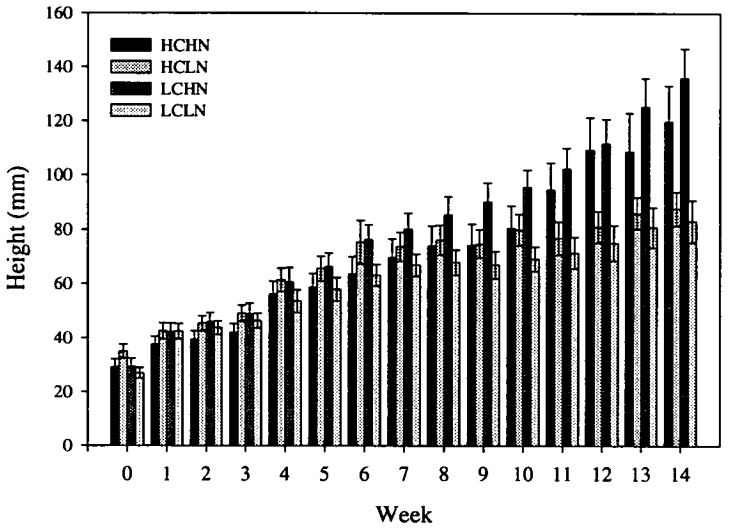


Figure 2.15 Growth of *C. odorata* in 1996. Mean values of plant height are shown for each CO_2 and nutrient treatment and week of experiment. Plant treatments were as follows: HCHN = elevated CO_2 concentration, high rate of nutrient application, HCLN = elevated CO_2 concentration, low rate of nutrient application, LCHN = ambient CO_2 concentration, high rate of nutrient application, LCLN = ambient CO_2 concentration, low rate of nutrient application. Mean values are shown for each treatment $\pm$ one standard error of the mean. $n = 27$ per treatment for weeks 0 - 3, $n = 23$ for weeks 4 - 5, $n = 19$ for weeks 6 - 10, $n = 15$ for weeks 11 - 14.

Investigation of the changes in internode length over time shows little difference between treatments with plants reaching maximum internode length at about seven weeks (Fig. 2.16). A similar pattern was observed in the change of tagged rachis length with time, but maturity was reached about four weeks after the start of the experiment (Fig. 2.17, p. 87). As the number of leaves in each treatment represents the number of leaves which were present for the entire experiment, it became apparent that the plants in a high rate of nutrient supply lost double the number of leaves which were tagged at the start of the season than the plants in a low rate of nutrient supply. This was most likely a result of an accelerated ontogeny, such that the lower leaves were abscised earlier in the experiment.

Neither CO_2 concentration nor nutrient treatment had a significant ($p > 0.05$) effect on the allometric plots of rachis length versus leaf area (Fig. 2.18, p. 88). The overall allometric relationship between rachis length and the square root of leaf area was very tight ($r^2 = 0.94$) and was used for estimation of leaf area in Chapter Three. The tight allometric relationship ($r^2 = 0.90$) between plant height and dry mass (Fig. 2.2, p. 62) was used to infer starting masses of plants for use in calculations of RGR and nutrient addition rate.

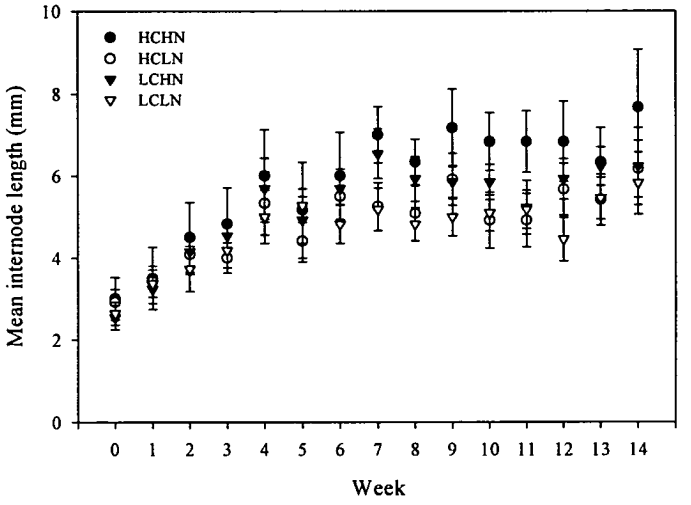


Figure 2.16 Growth of *C. odorata* internodes in 1996. For each plant, the rachis of the uppermost leaf at the start of the experiment was tagged and the extension of the internode immediately below was monitored. Values are shown for the internodes below leaves which persisted for the entire experiment. Mean values of internode length, plus or minus one standard error, are shown for each CO_2 concentration and nutrient treatment and week of experiment. Plant treatments were as follows: HCHN (elevated CO_2 concentration, high rate of nutrient application, $n = 6$), HCLN (elevated CO_2 concentration, low rate of nutrient application, $n = 12$), LCHN (ambient CO_2 concentration, high rate of nutrient application, $n = 7$) and LCLN (ambient CO_2 concentration, low rate of nutrient application, $n = 11$).

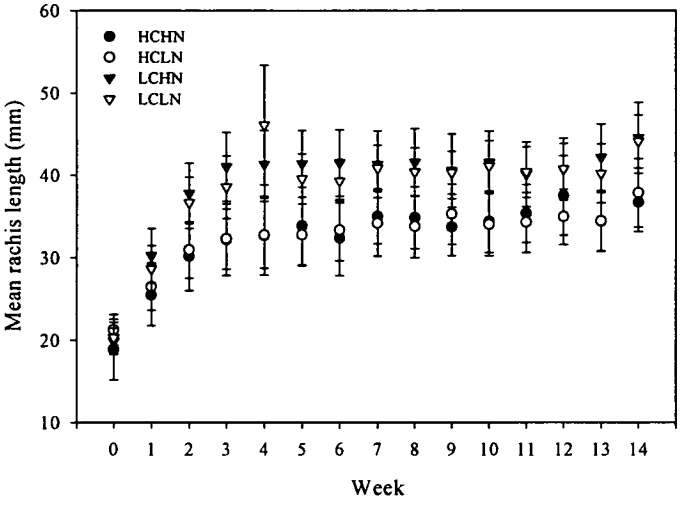


Figure 2.17 Growth of *C. odorata* leaves in 1996. For each plant, the rachis of the uppermost leaf at the start of the season was tagged and its extension monitored. Rachis lengths are shown only for leaves which persisted for the entire experiment. Mean values of rachis length are shown for each CO_2 concentration and nutrient treatment and week of experiment. Plant treatments were as follows: HCHN (elevated CO_2 concentration, high rate of nutrient application, n = 6), HCLN (elevated CO_2 concentration, low rate of nutrient application, n = 12), LCHN (ambient CO_2 concentration, high rate of nutrient application, n = 7) and LCLN (ambient CO_2 concentration, low rate of nutrient application, n = 11). Mean values are shown for each treatment $\pm$ one standard error of the mean.

C. odorata plants grown in either Perlite or Hydroleca™ in 1997 to investigate differences in plant ability to thrive in the two different growth media were similar. There was no significant difference in either average plant height or total dry mass of plants with growth medium ($p > 0.05$). Average plant height increases ($\pm$ one standard error of the mean) were 73 $\pm$ 18 % in Hydroleca™ and 74 $\pm$ 14 % in Perlite. Mean increases in biomass were 343 $\pm$ 103 % in Hydroleca™ and 319 $\pm$ 109 % in Perlite. Note that initial biomass was estimated from the allometric relationship of plant height with dry mass (Fig. 2.2, p. 62).

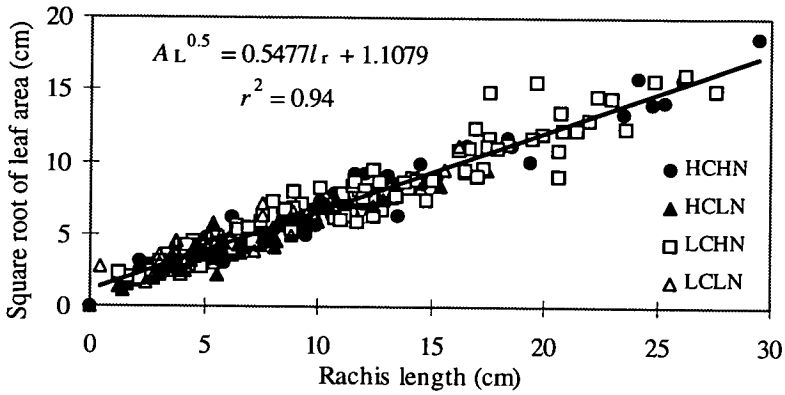


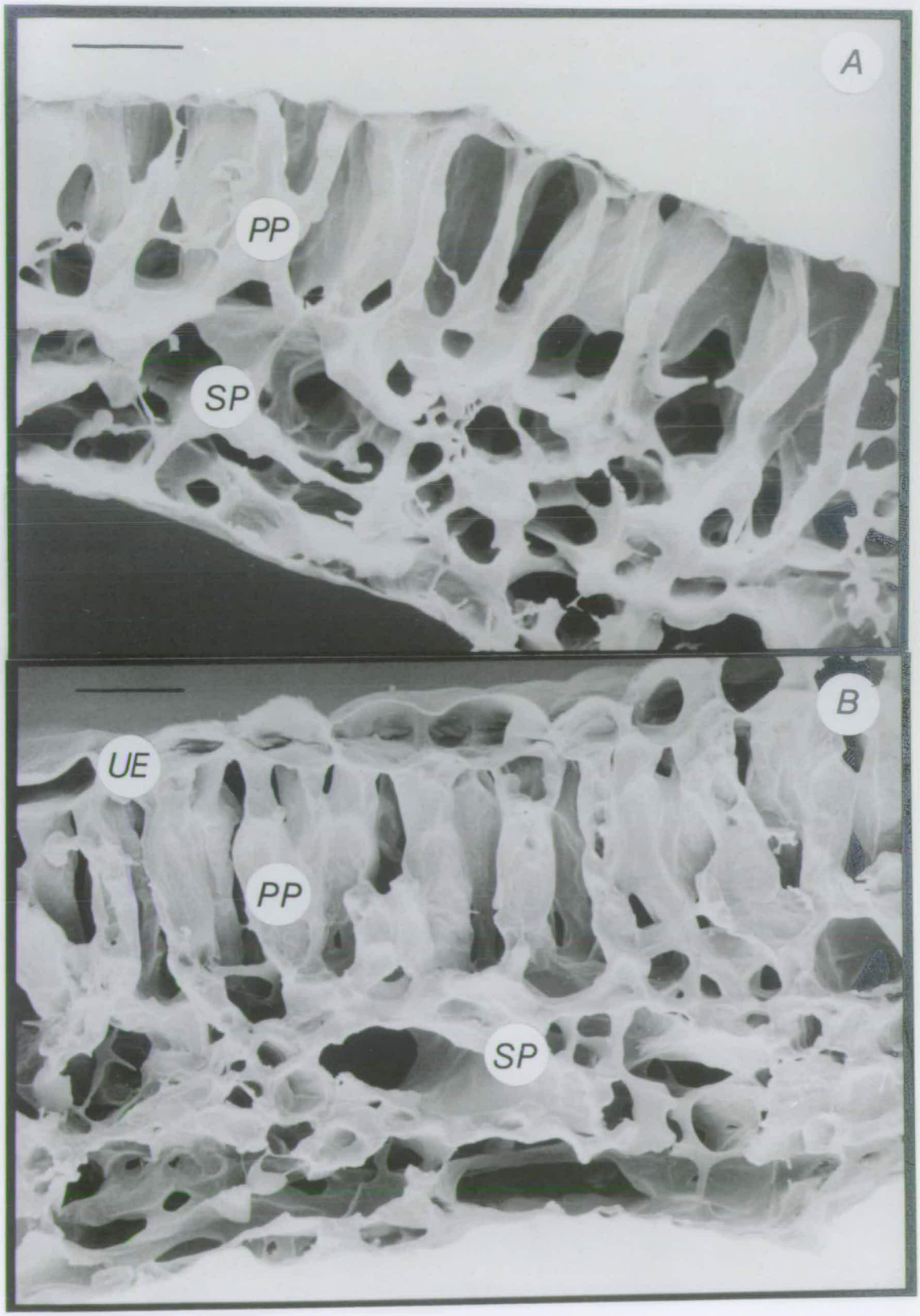
Figure 2.18 The allometric relationship between rachis length and the square root of leaf area of *C. odorata* at final harvest, 1996. $A_L^{0.5}$ = square root of leaf area. l_r = rachis length. Data from all plants of all CO_2 concentration and nutrient application rate treatments have been combined to establish the allometric relationship but leaves of individual treatments are represented by different symbols. CO_2 concentration and nutrient treatments were as follows: HCHN (elevated CO_2 concentration, high rate of nutrient application), HCLN (elevated CO_2 concentration, low rate of nutrient application), LCHN (ambient CO_2 concentration, high rate of nutrient application) and LCLN (ambient CO_2 concentration, low rate of nutrient application). All fully developed leaves on each plant (60 plants in total) have been included.

Leaf anatomy of *C. odorata*

The leaves of *C. odorata* were hypostomatous. Stomatal density did not differ significantly with CO_2 concentration or nutrient treatment ($p > 0.05$) in either 1995 or 1996 (Tables 2.2, p. 79 and 2.3, p. 84).

No anatomical differences were observed between leaves in elevated and ambient CO_2 concentrations. Although the palisade cells appear to occupy a proportionally larger volume of the mesophyll tissue than the spongy cells in leaves of plants grown in elevated CO_2 concentration (Fig. 2.19), the sample size was too small to test for differences statistically. Resolution of the images was insufficient to observe differences in starch granule size or relative abundance.

Figure 2.19 SEM micrographs of transverse sections of one leaflet from a *C. odorata* plant grown in ambient CO_2 concentration (A) and another plant grown in elevated CO_2 concentration (B) in 1996. Samples were prepared by freeze-drying and cryo-sectioning. Cells labelled are as follows: UE = upper epidermis. PP = palisade parenchyma. SP = spongy parenchyma. Note that the upper epidermis was removed from A during tissue fracture. Scale bars in top left hand corner represent 20 μm.


A
PP
SP
B
UE
PP
SP

Phytochemistry of *C. odorata*

In 1995, leaves of *C. odorata* appeared to have a higher concentration of total mono- and oligosaccharide in elevated CO_2 concentration although this difference was not statistically significant ($p > 0.05$). There were no differences between CO_2 treatments in chlorophyll and starch concentration ($p > 0.05$). Mono- and oligosaccharide, starch and chlorophyll concentration were all higher in leaves from plants grown in high nutrient application rates when compared with those in low nutrient application rates ($p < 0.05$, Table 2.4).

Table 2.4 Leaf concentrations of chlorophyll and carbohydrate components of *C. odorata* at final harvest, 1995. Chlorophyll is expressed as mass/unit leaf area for chlorophyll *a* and chlorophyll *b*. All concentrations are averages of all samples analysed chemically, plus or minus one standard error. HCHN = elevated CO_2 concentration, high rate of nutrient application, HCLN = elevated CO_2 concentration, low rate of nutrient application, LCHN = ambient CO_2 concentration, high rate of nutrient application and LCLN = ambient CO_2 concentration, low rate of nutrient application. Means with different superscripts within the same row are significantly different ($p < 0.05$).

	HCHN	HCLN	LCHN	LCLN
Chlorophyll *a*	0.28 ± 0.01^{a}	0.14 ± 0.01^{b}	0.29 ± 0.01^{a}	0.13 ± 0.01^{b}
($g\ m^{-2}$)	$n = 14$	$n = 14$	$n = 14$	$n = 14$
Chlorophyll *b*	0.12 ± 0.01^{a}	0.06 ± 0.005^{b}	0.13 ± 0.005^{a}	0.05 ± 0.004^{b}
($g\ m^{-2}$)	$n = 14$	$n = 14$	$n = 14$	$n = 14$
Starch ($mg\ g^{-1}$)	15.2 ± 6.1^{a}	30.2 ± 6.9^{b}	7.7 ± 1.1^{a}	25.2 ± 7.5^{b}
	$n = 14$	$n = 12$	$n = 14$	$n = 14$
Total mono +	7.95 ± 1.06^{a}	5.43 ± 0.54^{bc}	6.52 ± 0.60^{b}	4.75 ± 0.38^{c}
oligosaccharide	$n = 13$	$n = 12$	$n = 13$	$n = 14$
($mg\ g^{-1}$)				
Sucrose	1.58 ± 0.37^{a}	1.36 ± 0.36^{a}	1.02 ± 0.23^{a}	1.15 ± 0.27^{a}
($mg\ g^{-1}$)	$n = 13$	$n = 9$	$n = 13$	$n = 9$

In 1996 sucrose and starch concentration differed neither with nutrient application ($p > 0.05$) nor CO_2 concentration treatment ($p > 0.05$), and chlorophyll concentration was reduced only with a low rate of nutrient application ($p < 0.05$, Table 2.5).

Table 2.5 Leaf concentrations of chlorophyll and carbohydrate components of *C. odorata* at final harvest, 1996. Chlorophyll is expressed as mass/unit leaf area of chlorophyll *a* and chlorophyll *b*. All concentrations are averages, plus or minus one standard error. HCHN = elevated CO_2 concentration, high rate of nutrient application, HCLN = elevated CO_2 concentration, low rate of nutrient application, LCHN = ambient CO_2 concentration, high rate of nutrient application and LCLN = ambient CO_2 concentration, low rate of nutrient application. Means with different superscripts within the same row are significantly different ($p < 0.05$). The SPAD estimate of chlorophyll is based on the amount of light transmitted by intact leaves at two different wavelengths and was measured by a portable chlorophyll meter (SPAD-502, Minolta).

	HCHN	HCLN	LCHN	LCLN
Chlorophyll *a*	0.22 ± 0.01^{a}	0.17 ± 0.01^{b}	0.23 ± 0.01^{a}	0.15 ± 0.01^{b}
($g\ m^{-2}$)	$n = 14$	$n = 14$	$n = 15$	$n = 15$
Chlorophyll *b*	0.10 ± 0.006^{a}	0.07 ± 0.005^{b}	0.10 ± 0.005^{a}	0.07 ± 0.005^{b}
($g\ m^{-2}$)	$n = 14$	$n = 14$	$n = 15$	$n = 15$
Starch ($mg\ g^{-1}$)	31.1 ± 5.5^{a}	46.1 ± 12.7^{a}	25.6 ± 4.6^{a}	19.2 ± 3.4^{a}
	$n = 10$	$n = 9$	$n = 14$	$n = 7$
Total mono +	7.47 ± 0.58^{a}	6.09 ± 0.30^{b}	7.04 ± 0.6^{a}	5.54 ± 0.94^{b}
oligosaccharide	$n = 13$	$n = 9$	$n = 13$	$n = 9$
($mg\ g^{-1}$)				
Sucrose	0.82 ± 0.26^{a}	1.43 ± 0.23^{a}	0.75 ± 0.2^{a}	0.79 ± 0.29^{a}
($mg\ g^{-1}$)	$n = 13$	$n = 9$	$n = 13$	$n = 9$
SPAD estimate	25.93 ± 0.73^{a}	18.60 ± 0.5^{b}	27.38 ± 0.38^{a}	18.57 ± 0.39^{b}
of chlorophyll	$n = 13$	$n = 13$	$n = 13$	$n = 13$
(SPAD units)				

Final nutrient concentrations of leaves, stems and roots differed ($p < 0.05$) only with nutrient treatment in both years ($p < 0.05$, Tables 2.6 & 2.7).

Table 2.6 Summary of root, stem and leaf nutrient concentrations of *C. odorata* at the end of the 1995 experiment. All estimates are given as mean mg g^{-1} plus or minus one standard error. $n = 14$ for each of the CO_2 concentration and nutrient treatments, which were as follows: HCHN (elevated CO_2 concentration, high rate of nutrient application), HCLN (elevated CO_2 concentration, low rate of nutrient application), LCHN (ambient CO_2 concentration, high rate of nutrient application) and LCLN (ambient CO_2 concentration, low rate of nutrient application).

Nutrient	N	P	K	Ca	Mg
Root					
HCHN	41.7 ± 1.9	2.4 ± 0.2	25.6 ± 1.84	5.0 ± 0.3	3.0 ± 0.2
HCLN	15.3 ± 1.1	1.2 ± 0.04	15.5 ± 0.56	4.1 ± 0.2	5.5 ± 0.1
LCHN	37.9 ± 4.2	2.3 ± 0.2	24.7 ± 1.91	5.4 ± 0.5	3.3 ± 0.4
LCLN	15.7 ± 0.9	1.3 ± 0.1	18.6 ± 0.87	4.1 ± 0.3	5.9 ± 0.3
Stem					
HCHN	28 ± 0.09	2.6 ± 0.2	0.4 ± 0.02	0.1 ± 0.00	0.2 ± 0.01
HCLN	18 ± 0.23	2.4 ± 0.2	0.4 ± 0.03	0.2 ± 0.01	0.2 ± 0.02
LCHN	29 ± 0.11	3.0 ± 0.2	0.5 ± 0.03	0.1 ± 0.00	0.2 ± 0.01
LCLN	11.8 ± 0.15	2.3 ± 0.22	0.4 ± 0.01	0.2 ± 0.01	0.2 ± 0.01
Leaf					
HCHN	14.4 ± 0.05	2.2 ± 0.1	15.9 ± 1.0	2.76 ± 1.0	3.6 ± 0.1
HCLN	5.2 ± 0.05	1.2 ± 0.1	6.0 ± 0.9	6.57 ± 1.01	5.4 ± 0.6
LCHN	15.8 ± 0.06	2.3 ± 0.1	14.5 ± 1.1	3.08 ± 0.8	4.6 ± 0.3
LCLN	5.5 ± 0.07	1.4 ± 0.2	8.3 ± 1.5	6.23 ± 1.03	5.7 ± 0.6

Table 2.7 Summary of root, stem and leaf nutrient concentrations of *C. odorata* at the end of the 1996 experiment. All estimates are given as mean mg g^{-1} plus or minus one standard error. $n = 15$ for each of the CO_2 concentration and nutrient treatments, which were as follows: HCHN (elevated CO_2 concentration, high rate of nutrient application), HCLN (elevated CO_2 concentration, low rate of nutrient application), LCHN (ambient CO_2 concentration, high rate of nutrient application) and LCLN (ambient CO_2 concentration, low rate of nutrient application).

Nutrient	N	P	K	Ca	Mg
Root					
HCHN	19.4 ± 1.0	2.2 ± 0.2	28.6 ± 1.5	6.2 ± 0.2	6.1 ± 0.4
HCLN	11.5 ± 0.8	1.1 ± 0.0	25.5 ± 1.4	4.7 ± 0.2	7.1 ± 0.5
LCHN	18.1 ± 0.9	2.2 ± 0.1	28.8 ± 1.2	6.2 ± 0.3	6.0 ± 0.3
LCLN	10.4 ± 0.6	1.1 ± 0.1	24.1 ± 1.2	7.5 ± 2.4	7.3 ± 0.5
Stem					
HCHN	17.2 ± 1.6	2.3 ± 0.2	19.5 ± 1.0	15.9 ± 3.4	7.3 ± 0.7
HCLN	9.6 ± 1.1	0.9 ± 0.1	14.8 ± 1.3	7.7 ± 0.6	6.1 ± 0.6
LCHN	16.9 ± 1.2	2.2 ± 0.2	23.0 ± 1.7	12.6 ± 1.5	7.1 ± 0.6
LCLN	9.8 ± 1.4	1.2 ± 0.1	13.7 ± 1.2	12.3 ± 1.6	8.3 ± 1.1
Leaf					
HCHN	35.1 ± 2.7	3.0 ± 0.3	27.0 ± 1.5	17.7 ± 1.1	7.4 ± 0.8
HCLN	28.0 ± 1.3	1.8 ± 0.3	18.8 ± 1.3	22.7 ± 0.9	10.3 ± 1.3
LCHN	40.7 ± 1.5	3.3 ± 0.3	27.0 ± 0.2	19.5 ± 1.4	8.0 ± 1.3
LCLN	29.5 ± 2.2	2.0 ± 0.3	20.5 ± 2.0	23.2 ± 1.5	11.3 ± 1.1

Growth of *S. macrostachya*

There were no significant differences ($p > 0.05$) between CO_2 concentration treatments in biomass, height increase or leaf number and area of *S. macrostachya* (Figures 2.20 and 2.21).

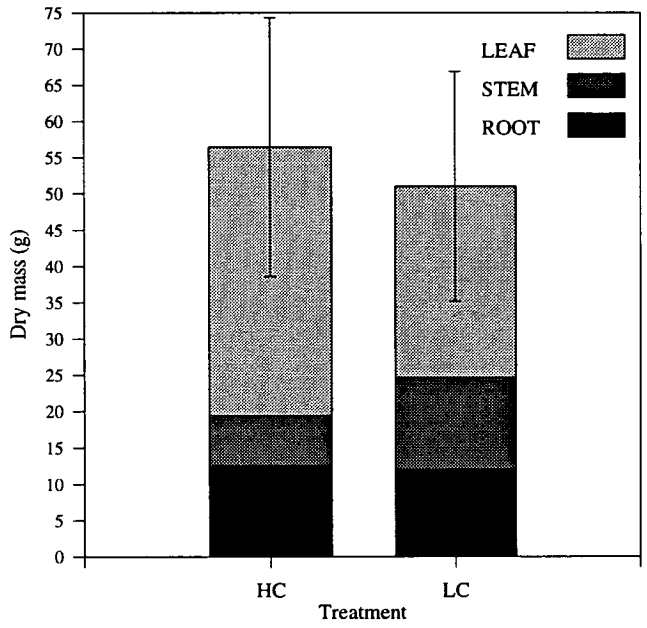


Figure 2.20 Mean values of dry mass of stem, leaf and root components of *S. macrostachya* plants grown in either elevated (HC) or ambient (LC) CO_2 concentrations in open top chambers. No attempt was made to control rate of nutrient supply. Mean values of average total dry mass are shown plus or minus one standard error. $n = 12$ per treatment.

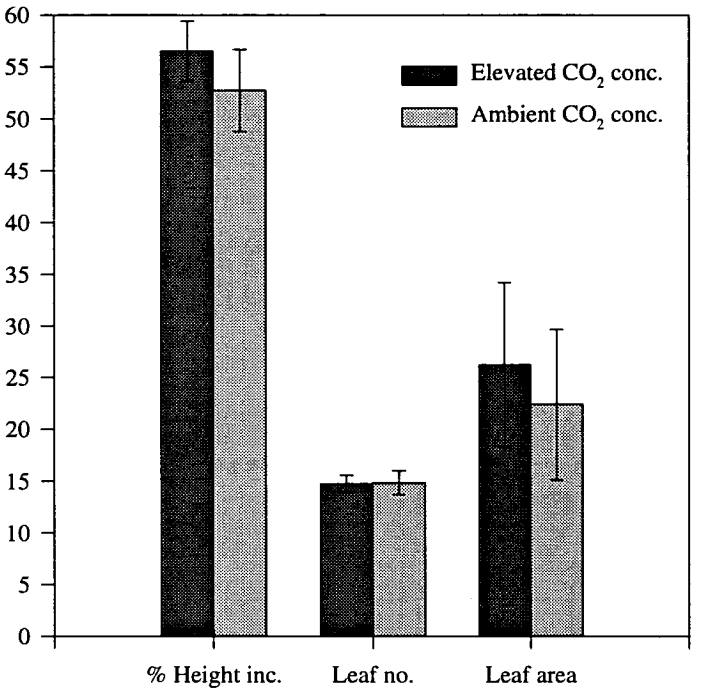


Figure 2.21 Morphology of *S. macrostachya* at final harvest grown in either ambient or elevated CO_2 concentration. No attempt was made to control rate of nutrient supply. Leaf areas are given as m^2 per 100 leaves. Height increase over the duration of the experiment is expressed as a percentage of original height. Means plus or minus one standard error are shown for each variable. n = 12 per treatment.

Leaf anatomy of *S. macrostachya*

Stomatal density was significantly increased ($p < 0.05$) in elevated CO_2 concentration. Leaves grown in elevated CO_2 concentration had an average stomatal density of 166 $\pm$ a standard error of 7 mm^{-2} whilst those on plants in ambient CO_2 concentration had an average stomatal density of 130 $\pm$ 9 mm^{-2}.

Phytochemistry of *S. macrostachya*

There were no significant differences in nutrient concentrations of either shoots or roots with elevated CO_2 concentration ($p > 0.05$, Table 2.8).

Table 2.8 Summary of root and shoot nutrient concentrations of *S. macrostachya* at the end of the 1996 experiment. All estimates are given as mg g^{-1}. Values are given as means in either ambient or elevated CO_2 concentrations plus or minus one standard error. n = 12 for each CO_2 concentration treatment.

Nutrient	N	P	K	Ca	Mg
Root					
Ambient	19.3 ± 1.4	4.4 ± 0.2	35.1 ± 1.2	6.2 ± 0.4	2.5 ± 0.1
Elevated	17.6 ± 1.8	4.0 ± 0.3	31.5 ± 3.1	6.1 ± 0.7	2.1 ± 0.3
Shoot					
Ambient	19.7 ± 1.0	3.0 ± 0.1	36.5 ± 2.3	15.9 ± 0.5	4.4 ± 0.2
Elevated	19.8 ± 0.7	3.2 ± 0.1	38.3 ± 4.8	16.7 ± 2.5	4.7 ± 0.2

DISCUSSION

Elevated CO_2 concentration did not have a strong stimulatory effect on the growth of *C. odorata* and *S. macrostachya*, particularly when compared with published results of other studies of growth of woody tree seedlings in elevated CO_2 concentration (Wullschleger *et al.* 1995). Possible explanations for the small size of the responses are explored.

Effects of elevated CO_2 concentration on *C. odorata* growth in 1995

Only the dry mass of *C. odorata* roots increased significantly in plants grown in elevated CO_2 concentration with a high nutrient supply (Fig. 2.8, p. 76). There was no statistical difference in mean RGR. This may be a feature of the timing of the harvests. A number of investigators have concluded that the stimulation of RGR with elevated CO_2 concentration occurs in the initial stages of exposure (Wulff & Strain 1982, Bazzaz 1990, Rey & Jarvis 1997). The first harvest was taken at 41 days and it is possible that by this time, RGR stimulation had already ceased. This may explain the overall lack of significance in RGR increase, calculated for the entire growing season.

Another possible explanation for the lack of difference in RGR may be compensations between NAR and LAR, which constitute calculated RGR. Mean NAR tended to increase with elevated CO_2 concentration while mean LAR tended to decrease with elevated CO_2 concentration (Fig. 2.9, p. 77). Although the decrease in LAR was not statistically significant ($p > 0.05$), it is hypothesised that while the rate of mass accumulation for a given leaf area (NAR) increased, the fraction of biomass apportioned to leaves (LAR) decreased, contributing to an overall non-significant increase in RGR with elevated CO_2 concentration ($p > 0.05$).

Plants grown at a low nutrient supply rate showed no difference in final biomass, yet the RGR of plants in elevated CO_2 conditions was higher. Because growth rate is exponential it seems likely that the increase in RGR would give a significant increase in biomass of plants grown in elevated CO_2 concentration, had the experiment been longer. It is hypothesised that the lack of significant difference in biomass at the end of the current experiment was a result of the large variation within a treatment, relative to the mean estimate of total dry mass.

The sources of within-treatment variation in the present experiment are thought to be a combination of environmental variation and genetic variation between plants. Environmental heterogeneity both within and between chambers may be sizeable (Fuhrer 1993; Potvin 1993). Although differences between provenances have been reported to account for considerable morphological variation in *C. odorata* (Newton *et al.* 1996), differences in dry mass were tested for between provenances and found to be not significant in this experiment ($p > 0.05$). Therefore, the observed variation is thought to be a result of genetic differences within a provenance, as well as a small amount of heterogeneity in PPFD and temperature within and between chambers (Figures 2.3 & 2.4, pp. 70-71). It is also thought that the highly significant nutrient effect has swamped the elevated CO_2 concentration effect in statistical tests.

Effects of elevated CO_2 concentration on *C. odorata* growth in 1996

There were no significant differences in either plant growth or morphology with elevated CO_2 concentration in 1996. In fact, high nutrient plants appeared larger in ambient than elevated CO_2 concentrations (Fig. 2.12, p. 81), but the difference was not significant. Plants were all of the same provenance (San Antonio) in 1996 confirming that a high degree of variation can occur within a provenance as well as in environmental conditions. Environmental variation between chambers was

smaller in both temperature and PPFD compared to 1995, with only chamber six being noticeably different to the other chambers (Figures 2.5 & 2.6, pp. 72-73).

Although the exact cause of the stunting in this experiment is unknown it has been reported that *C. odorata* plants are particularly vulnerable to induced bud dormancy as a result of short days or decreases in average night temperatures (Longman & Jeník 1974).

Interaction of nutrient limitation with elevated CO_2 concentration in *C. odorata*

Many authors have concluded that plant response to elevated CO_2 concentration is likely to be reduced by nutrient deficiency (reviewed by Ceulemans & Mousseau 1994). This is true for the present study only for root biomass where the increment was only significant in elevated CO_2 concentration with a high rate of nutrient supply. The relative growth rates tended towards a greater stimulation in elevated CO_2 with a low rather than a high rate of nutrient supply (Figures 2.10, p. 78 & 2.14, p. 83). A greater relative, rather than absolute, increase in plant growth rate in conditions of low nutrient supply, rather than high nutrient supply, is also predicted by a recent review of the literature (see Table 2.9 reproduced from the review by Lloyd & Farquhar 1996). It is proposed that the lack of statistically significant increase in the final biomass of *C. odorata* grown in elevated CO_2 with low nutrient supply is merely a function of the large variation within a treatment relative to final plant mass. Running the experiment for a longer period of time, to allow further increase in plant size, is predicted to reveal a larger relative increase in biomass of plants in elevated CO_2 with a low rather than a high rate of nutrient supply. There was no significant interaction shown in statistical tests between CO_2 concentration and nutrient supply rate ($p > 0.05$ in all cases) but this may again be a result of the

relatively large contribution of the nutrient effect when compared with the CO_2 concentration effect.

Table 2.9 Production ratios (growth rate at 2 x ambient CO_2 concentration/growth rate at ambient CO_2 concentration) in experiments where nitrogen nutrition has been varied (reproduced from Lloyd & Farquhar 1996).

Species	Production ratio at low nitrogen nutrition	Production ratio at high nitrogen nutrition	Reference
Abutilon theophrasti	1.0	1.3	(Zangerl & Bazzaz 1984)
Ambrosia artemisiifolia	1.1	1.0	(Zangerl & Bazzaz 1984)
Bromus mollis	1.0	1.4	(Larigauderie, Hilbert & Oechel 1988)
Chenopodium album	0.5	1.4	(Zangerl & Bazzaz 1984)
Eucalyptus calmadulensis	1.8	2.7	(Wong *et al.* 1992)
Eucalyptus cypellocarpa	1.9	3.1	(Wong *et al.* 1992)
Eucalyptus grandis	2.8	3.9	(Conroy, Milham & Barlow 1992)
Eucalyptus pauciflora	3.1	2.4	(Wong *et al.* 1992)
Eucalyptus pulverulenta	2.6	3.2	(Wong *et al.* 1992)
Glycine max	1.2	1.2	(Vessey, Henry & Raper 1990)
Gossypium hirstutum	2.4	2.5	(Wong 1979)
Gossypium hirstutum	2.1	1.5	(Wong 1990)
Picea glauca	1.3	1.5	(Brown & Higginbotham 1986)
Populus tremuloides	1.3	1.0	(Brown & Higginbotham 1986)
Polygonum pensylvanicum	2.0	1.7	(Zangerl & Bazzaz 1984)
Triticum aestivum	1.7	1.4	(Hocking & Meyer 1991)
Triticum aestivum	2.3	2.0	(Wong & Osmond 1991)
Xanthium occidentale	1.3	1.1	(Hocking & Meyer 1985)
Cedrela odorata (1995 data)	1.1	1.0	this study
Cedrela odorata (1996 data)	0.9	0.8	this study

It is important to separate the effects of nutrient limitation from those of sink limitation (Pettersson & McDonald 1994) and experiments which have not used Ingestad principles in nutrient application but have reported limits to CO_2 concentration response as a direct effect of nitrogen limitation, should be viewed with caution (Eamus & Jarvis 1989). Many studies of elevated CO_2 concentration effects using pot-grown plants have been criticised on the basis of small pot size (Arp 1991; Thomas & Strain 1991; Gunderson *et al.* 1993). Plants in the present experiments, however, were grown in large pots with no indication of pot binding. The use of the Ingestad nutrient regime ensured that plants were in a steady state with respect to nutrient supply and were, therefore, not sink-limited. One may conclude that *C. odorata* seedlings with a low rate of nutrient supply show a smaller absolute, but a larger relative, response to increases in atmospheric CO_2 concentration than plants grown at a high rate of nutrient supply.

Comparison of *C. odorata* growth in 1995 with that of 1996

Plants grew much larger in 1995 than 1996 (Figures 2.8, p. 76 & 2.12, p. 81). The experimental duration was 17 days longer but this could not account for mass accumulation approximately eight times that of final 1996 biomass. Using the average final mass and the average RGR calculated over the entire season, the expected increase in biomass with an extra 17 days experimental duration can be calculated as 1.94 g from 3.12 g to 5.06 g of final dry mass.

Were the environmental conditions responsible for growth limitation of C. odorata in 1996?

The difference in biomass between the two years can be largely explained by the more favourable growing conditions of the 1995 experiment. The chambers in the RBGE had a higher temperature (2 °C warmer on average) and PPFD (60 μmol m^{-2} s^{-1} higher on average). Also *D* was 5 % lower in the 1995 experiment ($p < 0.05$). Mean values of *D* for both years are very high (1.3 - 1.4 kPa) when compared with those of a previous study of an Amazonian rain forest in Brazil (Roberts *et al.* 1990). In the reported study, stomata showed a marked decline in conductance when exposed to a *D* of 0.6 kPa which is approximately half that of the present study. Values are at the high end of the range of *D* reported in an Amazonian rain forest in the current study where there was thought to be some drought stress occurring (Fig. 4.1, Chapter Four, p. 156). The increase in *D* in 1996, compared with 1995, may well be of biological significance as stomata can be very sensitive to even small changes in *D* (Jones 1992). It is thought that the most important difference in the growing conditions of the two years is the number of sunshine hours. 1995 had an average of a 64 % greater total of sunshine hours per month than in 1996. It is thought that the greater number of sunshine hours more closely approximates the conditions that would naturally occur in a tropical region.

In 1996 the high nutrient plants in the ambient CO_2 concentration treatment appeared larger than those in the elevated CO_2 treatment. This may be a result of the poorer overall growing conditions so that plants the extra atmospheric CO_2 could not be utilised. For example, non-saturating light conditions have been shown to reduce the effect of CO_2 concentration in some experiments (Mousseau & Saugier 1992). *C. odorata* is a fast-growing pioneer species, the young trees of which are reported to be highly intolerant of overhead shade (Newton *et al.* 1993). Bazzaz *et al.* (1990) found shade-intolerant tree species less responsive to elevated CO_2 concentration than the

shade-tolerant species of their investigation. They proposed that the low peak light intensity of their experiment was limiting the shade-intolerant species and they, therefore, showed a proportionally smaller CO_2 concentration response. However, experimental data have shown that *C. odorata* does not show the physiological characteristics of a shade-intolerant species and may actually be depressed by extremely high PPFD (Ramos & Grace 1990). Tree response to the interaction of limiting resources is complex, particularly with respect to light and CO_2 concentration, and it has also been observed that elevated CO_2 concentration may show the largest effect at low light (Bazzaz & Miao 1993).

Was the nutrient regime responsible for growth limitation of C. odorata in 1996?
Another possible limit to growth in 1996 was a problem with the nutrient regime. Although the nutrient solution was made afresh in 1996 the recipe used for the first 72 days of the experiment was the same as that used for all of 1995. A change in the stock solution after 72 days did not immediately improve the performance of the plants, but it is usual for there to be a lag phase after a change in the nutrient supply, while the plants adjust to the new medium (Ingestad & Agren 1992).

Results suggested that the nutrient regime used in 1995 was sufficient to maintain a high nutrient RGR of 5 % per day (Fig. 2.9, p. 77), as predicted. There were no indications that this nutrient recipe or the exponential feeding regime were in any way limiting of plant growth in 1995. However, the watering strategy differed between the years. In 1995 staff of the RBGE watered the plants daily by means of an irrigation system. Water in plant saucers frequently ran over. Watering was carefully controlled in 1996 to ensure no loss of nutrient solution from saucers. Seedlings clearly lagged behind in their growth rate in 1996 - the growth of the high nutrient plants was around 3.5 % per day. Because a little extra nutrient was added to high nutrient plants in both years (in case 5 % was an underestimate of the maximum RGR extrapolated from the experiment of Ramos & Grace 1990) it could

be that the solution left sitting in the saucers was increasingly concentrated in 1996, burning the roots of the plants and limiting their growth.

The growth medium was changed in 1996 to a hydroponic support of expanded clay as opposed to Perlite. Results from the 1997 experiment, testing differences in plant growth in these two media, suggest that the growth medium was not in itself responsible for differences in plant growth between the two years.

Were C. odorata seedlings already too mature to respond fully to elevated CO_2 concentration by the time of their exposure?

It is possible that the lack of observed CO_2 concentration response in 1996 was the result of a lag between time of germination and exposure to elevated CO_2 concentration. There is a lot of evidence suggesting that growth increment with elevated CO_2 concentration is determined early in the period of exposure to elevated CO_2 concentration (Wulff & Strain 1982; Bazzaz 1990). Plants show an initial stimulation of RGR and the subsequent growth that occurs in this extra tissue laid down at the beginning leads to an enhanced compound interest growth pattern, regardless of whether or not the actual CO_2 concentration effect is sustained (Norby *et al.* 1996).

It has also been suggested that increase in atmospheric CO_2 concentration causes a general acceleration of ontogeny which may be more important than a specific CO_2 concentration response (Gunderson & Wullschleger 1994; Rey & Jarvis 1997). However, in this experiment there was a lag between germination and exposure of seedlings to elevated CO_2 concentration. This lag was particularly pronounced in the second year of the experiment (1996) because of technical difficulties with the system. It is possible that seedlings had entered the exponential growth phase of their development and were therefore less responsive to the elevated CO_2

concentration. This is consistent with a lack of statistically significant response in *S. macrostachya* which was grown in ambient CO_2 concentration conditions for a year before exposure to elevated CO_2 concentration.

Did acclimation occur in C. odorata plants in 1996?

Did the plants grown in elevated CO_2 concentration in 1996 show an acclimation response? Many authors have reported lack of responsiveness at the end of the growing season, or even depression of photosynthesis of plants in an elevated CO_2 concentration, as an acclimation response (Wulff & Strain 1982; DeLucia *et al.* 1985; Reekie & Bazzaz 1989; Socias *et al.* 1993). In the majority of these studies increases in photosynthetic rates and plant growth were seen in plants grown in elevated CO_2 concentration at the start of the experiments and these differences slowly declined over time. There was no increase in mean NAR for the whole season in elevated CO_2 concentration in 1996, suggesting that there was no net stimulation of photosynthesis and that acclimation had occurred. In addition, photosynthetic parameters (discussed in a later chapter) used to test differences in photosynthetic rates showed signs of down-regulation in elevated CO_2 concentration.

There was no secondary evidence of acclimation such as starch build-up in the leaves (Table 2.5, p. 91) or a significant decrease in SLA (Fig. 2.13, p. 82). Neither was there a significant decline in foliar N concentrations (Table 2.7, p. 93) which would have confirmed reallocation of nitrogen away from leaves to other organs (Bowes 1991). On balance, however, it appears as if acclimation did occur and that there was something peculiar about the 1996 experiment which triggered this acclimation in comparison with the 1995 experiment.

Effects of elevated CO_2 concentration on *S. macrostachya*

S. macrostachya plants showed no significant increase in plant height or mass on exposure to an elevated concentration of CO_2 (Figures 2.20 & 2.21, pp. 94-95). Again there was a trend towards stimulation of growth but this was not significant because of the large amount of variation within the CO_2 treatments. This variation in *S. macrostachya* plants may be accounted for by large differences in size of the one year-old seedlings before the CO_2 concentration treatment commenced.

Surprisingly, stomatal density was increased with exposure to elevated CO_2 concentration ($p < 0.05$). This is not an uncommon result according to Woodward and Kelly (1995), but these authors expect that plants will decrease their stomatal density in response to elevated CO_2 concentration as a result of a reduced necessity to maximise the influx of CO_2.

Are tropical plants less responsive to elevated concentrations of CO_2?

Literature reviews of the extent of growth stimulation in elevated CO_2 concentration have consistently ranked tropical trees as the least responsive of tree species studied (Luxmoore *et al.* 1993; Wullschleger *et al.* 1995). On average, growth is stimulated by 25 % in tropical trees as opposed to 38 % for trees of the boreal region, for example (Luxmoore *et al.* 1993). In the present experiment, the total growth increment of *C. odorata* in elevated CO_2 concentration was not statistically significant. An inherent lack of tropical species responsiveness could account for some of the lack of statistical significance of both species responses, but, given a longer experimental duration, an increased number of replicates, and with more careful control of *S. macrostachya* nutrient application, to ensure that nutrient supply rate is non-limiting, it is possible that the effect of CO_2 concentration on growth

would have been significant in this species. The growth of *C. odorata* was stimulated by an elevated CO_2 concentration but the statistical significance of this effect appears to have been reduced as a result of the large nutrient effect in 1995. It is suggested that growth in 1996 was impeded by some other factor.

In conclusion, growth of *C. odorata* seedlings was stimulated in the short term by elevated CO_2 concentration, where other environmental variables were not limiting to growth response. This effect was larger in conditions of a stable supply of nutrient at a high rate. There was no initial change in plant development, morphology or leaf anatomy, nor were there any changes in phytochemicals or tissue nutrient concentrations with elevated CO_2 concentration. *S. macrostachya* showed no significant response to elevated CO_2 concentration but no attempts were made to control rate of nutrient supply so sink limitations and nutrient deficiency can not be ruled out as causes of the lack of response.

CONCLUSIONS

1) Elevated CO_2 concentration stimulated root biomass increment of *C. odorata* seedlings significantly, with a trend to an increase in total biomass increment. This seemed to depend on other growth conditions being optimal, as was assumed to be the case in 1995 but not 1996.

2) *C. odorata* plants grown at stable high rates of nutrient supply in 1995 showed a bigger absolute growth response to elevated CO_2 concentration than those grown at low rates of nutrient supply.

3) The relative growth rates of *C. odorata* plants grown in elevated CO_2 concentration, appeared to have been stimulated more at low rates of nutrient supply than plants grown in elevated CO_2 concentration at high rates of nutrient supply.

4) There were no other significant manifestations of plant response to elevated CO_2 concentration in either year, such as changes in plant morphology or development, leaf anatomy or foliar chemical concentration.

5) In 1996 *C. odorata* lacked the capacity to respond to an elevated CO_2 concentration. It is possible that an inappropriate nutrient regime exacerbated the poor growth which resulted from sub-optimal growing conditions. The nutrient supply rate was greater than the plant growth rate, allowing a potentially deleterious build-up of nutrient in solution.

6) Where growth of *C. odorata* was limited, possibly by a combination of reduced numbers of sunshine hours, high saturation deficit and excessive concentrations of nutrient, as in 1996, acclimation to elevated concentrations of CO_2 was triggered.

7) *S. macrostachya* showed no statistically significant responses to an elevated concentration of CO_2. It is possible that the correct application of an Ingestad nutrient regime and the inclusion of plants in the experiment from the time of germination would have reduced the amount of intra-treatment variability sufficiently to show a statistically significant growth increase in response to elevated CO_2 concentration.

Chapter Three:
Effects of an elevated concentration of atmospheric CO_2 on the gas exchange of a tropical tree, *Cedrela odorata* L., in its seedling stage

INTRODUCTION

The analysis of leaf gas exchange can elucidate both direct and indirect responses of photosynthesis to CO_2 enrichment (Sage 1994), and has been widely used in studies on herbaceous and tree species. Gas exchange analysis enables quantification of the effect of elevated CO_2 concentration on leaf physiology. This is vital if experimental effects are to be collated and used in a predictive capacity. The majority of authors report an increase in photosynthesis at elevated CO_2 concentrations (Radin *et al.* 1987; Norby *et al.* 1992; Silvola & Ahlholm 1992; Gunderson *et al.* 1993), the average increase for tree seedlings being about 50 % following doubling of the CO_2 concentration (reviewed in Cure & Acock 1986). There are very few studies of the effect of elevated CO_2 concentration on tropical tree species (Hogan *et al.* 1991). Whilst increases in photosynthesis with elevated CO_2 concentration have been reported, in many cases this stimulation of photosynthesis declines or even disappears after a lengthened period of exposure to elevated atmospheric CO_2 concentration (Gaudillère & Mousseau 1989; Hogan *et al.* 1991; Mousseau &

Saugier 1992; Grulke *et al.* 1993). This decline may represent a down-regulation of photosynthesis, perhaps involving changes at a biochemical level that improve the overall performance of the plant. The process has been termed "acclimation" (Sage 1994).

Photosynthetic responses to elevated CO_2, particularly acclimation, can be investigated by examining the relationship between assimilation rate (A) and intercellular CO_2 concentration (C_i) (Hogan *et al.* 1991). The interpretation of the A/C_i curve is now well established (Farquhar *et al.* 1980) and enables an analysis to be made of the acclimation response (Sage 1994). For example, an observed decline in the initial slope of the A/C_i curve suggests that acclimation in the form of a reduction in Rubisco activity has occurred (Wong 1979; Stitt 1991). The nature and existence of the acclimation response is controversial. It has been suggested, for example, that acclimation may be merely an artefact of growth conditions particularly where nutrient limitation is suspected (Sage 1994). Therefore nutrient interaction with elevated CO_2 concentration should be investigated through the use of A/C_i curves or an equivalent method which details the photosynthetic response to CO_2 in both ambient and elevated concentrations of CO_2 (Gunderson & Wullschleger 1994). In addition to estimating the acclimation response, the derived parameters from the A/C_i curve can be used in models of photosynthesis and can therefore be directly perturbed to estimate photosynthesis in an elevated CO_2 atmosphere.

The decrease in photorespiration with an increase in intercellular CO_2 concentration is arguably the most important effect of an increased concentration of atmospheric CO_2 (Drake *et al.* 1997). However, photosynthetic responses should be viewed as only partly responsible for growth determination. Other processes may be equally or even more important, such as respiration and the allocation of dry matter between leaf, stem and root tissues (Körner 1991). It has been estimated that up to 50% of all

carbon fixed in photosynthesis is respired (Kira 1975), yet, plant respiration in relation to an elevated CO_2 concentration remains relatively unresearched and poorly understood (Amthor 1991). Both increases and decreases in dark respiration of leaves with elevated CO_2 concentration have been reported, making predictions unclear (reviewed by. Amthor 1995).

Some of the confusion may be resolved by separating the direct effects of an elevated CO_2 concentration on plant respiration from the indirect effects (Drake *et al.* 1997). Elevated CO_2 concentration directly inhibits some of the enzymes involved in mitochondrial electron transport (Gonzàlez-Meler *et al.* 1996), explaining the 20 % average instantaneous decrease in respiration upon plant exposure to a CO_2 concentration twice that of ambient (Drake *et al.* 1997). Although a decrease in leaf Rubisco content should correlate with a decrease in leaf respiration, this decrease in leaf respiration is unlikely to be significant when expressed as a proportion of leaf mass, which is expected to increase with elevated CO_2 concentration (Drake *et al.* 1997).

Little detailed work has been done on changes to root and associated soil microbial respiration which could be a major factor if mycorrhizal colonisation is increased with elevated CO_2 concentration (Rogers *et al.* 1994). Exudation may account for as much as 18 % of whole plant dry matter (Barber & Martin 1976), an increase in which would contribute to an increase in soil carbon storage and CO_2 efflux.

In this chapter I present A/C_i curves of tropical forest seedlings of *Cedrela odorata* L. grown in both ambient and elevated CO_2 concentration with either high or low rates of nutrient application. In addition, whole-plant gas exchange was measured in both ambient and elevated CO_2 concentration for plants which had been grown in either of these concentrations, for a thorough investigation of any acclimation response.

METHODS

Plant material and growth conditions

For full details of plant material and growth conditions refer to Chapter Two. The same plants were used as in the previous chapter where growth and phytochemical response to elevated CO_2 concentration were measured.

Nutrient regime

Seedlings of *C. odorata* were supplied with nutrients at a rate proportional to that of their growth. The effect of the interaction of nutrient limitation with elevated CO_2 concentration on photosynthesis was only investigated in 1995. For full details of the nutrient regime refer to Chapter Two.

Photosynthesis measurement

In 1995 the investigation took the form of measurements of rate of net photosynthesis (A) for a range of intercellular CO_2 concentrations (C_i) and photosynthetic parameters were derived from the resulting A/C_i curves (Harley *et al.* 1992). A/C_i curves were obtained for all four treatments, namely ambient or elevated CO_2 concentration with high or low rate of nutrient supply.

In 1996 three types of gas exchange measurements were made. A/C_i curves were obtained for plants of high nutrient supply only of both elevated and ambient CO_2 concentrations. Second, spot measurements of photosynthesis were made on plants of all high nutrient treatments at both concentrations of CO_2 *i.e.* at both the growth

and the reciprocal concentration of CO_2. Third, whole-plant gas exchange was measured using a custom-built chamber in order to obtain the daily carbon balance.

A/C_i curves

A/C_i curves were obtained in weeks 9-10 of the 1995 experiment and in week 11 of the 1996 experiment. C_i was adjusted in the range 18 to 1300 μmol mol^{-1} by using an external source of CO_2 at a high concentration and varying the concentration supplied to the sample chamber through the use of the scrubber tube. The resultant changes in A were measured using a portable gas exchange system (LCA-3, Analytical Development Company, Hoddesdon, UK). Photosynthesis was allowed to equilibrate to each new value of C_i for between five and ten minutes by allowing values of A to stabilise before the measurements were recorded. Measurements were started at the operational C_i (approximately 225 μmol mol^{-1}) and then made at decreasing C_i intervals of approximately 50 μmol mol^{-1}. Once C_i was estimated to be around 0 μmol mol^{-1} it was subsequently restored to the operational C_i and then increased in intervals of approximately 200 μmol mol^{-1} until saturation was reached. In all cases an artificial light source (with a PPFD of *ca* 1100 μmol m^{-2} s^{-1}) was used to ensure light saturation, which was assumed to occur at about 800 μmol m^{-2} s^{-1} for this species (Ramos & Grace 1990). Air was taken from within an open-top chamber and then bubbled through water to ensure a relative humidity of about 75 % in the leaf cuvette. No attempt was made to control leaf temperature.

In 1995 the terminal leaflet of the uppermost fully expanded, but non-senescent, leaf was measured on each of four plants selected at random from each of the four nutrient/CO_2 treatments. In 1996 the terminal leaflet of the uppermost fully expanded, but non-senescent, leaf was measured on each of eight plants in either ambient or elevated CO_2 concentration with the high nutrient treatment. In 1996

A/C_i curves were not obtained for the low nutrient treatments as leaves did not appear to be fully developed at the time of A/C_i measurement.

The Farquhar *et al.* model (1980) was fitted to the A/C_i data, using an Excel spreadsheet (de Pury & Farquhar 1997) designed specifically for obtaining estimates for the photosynthetic parameters of J_{max}, V_{cmax} and R_d (defined below). Because light response curves were not measured, the convexity parameter θ, was taken as 0.67, an average value for broadleaf species (Farquhar & Wong 1984). Derived photosynthetic parameters were then compared between treatments.

Model equations:

An observed value of A can be defined for a given value of C_i, as the minimum value of two overlaid curves (Fig. 3.1, p. 122). These are the curves of A_j (rate of photosynthesis limited by ribulose-1,5-bisphosphate (RuBP) regeneration) and A_v (rate of photosynthesis limited by rate of ribulose 1, 5 bisphosphate carboxylase-oxygenase enzyme (Rubisco) activity) and the saturation value for each of these processes has been defined as J_{max} and V_{cmax} respectively. The rate of RuBP regeneration is determined by the velocity of electron transport in the light reactions while the behaviour of Rubisco in the dark reactions can be described by Michaelis-Menten enzyme kinetics. Thus J_{max} is the maximum rate of electron transport and V_{cmax} the maximum velocity of the Rubisco enzyme. R_d, the so-called day respiration rate, is defined as the rate of dark respiration in the light (Farquhar *et al.* 1980).

A full description of the model can be found in Chapter One, with model equations 1.1 - 1.4 given on pp. 17-18.

Further equations of electron transport dependence on irradiance and other Rubisco parameters on temperature, which are used by this fitting procedure are published elsewhere (de Pury & Farquhar 1997). These are used to extrapolate the observed response to that at 25 °C. Parameters reported by this study are therefore those for a leaf at 25 °C.

Spot measurements of photosynthesis in 1996

Spot measurements of photosynthesis were made in week 7 of the experiment. Measurements of A were made using a portable photosynthesis analyser (LCA-3, Analytical Development Company, Hoddesdon, UK). One leaf on each of six individual plants grown at elevated CO_2 concentration was measured instantaneously at 700 μmol mol^{-1} CO_2 concentration and then at 350 μmol mol^{-1} concentration. Thus a comparison could be made between current photosynthesis and the potential photosynthetic rate at the reciprocal CO_2 concentration. All leaves were measured using an artificial light source supplying a PPFD of about 1100 μmol m^{-2} s^{-1}.

Measurements of stomatal conductance (g_s) were made simultaneously with A using the same apparatus to enable comparisons between treatment concentrations of CO_2.

Whole-plant gas exchange in 1996

Whole-plant gas exchange of eight high nutrient plants from each of the elevated and ambient CO_2 concentration treatments, was measured using a purpose-built acrylic box (Barton 1997). These measurements occurred throughout weeks 8 - 15 of the experiment with plants selected for measurement coming from a growth concentration of alternately ambient then elevated CO_2. The box was divided into an upper and lower chamber to allow separate measurement of above and below-ground processes (Appendix 5). Each plant was kept in the box for 48 hours and was fully watered at the start of the measurement period. During the first 24 hours, gas exchange of both above and below ground parts of the plant was measured at the

growth CO_2 concentration. During the second 24 hours, gas exchange was measured at the reciprocal CO_2 concentration *ie.* 350 μmol mol^{-1} for plants grown at elevated CO_2 concentration and 700 μmol mol^{-1} for plants grown at ambient CO_2 concentration.

The treatments were, therefore, described as plants grown at elevated CO_2 concentration and measured at ambient CO_2 concentration (E350), plants grown at elevated CO_2 concentration and measured at elevated CO_2 concentration (E700), plants grown at ambient CO_2 concentration and measured at ambient CO_2 concentration (A350), plants grown at ambient CO_2 concentration and measured at elevated CO_2 concentration (A700).

Both the upper and lower chambers were sealed at the start of each cycle of measurements, although only one chamber was measured at a given measurement time. Measurements were taken of the change in CO_2 concentration in the upper chamber on every hour and half hour. Measurements were taken of the change in CO_2 concentration in the lower chamber at every 15 and 45 minutes past the hour. Each chamber was measured for a period of five minutes as a closed system for the measurement duration. At the end of this measurement period both chambers were fully flushed out for ten minutes before the next measurement. Full details of measurement and control of the CO_2 concentration are published elsewhere (Barton 1997).

The rate of gas exchange per plant was calculated from a knowledge of the rate at which the CO_2 of the closed chamber was depleted or enriched. The CO_2 concentration of air within each chamber was recorded every 20 seconds. These rates of change were usually linear, and could be described by a linear regression equation fitted to the data. Rate of change in CO_2 concentration was later corrected

for chamber volume and leaks to convert fluxes to estimates for a single plant. Upper chamber volume was 0.165 m^3 and lower chamber volume was 0.048 m^3.

The chamber was run at 700 μmol mol^{-1} with no plant in it for 24 hours on two separate days. Average values of the observed leakage were used to correct plant fluxes for changes in CO_2 concentration that were independent of plant behaviour. It was assumed that this leakage was proportional to the concentration of CO_2 inside the chamber at the time of measurement and that the relationship between CO_2 concentration and leakage was linear. Estimates of net above-ground photosynthesis, below-ground respiration and of whole-plant CO_2 flux were, therefore, obtained.

In addition to CO_2 concentration, ambient conditions in both chambers were measured. In the upper chamber PPFD was measured using a PPFD sensor (Macam, Livingstone, UK), and relative humidity and chamber air temperature were measured using a relative humidity/temperature probe (HMP 35A, Vaisala, Helsinki, Finland). Leaf temperature was measured using the average value given by three small wire thermistors, coiled round the petioles and bent to touch the underside of three leaflets. In the lower chamber soil temperature was measured using a platinum resistance thermometer (PRT, RS components, Corby, UK).

Total leaf area for each plant at the time of measurement was estimated using an allometric relationship between leaf area and rachis length, which was determined from the plant harvests (Fig. 2.14, p. 83). Rachis lengths of all leaves on a plant were, therefore, measured at the time of gas exchange measurement. Root dry mass was also estimated from the allometric relationship between plant height and root mass (Fig. 3.5, p. 129).

The relationship between photosynthesis and PPFD was investigated by plotting all daytime measurements of A against PPFD from all plants of the same growth and

measurement CO_2 concentration. A hyperbolic light response function (France & Thornley 1978) was fitted to the data from each measurement condition and parameters compared. The function is given by:

$$A = \frac{A_{max} \alpha Q}{(A_{max} + \alpha Q)} - R_d \qquad (3.1)$$

where

A_{max} = maximum rate of photosynthesis (μmol m^{-2} s^{-1}),

α = apparent quantum efficiency (μmol m^{-2} s^{-1}), calculated as the initial slope of A against PPFD,

Q = the photosynthetic photon flux density, PPFD, (μmol photons m^{-2} s^{-1}),

and

R_d = rate of dark respiration in the light (μmol m^{-2} s^{-1}).

Another estimate of the apparent quantum efficiency (α) was made from the initial slope of the of the light response curve over a PPFD range of 0 to 50 μmol m^{-2} s^{-1} (Leverenz 1987) and this was also compared between measurement conditions. It was expected that this would be a more reliable fit to the data as it was only using the linear portion of the curve where there was proportionally less scatter.

Statistical analysis

1995

The goodness of fit of the A/C_i curves was tested by regressing predicted versus observed values of A and testing the product moment correlation coefficient, r, for statistical significance (Fowler & Cohen 1990). The range of the coefficient of determination, r^2, is given for the fitted curves. Parameters obtained from the A/C_i curves of *C. odorata* in 1995 were tested for significant differences using a two way analysis of variance (Fowler & Cohen 1990).

1996

In 1996 A/C_i curve fit was assessed in the same way as 1995. The range of r^2 is given for the goodness of fit of the curves. As curves were only fitted to two treatments (ambient and elevated CO_2 concentration, both with a high rate of nutrient supply) a t-test for comparing means of small samples was used to examine differences between fitted parameters (Fowler & Cohen 1990).

Differences in instantaneous photosynthetic rates between ambient and elevated growth concentrations of CO_2 and between ambient and elevated measurement concentrations of CO_2 were tested using a two way analysis of variance.

Differences in instantaneous measurements of g_s between ambient and elevated growth concentrations of CO_2 and between ambient and elevated measurement concentrations of CO_2 were tested using a two way analysis of variance. In addition, the relationship between g_s and A was investigated using linear regression.

Differences between growth and measurement concentration treatments in whole-plant CO_2 uptake and average rates of photosynthesis and respiration were tested using two way analysis of variance.

Differences between light response curves fitted to PPFD versus *A* data were tested using comparison of non-linear regressions (Mead & Curnow 1983; Potvin, Lechowicz & Tardif 1990). Differences in the initial slopes (α) were investigated further using an analysis of covariance in SAS (SAS Institute Inc. 1990).

RESULTS

A/C_i curves

A/C_i curves fitted to data from *C. odorata* plants showed a good fit (Fig. 3.1). In 1995 the range of r^2 from comparisons of predicted versus observed values of A was from 0.78 to 0.98 with 90 % of the relationships having an r^2 of greater than 0.80. In 1996 r^2 ranged from 0.95 to 0.99.

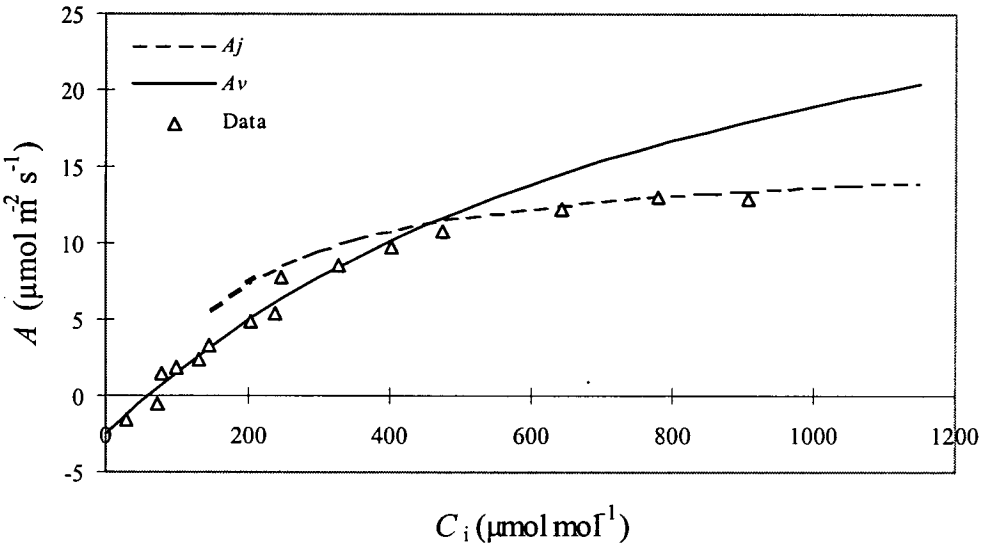


Figure 3.1 An example of a curve fitted to A/C_i data using the Farquhar *et al.* (1980) model. Data from one leaf of a *C. odorata* plant grown in ambient CO_2 concentration with a high rate of nutrient supply are shown as single points (Δ) with the modelled lines of A_j and A_v (de Pury & Farquhar 1997).

In 1995 there were statistically significant differences between A/C_i curves of different nutrient treatments but not between CO_2 concentration treatments (Fig. 3.2, p. 124). Estimates of V_{cmax} and J_{max} differed significantly between plants grown at low and high rates of nutrient application ($p < 0.05$). Estimates of V_{cmax}, J_{max} and R_d (Table 3.1) did not differ significantly between the ambient and elevated CO_2 concentration treatments ($p > 0.05$).

Table 3.1 Mean estimates of parameters of *C. odorata* plants in 1995 derived from A/C_i curves using the Farquhar *et al.* (1980) model. Mean values for parameters for each CO_2 and nutrient treatment are shown plus or minus one standard error. Treatments were as follows: HCHN (elevated CO_2 concentration, high rate of nutrient application), HCLN (elevated CO_2 concentration, low rate of nutrient application), LCHN (ambient CO_2 concentration, high rate of nutrient application) and LCLN (ambient CO_2 concentration, low rate of nutrient application). $n = 5$ for each high nutrient treatment and 4 for each low nutrient treatment. Means with different superscripts in the same column are significantly different ($p < 0.05$).

Treatment	J_{max} (μmol m^{-2} s^{-1})	V_{cmax} (μmol m^{-2} s^{-1})	R_d (μmol m^{-2} s^{-1})
HCHN	76.8 ± 3.9^a	27 ± 5.81^a	-0.42 ± 0.36^a
HCLN	44.25 ± 6.7^b	15 ± 3.0^b	-0.35 ± 0.15^a
LCHN	75 ± 12.4^a	31.2 ± 4.6^a	-0.2 ± 0.17^a
LCLN	28 ± 4.8^b	14.75 ± 2.2^b	-0.58 ± 0.13^a

In 1996 fitted curves for plants grown at ambient and elevated CO_2 concentration, always at high nutrient supply (Fig. 3.3, p. 125), differed significantly in the mean derived values of both V_{cmax} and J_{max}, which were significantly lower ($p < 0.05$) in plants growing in elevated CO_2 concentration at the time of A/C_i measurement (Table 3.2).

Table 3.2 Mean estimates of parameters of *C. odorata* plants in 1996 derived from A/C_i curves using the Farquhar *et al.* (1980) model. Mean values for parameters in both CO_2 concentrations with a high rate of nutrient supply are shown plus or minus one standard error. $n = 8$ per treatment. Means with different superscripts in the same column are significantly different ($p < 0.05$).

CO_2 treatment	J_{max} (μmol m^{-2} s^{-1})	V_{cmax} (μmol m^{-2} s^{-1})	R_d (μmol m^{-2} s^{-1})
Elevated	61.9 ± 10.3^a	23.3 ± 2.4^a	-0.09 ± 0.07^a
Ambient	91.4 ± 6.9^b	38.1 ± 3.6^b	-0.36 ± 0.15^a

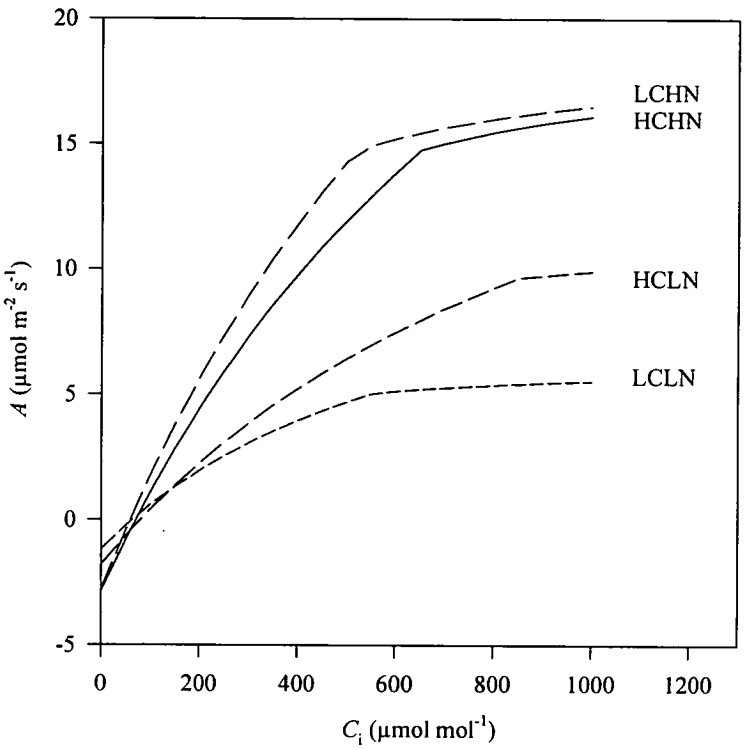


Figure 3.2 Assimilation rate/intercellular CO_2 concentration (A/C_i) curves for 1995 *C. odorata* data. Curves were derived from estimates of photosynthesis model parameters J_{max}, V_{cmax}, R_d (Farquhar *et al.* 1980). The estimated average curve per treatment is shown. Treatments were as follows: HCHN (elevated CO_2 concentration, high rate of nutrient application), HCLN (elevated CO_2 concentration, low rate of nutrient application), LCHN (ambient CO_2 concentration, high rate of nutrient application) and LCLN (ambient CO_2 concentration, low rate of nutrient application). n = 5 per high nutrient treatment and 4 per low nutrient treatment.

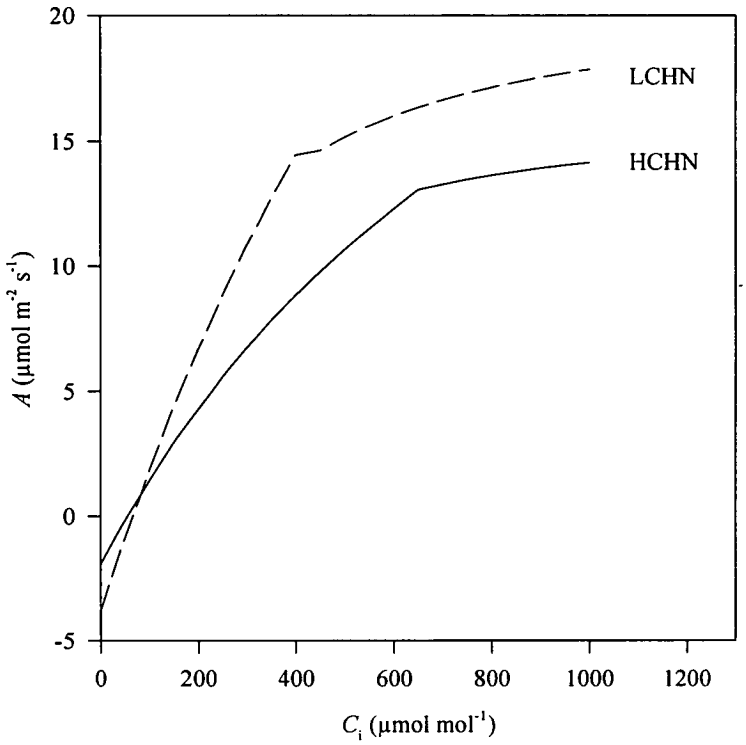


Figure 3.3 Assimilation rate/intercellular CO_2 concentration (A/C_i) curves for 1996 *C. odorata* data. Curves were derived from estimates of photosynthesis model parameters J_{max}, V_{cmax}, R_d (Farquhar *et al.* 1980). The estimated average curve per treatment is shown. $n = 8$ per treatment. Treatments were as follows: HCHN (elevated CO_2 concentration with high rate of nutrient application) and LCHN (low CO_2 concentration with high rate of nutrient application).

Spot measurements of photosynthesis

Instantaneous rates of photosynthesis did not differ between measurement CO_2 concentrations ($p > 0.05$) for *C. odorata* plants grown in 1996 in elevated CO_2 concentration (Table 3.3). However, A700 plants showed a significant increase in rate of photosynthesis when compared with A350 plants ($p < 0.05$). In addition, A700 plants showed a significantly higher rate of photosynthesis than E350 plants ($p < 0.05$).

Table 3.3 Mean values plus or minus one standard error of instantaneous photosynthesis measurements of *C. odorata* plants in 1996. Plants from a high rate of nutrient application and either ambient or elevated CO_2 concentration treatment were measured at both the CO_2 concentration of growth and the reciprocal concentration. PPFD was *ca* 1100 μmol m^{-2} s^{-1}. n = 6 per treatment. Means with different superscripts across either rows or columns are significantly different ($p < 0.05$).

CO_2 growth treatment	Instantaneous photosynthesis at 350 μmol mol^{-1} (μmol m^{-2} s^{-1})	Instantaneous photosynthesis at 700 μmol mol^{-1} (μmol m^{-2} s^{-1})
Elevated	5.14 ± 0.29^{a}	6.90 ± 0.66^{ab}
Ambient	5.01 ± 0.44^{a}	7.45 ± 0.65^{b}

Simultaneous measurements of stomatal conductance (g_s) were made at the time of photosynthesis measurement. There was no significant difference in g_s between plants grown in either ambient or elevated CO_2 concentration when compared at the respective measurement concentrations ($p > 0.05$, Table 3.4). Neither was there a significant difference when g_s values for a single growth CO_2 concentration were combined ($p > 0.05$) to give mean g_s values of 138 ± 13 mmol m^{-2} s^{-1} and 119 ± 11 mmol m^{-2} s^{-1} in ambient and elevated CO_2 concentration, respectively. There did appear to be a trend, however, towards a decrease in instantaneous g_s when measured at elevated as opposed to ambient CO_2 concentration for plants grown in both CO_2 concentrations (Fig. 3.4).

Table 3.4 Mean values plus or minus one standard error of instantaneous stomatal conductance (g_s) measurements of *C. odorata* plants in 1996. These were obtained whilst measuring photosynthesis simultaneously. Plants from a high rate of nutrient application and either ambient or elevated (700 μmol mol^{-1}) CO_2 concentration were measured at both the CO_2 concentration of growth and the reciprocal concentration. PPFD was *ca* 1100 μmol m^{-2} s^{-1}. $n = 6$ per treatment. Means with different superscripts across columns or rows are significantly different ($p < 0.05$).

CO_2 growth treatment	g_s at 350 μmol mol^{-1} (mmol m^{-2} s^{-1})	g_s at 700 μmol mol^{-1} (mmol m^{-2} s^{-1})
Elevated	124.3 ± 15.5^{a}	113.3 ± 17.4^{a}
Ambient	152.8 ± 18.8^{a}	122.2 ± 17.1^{a}

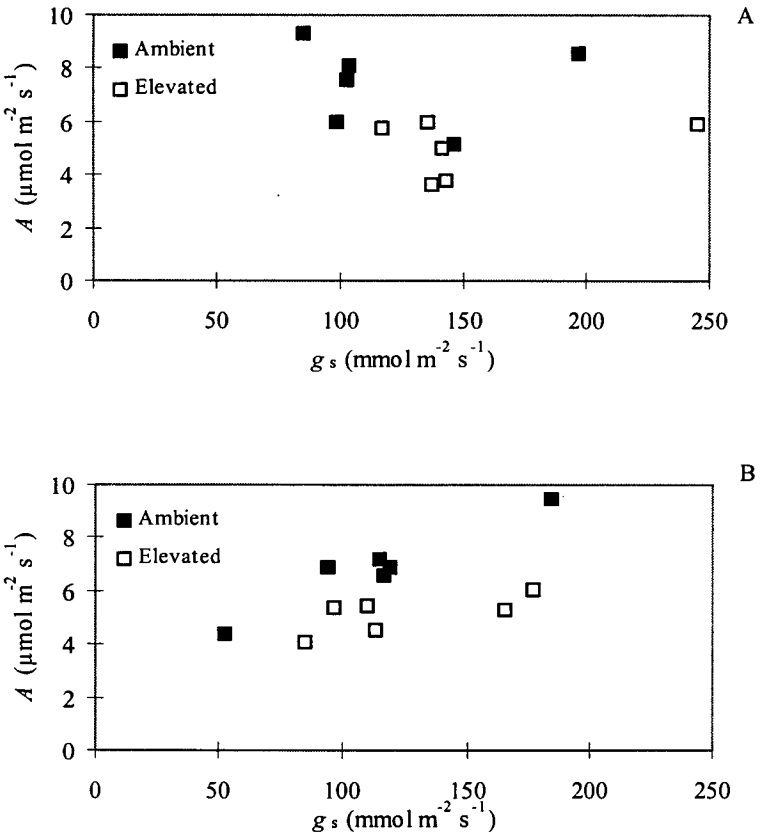


Figure 3.4 Relationships between stomatal conductance (g_s) and photosynthetic assimilation rate (A). A. Plants grown in ambient CO_2 concentration and measured at both ambient and elevated CO_2 concentrations. B. Plants grown in elevated CO_2 concentration and measured at both ambient and elevated CO_2 concentrations. Data were collected while making instantaneous measurements of photosynthesis. $n = 6$ per measurement concentration.

Whole-plant gas exchange

Gas exchange measurements show a significant difference in whole-plant CO_2 flux between A700 plants and E350 plants only ($p < 0.05$, Table 3.5). There were no significant differences between rates of mean above-ground uptake of CO_2 or below-ground efflux ($p > 0.05$). The allometric relationship between root mass and plant height (Fig. 3.5) was used to infer root mass at the time of whole-plant gas exchange measurement without destructive harvesting. Estimated values of root mass and leaf area at the time of gas exchange measurement are given in Table 3.6.

Table 3.5 Mean whole-plant fluxes of CO_2 for *C. odorata* plants grown in either ambient or elevated concentrations of CO_2 in 1996. All plants were selected from those receiving a high rate of nutrient application. Fluxes are given for a period of 24 hour exposure to an atmospheric CO_2 concentration of either 350 or 700 μmol mol^{-1}. All values have been corrected for leaks in the chambers and are shown as means plus or minus one standard error. Average below-ground carbon flux was calculated per gram of root and average above-ground flux was calculated per m^2 of leaf area. $n = 8$. Note that the same plants were kept in the chamber for 24 hours at one CO_2 concentration (that of growth) and then 24 hours at the reciprocal (to growth) concentration. Means with different superscripts in the same column are significantly different ($p < 0.05$). Measurement conditions were as follows: E700 = plants grown at elevated CO_2 concentration (700 μmol mol^{-1}) and gas exchange measured at 700 μmol mol^{-1}. E350 = the same plants grown at elevated CO_2 concentration but measured at 350 μmol mol^{-1}. A700 = plants grown at ambient CO_2 concentration (350 μmol mol^{-1}) and measured at 700 μmol mol^{-1}. A350 = the same plants grown at ambient CO_2 concentration and also measured at 350 μmol mol^{-1}.

Measurement	Average below-ground CO_2 flux (μmol $g^{-1} s^{-1}$)	Average above-ground CO_2 flux (μmol $m^{-2} s^{-1}$)	Net CO_2 uptake (mmol plant $^{-1}$ day^{-1})
E350	-0.014 ± 0.003^{a}	1.02 ± 0.14^{a}	1.6 ± 0.5^{a}
A350	-0.015 ± 0.003^{a}	1.40 ± 0.19^{a}	4.6 ± 1.4^{ab}
E700	-0.017 ± 0.002^{a}	2.00 ± 0.16^{a}	4.3 ± 0.6^{ab}
A700	-0.017 ± 0.003^{a}	2.14 ± 0.28^{a}	7.6 ± 1.9^{b}

Table 3.6 Root dry masses and leaf areas used to convert changes in chamber CO_2 concentration to above- and below-ground CO_2 fluxes. Values are given for all plants used in this experiment plus the mean values per treatment plus or minus one standard error.

Plant	Root dry mass (g)	Leaf area (m^2)
Plants grown at elevated CO_2 concentration		
1.6	0.92	0.027
1.9	1.58	0.053
3.7	1.81	0.036
3.8	1.41	0.027
6.4	1.33	0.031
6.5	1.95	0.045
6.6	2.04	0.072
6.7	1.81	0.031
Mean values $\pm$ 1 SE	1.61 $\pm$ 0.13	0.040 $\pm$ 0.006
Plants grown at ambient CO_2 concentration		
2.5	1.87	0.045
2.6	1.08	0.042
2.6	1.84	0.063
4.4	1.95	0.082
4.5	1.61	0.045
4.8	0.98	0.032
5.6	1.31	0.060
5.9	1.44	0.039
Mean values $\pm$ 1 SE	1.51 $\pm$ 0.13	0.051 $\pm$ 0.006

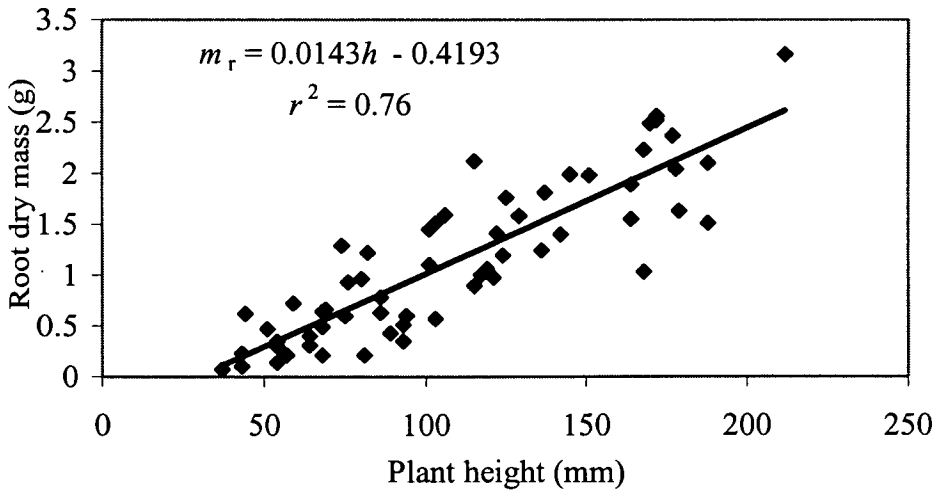


Figure 3.5 Relationship between root dry mass and plant height of *C. odorata* plants in 1996. m_r = root dry mass. h = height. All CO_2 concentration and nutrient treatments are combined from the final harvest (n = 60).

An example of flux data for one plant grown and measured at ambient CO_2 concentration (350 μmol mol^{-1}) for 24 hours is shown in Figures 3.6 and 3.7.

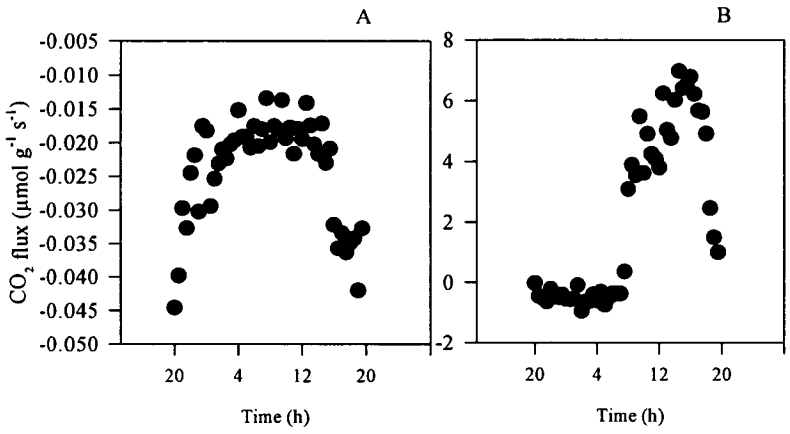


Figure 3.6 An example of flux data from one *C. odorata* plant grown and measured at ambient CO_2 concentration in 1996. Measurements were collected every 15 minutes, alternatively from either the upper or lower chamber, so that each chamber was sampled every half-hour. A. Total below-ground CO_2 flux, measured in the lower chamber in an expanded clay medium in a pot, is expressed per g of root dry mass. B. Total above-ground flux, measured in the upper chamber, is expressed per m^2 of leaf area. Uptake of CO_2 is represented as a positive flux and efflux is represented as a negative flux.

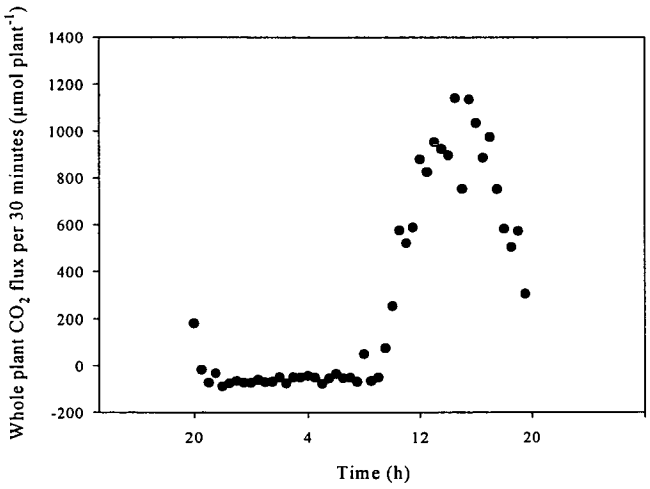


Figure 3.7 A sample of whole-plant flux data from one *C. odorata* plant grown and measured at ambient CO_2 concentration in 1996. Measurements were collected in each chamber every half hour so net flux for the plant is shown as a 30 minute estimate. Uptake of CO_2 is represented as a positive flux and efflux represented as a negative flux.

Average values of both PPFD and temperature (Fig. 3.8) were similar for the four treatments so it was not necessary to standardise fluxes to allow for variations.

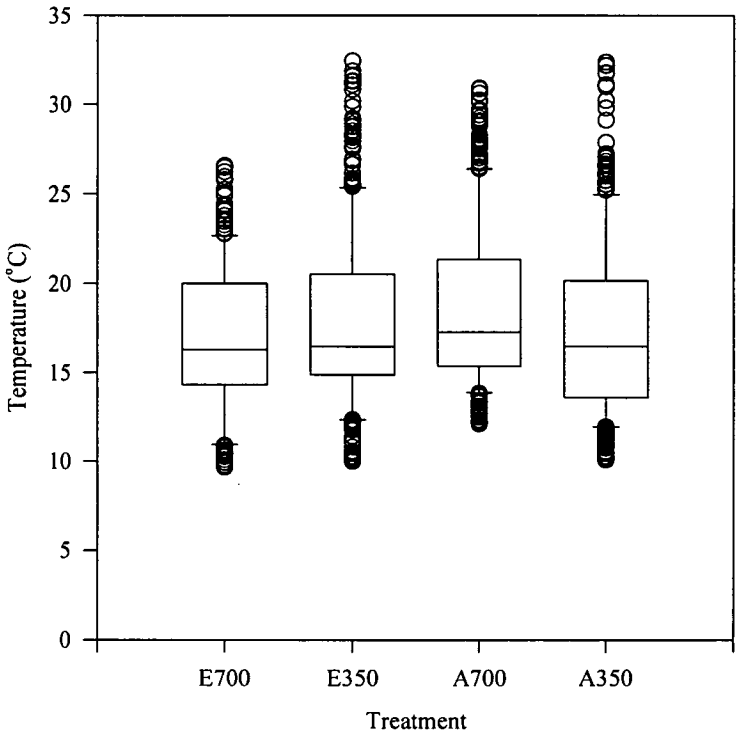


Figure 3.8 Box plots of average soil temperature in each of the measurement conditions during the course of whole-plant gas exchange measurements. The mean of each data set is shown as the central bar of the box with the delimiters of the box representing the 25th and 75th percentiles. Capped bars represent the 10th and 90th percentiles with values lying outside of these percentiles shown as open circles. Treatments were as follows: E700 = plants grown in elevated CO_2 concentration (700 μmol mol^{-1}) and gas exchange measured at 700 μmol mol^{-1}. E350 = the same plants grown in elevated CO_2 concentration but measured at 350 μmol mol^{-1}. A700 = plants grown in ambient CO_2 concentration (350 μmol mol^{-1}) and measured at 700 μmol mol^{-1}. A350 = the same plants grown in ambient CO_2 concentration and also measured at 350 μmol mol^{-1}.

On average, a good relationship was observed between soil temperature and respiration for the lower chamber (Fig. 3.9), and PPFD and photosynthesis in the

upper chamber (Fig. 3.10, p. 134). The relationship of soil CO_2 efflux with temperature can be described by the function:

$$R = R_0 e^{kT}$$

where R = respiration (soil CO_2 efflux in this case),

R_0 = respiration at 0 °C,

k = rate constant, and

T = soil temperature (°C)

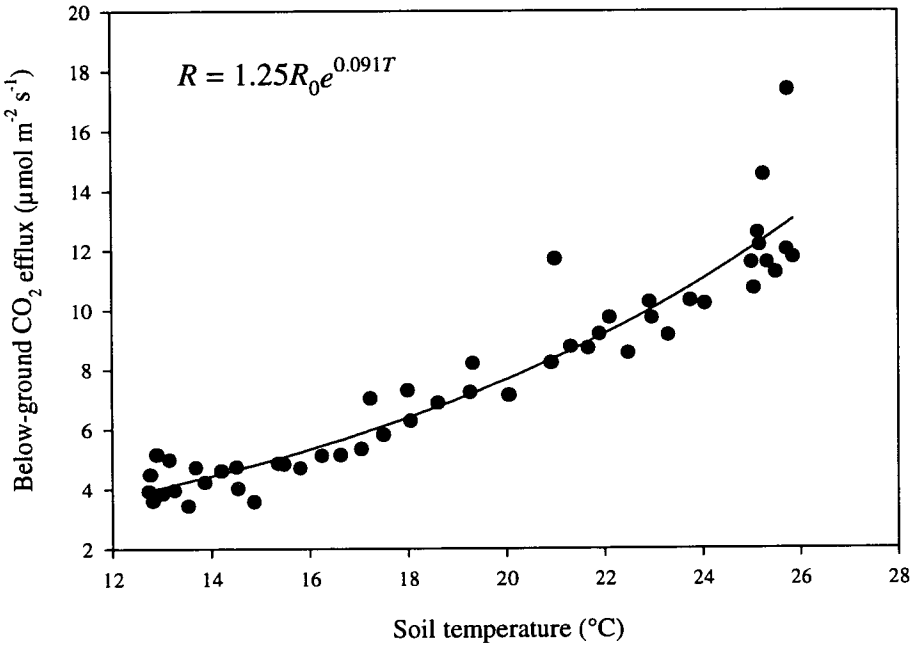


Figure 3.9 An example of the response of below-ground CO_2 efflux to increases in soil temperature of a *C. odorata* plant grown in elevated CO_2 concentration in 1996. The CO_2 efflux was assumed to be largely microbial and root respiration and is expressed per m^2 of soil surface area. Total below-ground respiration was measured at ambient CO_2 concentration in the lower compartment of a whole-plant gas exchange chamber. These data were collected every half an hour over a 24 hour period. Also shown is the regression equation for the respiration response function to temperature (r^2 = 0.9). Note that the meteorological sign convention has been used in this graph for the purpose of fitting the temperature/respiration function.

Light response curves fitted to the data did not differ significantly from one another ($p > 0.05$, Fig. 3.10, Table 3.7).

Fitted values for R_d and α obtained by linear regression of the initial slopes of the light response curves are shown in Table 3.8, p. 135. α did not differ significantly between treatments ($p > 0.05$, Fig. 3.11, p. 135).

Table 3.7 Values of A_{max} and α of the curves fitted to photosynthetic light response data, one average curve per treatment. A_{max} = maximum rate of photosynthesis. α = apparent quantum efficiency. Data were obtained from whole-plant gas exchange measurement of each of 8 plants per treatment which were subsequently combined for the purpose of fitting the curve. The model fitted to the data was an empirical light response function (France & Thornley 1978). Measurements were as follows: E700 = plants grown in elevated CO_2 concentration (700 µmol mol^{-1}) and gas exchange measured at 700 µmol mol^{-1}. E350 = the same plants grown in elevated CO_2 concentration but measured at 350 µmol mol^{-1}. A700 = plants grown in ambient CO_2 concentration (350 µmol mol^{-1}) and measured at 700 µmol mol^{-1}. A350 = the same plants grown in ambient CO_2 concentration and also measured at 350 µmol mol^{-1}.

Treatment	A_{max} (µmol $m^{-2} s^{-1}$)	α	R_d (µmol $m^{-2} s^{-1}$)
E350	5.11 ± 0.23	0.06 ± 0.01	-0.4 ± 0.21
A350	5.34 ± 0.34	0.09 ± 0.02	-0.46 ± 0.34
E700	8.24 ± 0.29	0.06 ± 0.01	0.01 ± 0.2
A700	8.61 ± 0.67	0.13 ± 0.05	-0.89 ± 0.71

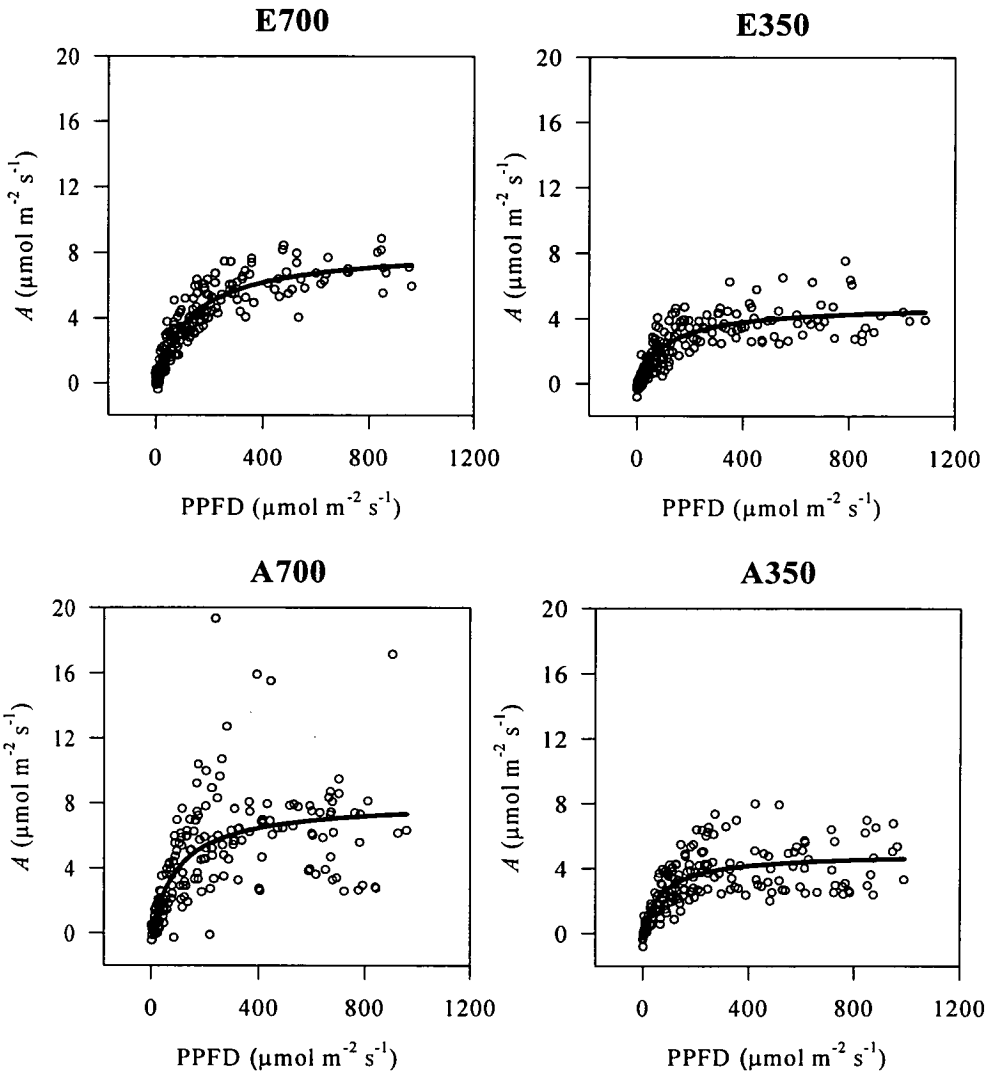


Figure 3.10 Response of above-ground photosynthesis (*A*) to photosynthetic photon flux density (PPFD) of four treatments measured in a whole-plant gas exchange chamber. The gas exchange of each plant was measured for 24 hours at each CO_2 concentration giving a total of 48 hours in the chamber. Curves show measured values (open circles) of photosynthesis for eight plants per treatment plus modelled relationship (solid line) between PPFD and *A* for the same eight plants combined. Treatments were as follows: E700 = plants grown in elevated CO_2 concentration (700 μmol mol^{-1}) and gas exchange measured at 700 μmol mol^{-1}. E350 = the same plants grown in elevated CO_2 concentration but measured at 350 μmol mol^{-1}. A700 = plants grown in ambient CO_2 concentration (350 μmol mol^{-1}) and measured at 700 μmol mol^{-1}. A350 = the same plants grown in ambient CO_2 concentration and also measured at 350 μmol mol^{-1}.

Table 3.8 Values of α and R_d parameters obtained from linear regression of initial (PPFD < 50 μmol m^{-2} s^{-1}) slope of light response curves. Data were obtained from whole-plant gas exchange measurement of each of 8 plants per treatment which were subsequently combined for the purpose of fitting the curve. Treatments were as follows: E700 = plants grown in elevated CO_2 concentration (700 μmol mol^{-1}) and gas exchange measured at 700 μmol mol^{-1}. E350 = the same plants grown in elevated CO_2 concentration but measured at 350 μmol mol^{-1}. A700 = plants grown in ambient CO_2 concentration (350 μmol mol^{-1}) and measured at 700 μmol mol^{-1}. A350 = the same plants grown in ambient CO_2 concentration and also measured at 350 μmol mol^{-1}.

Treatment	α	R_d (μmol m^{-2} s^{-1})
E350	0.034 ± 0.004	-0.22 ± 0.10
A350	0.039 ± 0.004	-0.094 ± 0.10
E700	0.053 ± 0.007	-0.0069 ± 0.18
A700	0.043 ± 0.007	-0.0032 ± 0.18

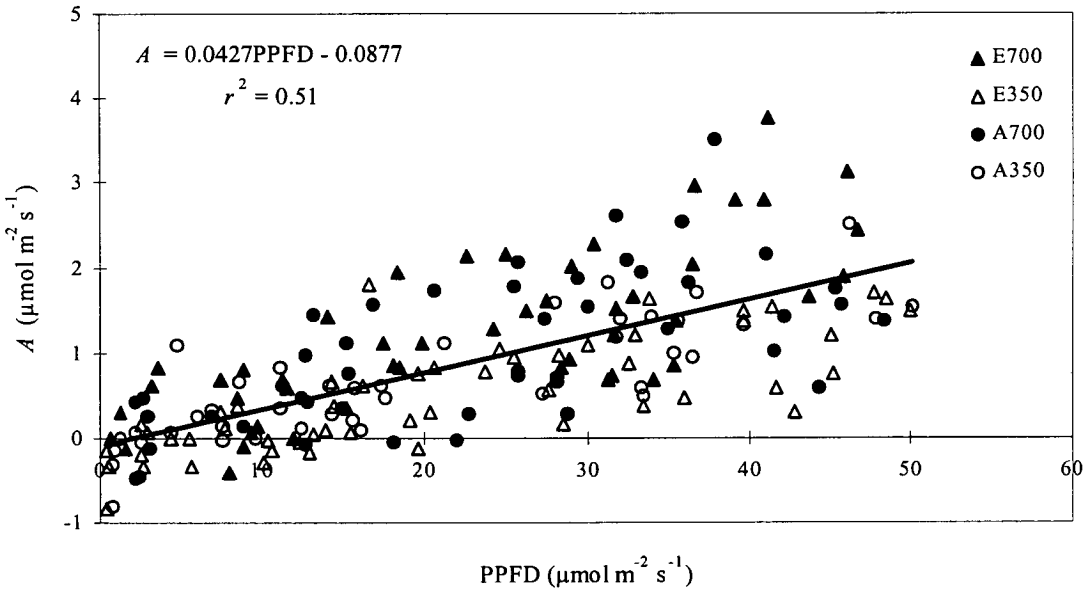


Figure 3.11 Comparison of apparent quantum efficiencies (α) represented by the initial slopes of the photosynthetic light responses of *C. odorata* plants grown in either ambient or elevated CO_2 concentration in 1996. There was no significant difference in α ($p < 0.05$) between treatments so the regression line shown is that of the data combined. *A* was measured simultaneously with PPFD in a whole-plant gas exchange chamber. Data for eight *C. odorata* plants per treatment is shown for PPFD values of 50 μmol m^{-2} s^{-1} or less. Treatments were as follows: E700 = plants grown in elevated CO_2 concentration (700 μmol mol^{-1}) and gas exchange measured at 700 μmol mol^{-1}. E350 = the same plants grown in elevated CO_2 concentration but measured at 350 μmol mol^{-1}. A700 = plants grown in ambient CO_2 concentration (350 μmol mol^{-1}) and measured at 700 μmol mol^{-1}. A350 = the same plants grown in ambient CO_2 concentration and also measured at 350 μmol mol^{-1}.

DISCUSSION

Acclimation

C. odorata seedlings appeared to show an acclimation response to elevated CO_2 in 1996 but not in 1995. In 1995 parameters derived from A/C_i curves did not significantly differ ($p > 0.05$) between CO_2 treatments (Table 3.1, p. 123). Plants grown in elevated CO_2 concentration appeared to be still responding to the higher-than-ambient concentration of CO_2 in the same way as the plants grown in ambient CO_2 concentration. This agrees with the results of other investigators who found no change in A/C_i curves with elevated CO_2 concentration (Pettersson & McDonald 1992; Gunderson *et al.* 1993).

Values of J_{max} and V_{cmax} did differ between nutrient treatments, however, and this suggests that a low rate of nutrient application resulted in down-regulation of photosynthesis, with nutrient-limited plants showing a reduced photosynthetic capacity. This reduction in photosynthetic capacity did not, however, increase the likelihood of acclimation to elevated CO_2 concentration. Plants growing in low nutrient conditions in elevated CO_2 concentration did not show signs of acclimation when compared with those in ambient CO_2 concentration (Table 3.1, p. 123). Evidence from the literature for low nutrient triggering of acclimation is inconsistent (reviewed by Pettersson & McDonald 1994) and has generally used the growth concentration to test for acclimation instead of comparing Rubisco activity at both ambient and elevated CO_2 concentration for plants grown in both concentrations (Gunderson & Wullschleger 1994).

There was more evidence for photosynthetic acclimation to elevated CO_2 concentration in the 1996 experiment. Instantaneous photosynthesis measurements taken seven weeks after the start of the experiment showed that plants grown in

elevated CO_2 concentration had a smaller stimulation of photosynthesis when measured at elevated CO_2 concentration than their ambient-grown counterparts (Table 3.3, p. 126). That is, the rate of photosynthesis did not differ significantly between E700 and E350 suggesting no net stimulation at this time ($p > 0.05$). Data from A/C_i curves taken 11 weeks after the start of the experiment seem to support the suggestion of acclimation with a difference in photosynthetic capacity between the two treatments (Table 3.2, p. 123).

In 1996, both V_{cmax} and J_{max} were significantly ($p < 0.05$) lower in the elevated CO_2 concentration treatment than in the ambient treatment. Values of R_d did not differ between CO_2 concentration treatments ($p > 0.05$). This suggests that Rubisco activity was reduced and resources were allocated away from this now non-limiting step of assimilation. A recent review of A/C_i responses of photosynthesis to elevated CO_2 concentration also showed that most species show a decrease in V_{cmax}, suggesting acclimation as widespread (Gunderson & Wullschleger 1994).

Growth conditions

Why was an acclimation response observed in one year only for the same plant species? What was the trigger that caused a reallocation of plant resources in 1996? Given the literature of unpredictability in acclimation response to elevated CO_2 concentration, it seems likely that growth conditions are the most important determinant of changes in A/C_i relationship with elevated CO_2 (Sage 1994). The observed lack of acclimation may, therefore, result from the more favourable growing conditions of the 1995 experiment, particularly the higher number of total sunshine hours per month (discussed in Chapter Two). There was no perceived stimulus for the plant to reallocate resources away from Rubisco towards another process more limiting to growth.

There is a strong inverse relationship between atmospheric vapour pressure deficit (D) and g_s (Schulze *et al.* 1972; Fanjul & Jones 1982). D was sufficiently high in both years to have reduced g_s. However, D was higher in 1996 and the instantaneous measurements of g_s are at the low end of those previously reported for *C. odorata* grown in its natural habitat (McWilliam *et al.* 1996). Unfortunately g_s was not measured in 1995 as a comparison. Although an elevated concentration of CO_2 has been reported to decrease g_s in some species (Idso 1991; Kellomäki & Wang 1997), there has been no change in other species (Gunderson *et al.* 1993; Bunce 1992; Stirling *et al.* 1997). The g_s of *C. odorata* did not change with elevated CO_2 concentration in the present study (Table 3.4, p. 127), although, there was a trend towards a decrease in g_s with instantaneous exposure to an elevated CO_2 concentration (Fig. 3.4, p. 127).

There is some evidence to suggest that the extent of plant response to elevated CO_2 concentration is determined by the similarity of the experimental light environment to that in which the species is most likely to occur. Kubiske & Pregitzer (1997) showed that shade tolerant species had a larger response to elevated CO_2 concentration when grown in the shade and shade intolerant species responded more when grown in the sun. Other experimenters also concluded that low peak PPFD limited the response of shade-intolerant species to elevated CO_2 concentration (Bazzaz *et al.* 1990). More recent work suggests that the ability to acclimate to elevated concentrations of CO_2 may be related to ability to adapt to low PPFD (Kubiske & Pregitzer 1996). It was hypothesised that shade tolerant species are more able to alter the partitioning of nitrogen between Rubisco and the light harvesting processes of the thylakoid membranes and that this also enables them to acclimate to elevated CO_2 concentration with a larger increase in J_{max}. The authors stress the difference between this kind of reallocation and down-regulation resulting from sink limitation. *C. odorata* is thought to be shade-intolerant (Newton *et al.* 1993) so

perhaps it is less able to alter the nitrogen partitioning within the photosynthetic apparatus, and therefore capitalise on an elevated CO_2 concentration.

The present experiment was assumed to be free of sink limitation as large pot sizes were used (Thomas & Strain 1991). There is no evidence of acclimation in favour of the light harvesting complex in this experiment. *C. odorata* is a fast-growing pioneer species, the young trees of which are reported to be highly intolerant of overhead shade (Newton *et al.* 1993). In addition, it may be intolerant of extremely high values of PPFD (Ramos & Grace 1990). It is therefore possible that this species did not alter the partitioning of nitrogen within the chloroplast but within the plant as a whole. A poorer lighting regime in 1996, in conjunction with a higher vapour pressure deficit (discussed in Chapter Two) and an inappropriate nutrient regime, is hypothesised to have triggered this acclimation to elevated CO_2 concentration. It is assumed that in 1995 both the PPFD and the total number of sunshine hours were sufficiently high not to limit photosynthesis significantly.

Whole-plant fluxes

Because whole-plant CO_2 uptake did not differ significantly between E700 and E350 plants ($p > 0.05$) it would appear as if plants grown in elevated CO_2 concentration were assimilating no more CO_2 than if they had been growing in ambient conditions. This also suggests that acclimation has occurred such that photosynthetic capacity in the E700 plants has been reduced. These results are thought to give a better picture of the longer-term response when compared with instantaneous photosynthesis measurements which were made earlier in the experiment.

Some of the increased CO_2 uptake in A350 plants can be explained, however, by differences in leaf area, with plants grown in ambient CO_2 concentration having a larger leaf area and therefore larger overall uptake of CO_2 (Table 3.6, p. 129).

Values for below-ground respiration are a little high in comparison with other literature values for root-only respiration. In a study of beech root respiration maximum recorded rates of fine and coarse root respiration were 0.0098 and 0.0031 $\mu mol\ g^{-1}\ s^{-1}$, respectively (Gansert 1994). In another study investigating the influence of temperature on root respiration the maximum recorded value for stands of temperate hardwoods was 0.007 $\mu mol\ g^{-1}\ s^{-1}$ at 18 °C. Mean respiration rates in the current study lie in the range of 0.014 - 0.017 $\mu mol\ g^{-1}\ s^{-1}$ for coarse and fine roots combined. It is assumed that this discrepancy lies largely in the contribution of soil microbial respiration which could not be separated from root respiration. In addition, experimental errors plus the higher temperatures of the current experiment could have boosted estimated respiration rates beyond those previously measured in temperate tree species.

Indeed, when respiration was expressed on a soil surface area basis (Fig. 3.7, p. 130), its response to changes in temperature was very similar to that measured in a previous *in situ* study of Brazilian primary rain forest (Meir 1996). Values of R_0 and k in the present study were 1.25 and 0.09 respectively, compared with 0.81 and 0.083 in Meir's study. It is suggested that a study of root respiration using plants of greater size than in the current study would give more reliable estimates of these parameters, such that they could be used in models which "scale up" the response of a single species to that of a forest stand.

Some authors have suggested that root respiration is depressed by an increased concentration of atmospheric CO_2 (*e.g.* Burton *et al.* 1997) but this experiment shows no evidence of such a decrease (Table 3.5, p. 128). It is possible though that the error associated with the estimates of root respiration is sufficiently large to mask any treatment differences.

Gas exchange chamber errors

There is a larger error term associated with the figure for root flux of CO_2 because the signal of CO_2 change is smaller because of the small root mass. Therefore, leaks are more important. Because leakage was calculated as an average for the entire duration of the whole-plant gas exchange measurement some calculated leakage values will likely have drifted a little from their actual values. It is expected that each time the chamber was sealed the actual leakage varied. This small fluctuation in leakage will have been particularly important in the lower chamber where a leakage represents a potentially larger proportion of the observed CO_2 flux.

Light response curves

There were no significant differences between curves fitted to the light response of photosynthesis for each of the four treatments. α did not differ between treatments suggesting that neither plants grown at ambient nor elevated CO_2 concentration became more efficient when measured at elevated CO_2 concentration. Values obtained for R_d and α differed substantially according to the method used (compare Tables 3.7, p. 133 and 3.8, p. 135). Values obtained from linear regression are assumed to be more accurate than those obtained by fitting the rectangular hyperbola to the data as the regressions showed a better fit to the data and only two parameters were fitted at the one time. Indeed, the positive value obtained for R_d through fitting the rectangular hyperbola (Table 3.7, p. 133) differs from a study of tropical leaves *in situ* (Meir 1996).

Fitted values of R_d from linear regression also differ from fitted values of R_d from A/C_i curve measurement (compare Table 3.2, p. 123 with 3.8, p.135). There are some errors associated with each of these measurements. R_d values should theoretically be fitted from light response curves (Farquhar *et al.* 1980) so those fitted from A/C_i

curves are inherently less reliable. However, because there is some error associated with the leak correction applied to the whole-plant gas exchange data, the values of R_d fitted from whole-plant light response curves are expected to be only slightly more reliable. It is proposed that a study of individual leaf response to changes in PPFD would be the best way to fit these parameters (de Pury & Farquhar 1997) and to test for real differences in α.

Although further investigation of stomatal and light responses is warranted, it would appear as if *C. odorata* plants will not sustain an increased rate of photosynthesis with extended exposure to elevated CO_2 concentration in sub-optimal growth conditions. It is proposed, however, that where environmental conditions do not trigger an acclimation response, the increase in photosynthesis will be sustained.

CONCLUSIONS

1) *C. odorata* seedlings grown in elevated CO_2 concentration showed no acclimation in photosynthetic capacity over the course of the experiment in 1995; V_{cmax} was in the range of 27 to 31 μmol m^{-2} s^{-1} at a high rate of nutrient supply, and J_{max} was in the range of 75 to 77 μmol m^{-2} s^{-1}.

2) *C. odorata* seedlings appeared to show an acclimation in photosynthetic capacity to elevated CO_2 concentration in 1996, as indicated by a decrease in maximum carboxylation velocity, V_{cmax}, from 38 μmol m^{-2} s^{-1} at ambient CO_2 concentration to 23 μmol m^{-2} s^{-1} at elevated CO_2 concentration. The maximum rate of electron transport, indicated by J_{max}, also decreased from 91 to 62 μmol m^{-2} s^{-1} when exposed to an elevated CO_2 concentration.

3) This acclimation is likely to have been triggered by the sub-optimal growth conditions of the 1996 experiment. These were suggested both by the reduction in the number of sunshine hours and also by the lower mean temperature and PPFD. In addition, vapour pressure deficit was higher, and the nutrient regime inappropriate for the growth conditions, in the 1996 experiment.

Chapter Four:
Within-canopy photosynthesis of an undisturbed Amazonian rain forest

INTRODUCTION

Tropical forests are estimated to contain about 37 % of global vegetation and soil carbon (Dixon *et al.* 1994), suggesting a pivotal role for them in the global carbon balance. However, only a handful of authors have studied the responses of tropical woody species to elevated carbon dioxide (Oberbauer *et al.* 1985; Reekie & Bazzaz 1989; Hogan *et al.* 1991; Ziska *et al.* 1991; Körner & Arnone 1992). Tropical forest (including deciduous and montane forms) covers approximately 17.6 x 10^8 hectares of the earth's surface and contains 59 % of the forested area of the world (Dixon *et al.* 1994). It is estimated to have a net primary productivity of 30 Gt C yr^{-1}, which is half of the global total (Grace *et al.* 1997; Malhi *et al.* 1997). It is also the area of greatest change in land use cover (Raven *et al.* 1986; Houghton 1995), yet we have barely begun to characterise the difference in the atmosphere/biosphere interaction when the land is cleared. The need for ecophysiological study of the undisturbed

forest, so that we have a better understanding of the interaction between the atmosphere and the biosphere, is therefore all the more urgent.

A major obstacle to the prediction of ecosystem response to elevated CO_2 concentration is our currently scant understanding of ecosystem function (Eamus & Jarvis 1989). There is a strong need to collect more data on canopy photosynthesis as well as to explain the observed photosynthesis in terms of the changes in the variables which drive the process. There needs to be a model of whole-canopy photosynthesis which is driven by simple changes in within-canopy environment but can still account for the complexity of the interaction of all the variables which might respond to an elevated CO_2 concentration (McMurtrie & Wang 1993). Data are presented in this chapter to test our current understanding of the controlling variables of canopy photosynthesis. In addition, quantitative descriptions of *in situ* rain forest photosynthesis are given because it is envisaged that this is the point where variations in external CO_2 concentration could be incorporated into models of ecosystem response.

It has been shown that strong vertical gradients of stomatal conductance and maximum photosynthetic rate exist in Amazonian forest (Roberts *et al.* 1990; McWilliam *et al.* 1996; Meir 1996). This is most commonly linked to the concentration of foliar nitrogen (Field & Mooney 1986; Hirose & Werger 1987) and there is evidence that tree species have the ability to allocate nitrogen resources within a vertical profile in order to maximise photosynthetic activity at the sites of greatest light interception, that is, near the top of the canopy (Field 1983; Pons & Bergkotte 1996).

The theory predicting an optimal distribution of photosynthetic capacity through a canopy with respect to light interception depends on two major assumptions. The first is that the measurement of photosynthetic capacity indicates the maximum

photosynthetic output at natural limits of critical environmental parameters. This capacity is a result of natural selection whereby the cost of building and maintaining photosynthetic machinery dictates the extent of the investment (Field & Mooney 1986). Photosynthetic capacity is therefore determined by the time-averaged physical leaf conditions, which include light, soil nutrient status, water availability *etc* so that a measurement of photosynthetic capacity indicates the investment in photosynthesis for the conditions experienced by the leaf. This process should operate whether the leaf is in sun or shade for the majority of the time. The second assumption of the theory is that photosynthetic capacity can be predicted from nitrogen concentration of leaves and that this relationship holds across a wide range of species and within a multi-species canopy. The literature has previously been surveyed and the relationship between foliar nitrogen concentration and photosynthetic capacity has been shown to be highly conserved across light environments, leaf ages, leaf positions within a tree, ecological niches and plant functional types (Field 1983, Evans 1989, Sellers *et al.* 1992). However, the full range of interrelationships between photosynthetic capacity and critical environmental driving variables has not yet been defined in sufficient detail for any one canopy and a shortage of data exists on how well the nitrogen/photosynthesis relationship holds for a single forest canopy (Kull & Jarvis 1995). This chapter investigates the optimisation of photosynthetic capacity, as indicated by leaf nitrogen concentration, as an adequate interpretation of photosynthesis through a rain forest canopy in Brazil.

This "acclimation" to light significantly affects the design of models of canopy photosynthesis (Kull & Jarvis 1995) which are a vital tool for predicting physiological response to elevated atmospheric CO_2 concentration. In addition, there is a natural gradient of CO_2 concentration between the top and the bottom of a rain forest canopy (Medina *et al.* 1986). The complexity of the photosynthetic response to the interaction of light interception with CO_2 concentration is well established

(Evans 1996) but as yet unresolved. This suggests an urgent need for more empirical data before the models can evolve further (J. Lloyd, *pers comm.*, 1997).

The process of canopy acclimation to light environment may take a number of forms. It has been proposed that the photosynthetic system of a forest canopy responds to the decreasing levels of PPFD within a canopy using two mechanisms: "homogenisation" of the light environment and acclimation of the photosynthesis of individual leaves to their local light environment (Terashima & Hikosaka 1995). Homogenisation of the light environment usually involves changes in leaf inclination, leaf thickness and anatomical changes in the number and thickness of palisade as opposed to spongy mesophyll cells (Givnish 1988). The theory of an optimal distribution of photosynthetic capacity within a canopy has usually inferred that the strong relationship of nitrogen with photosynthetic capacity (Field & Mooney 1986) may be used to predict photosynthetic capacity from investigation of leaf nitrogen alone (Hirose & Werger 1987). However, more recent research suggests that each leaf acclimates fully to its average light environment using a number of mechanisms, the "optimal" distribution of leaf nitrogen incorporated in photosynthetic enzymes being only one of these acclimation mechanisms (Sellers *et al.* 1992). Much controversy still exists about whether nitrogen concentration can be used to indicate photosynthetic capacity, and if it can, does its distribution through a canopy actually follow an optimal distribution with respect to light environment (Kull & Jarvis 1995; Terashima & Hikosaka 1995)?

In the current study vertical profiles of photosynthetic activity have been obtained in an undisturbed tropical rain forest near Manaus, Brazil. This mixed-species, lowland *terra firme* forest covers 60 - 70 % of the Amazon (Pires 1978) and grows on deeply weathered, nutrient poor soils, usually oxisols or ultisols (Herrera 1985). In each canopy level A/C_i curves, as discussed in Chapter Three, were constructed to investigate species' instantaneous responses to changes in CO_2 concentration. An

updated version of the Farquhar *et al.* (1980) model was fitted to these data to allow quantitative comparison of the photosynthetic response to CO_2 concentration of leaves at different canopy levels. Daily variations in within-profile photosynthesis, stomatal conductance and micro-environment were also investigated. Foliar nitrogen concentrations were also measured to investigate the relationship between height of leaf insertion (level) in the canopy and the interaction between light intensity and CO_2 concentration. As far as I am aware, this is the first study of A/C_i profiles of dense Amazonian rain forest.

In this study I attempt to answer the following questions:
What is the quantitative difference in photosynthetic capacity among layers of a typical primary rain forest canopy? Is this distribution of photosynthetic capacity optimal for the prevailing environment? Is the distribution of photosynthetic capacity correlated with the distribution of nitrogen within a canopy? What is the predictive value of these data in models of physiological responses to increases in atmospheric CO_2 concentration?

METHODS

Site description

The study site was situated 60 km north of Manaus, Brazil, in a forest reserve, Reserva Biologica do Cuieiras (2°35'22" S, 60°06'55" W), belonging to INPA (Instituto Nacional de Pesquisas da Amazônia). Measurements were made at the ZF2 tower approximately 9 km west of the BR-174 road, accessible by an access road to a track close to the tower. This tower was 41.5 m high and also belonged to INPA.

The forest type is dense (above-ground dry biomass of 300-350 t ha^{-1}), lowland *terra firme* rain forest, believed to be undisturbed. Leaf area index of a nearby site has been estimated at 5 to 6 (McWilliam *et al.* 1993) and is assumed to be similar at this site. The soil may be described as nutrient poor oxisol or latisol containing a very high proportion of clay (Herrera 1985; Hodnett *et al.* 1996). Canopy height is approximately 30 m.

There were walkways on the tower at vertical intervals of four metres which allowed access to leaves for gas exchange measurement at these levels (Appendix 2). Photosynthesis was measured on the leaves of whichever two species were accessible from the tower at each of 8, 16, 20 and 24 m from the ground. In addition, leaves of two species were measured at ground level, less than 1 m from the ground, within a 10 m radius of the tower but avoiding the canopy gap it had created. The species measured were *Jacaranda copaia*, *Helicostylis sp.*, *Protium sp.*, *Goupia glabra*, *Memora sp.*, *Vochysia sp.*, *Inga sp.*, *Atolla otoleoides* and *Oenocarpus sp.* Details of the distribution of the species with canopy level are given in Table 4.1. Little information is available about the ecology of these species but most rain forest trees are climax species (Whitmore 1990). All of the species measured were indeed climax tree species with one or two notable exceptions; *Memora sp.* is a liana from

the Bignoniaceae family and the *Oenocarpus* measured was a seedling of an arborescent palm. *Inga sp.* was present as a canopy emergent it should be noted that this legume grows fast even in the shade.

Table 4.1 Species list of all plants measured for gas exchange properties through a vertical profile of primary Brazilian rain forest.

Canopy layer (m)	Species 1	Species 2
24	*Jacaranda copaia*	*Inga sp.*
20	*Helicostylis sp.*	*Inga sp.*
16	*Protium sp.*	*Vochysia sp.*
8	*Goupia glabra*	*Memora sp.*
ground level	*Atolla otoleoides*	*Oenocarpus sp.*

Vertical variation of gas exchange

Mean light environment

The vertical variation in photosynthetic photon flux density (PPFD) through the canopy was investigated in the month of December 1995 using fixed PPFD sensors. These were attached to horizontal poles which extended from the ZF2 data. PPFD was measured every second but values were averaged every ten minutes. Although there were initially several sensors at each of the canopy heights investigated, equipment failure reduced the number to one sensor per canopy level at some heights. The number of sensors reported per canopy level were therefore as follows: 32 m - one sensor, 28 m - one, 24 m - one, 16 m - four, 12 m - three, 8 m - three, 4 m - four, 0 m - eight.

Instantaneous measurement of photosynthesis over 24 hours

Variation in gas exchange among vertical canopy layers, and also with time of day, was investigated using portable gas exchange equipment to measure net CO_2 flux on a sample of leaves in the month of November, 1996. This is the start of the wet season so leaves were only measured on fine days. Measurements were made on at least four leaves on each of two trees per level three times during a 24 hour measurement period. This measurement period was split over two days with the first measurement being on the afternoon of day one.

The physiological variables of rate of photosynthetic assimilation (A) and stomatal conductance (g_s) were measured simultaneously with the environmental variables of photosynthetic photon flux density (PPFD) and vapour pressure deficit (D). Values of A, g_s and PPFD plus relative humidity (RH) and air temperature were measured using portable gas exchange equipment (LCA-3, Analytical Development Company, Hoddesdon, UK) and D was derived from temperature and relative humidity. Mean values per tree were compared between levels and times of day. The environmental variables measured by the LCA-3 were assumed to represent the *in situ* conditions for that leaf position only at the time of measurement.

In addition, a model predicting stomatal response to perturbations in environmental conditions was fitted to the data and assessed as to how appropriate a description it was. This was investigated because many models of canopy photosynthesis use the Ball, Woodrow & Berry (1987) model to describe stomatal response on a whole-stand scale (*e.g.* Harley & Baldocchi 1995; Leuning *et al.* 1995) and it is assumed to be a relationship which holds universally. A modified form of the original Ball *et al.* (1987) model was fitted and this model may be described as follows:

$$g_s = g_0 + \left(\frac{kAh_s}{c_s - \Gamma} \right) \qquad (4.1)$$

where g_0 = residual stomatal conductance when A and PPFD $\rightarrow$ 0,

k = slope constant,

h_s = relative humidity at the leaf surface,

c_s = CO_2 concentration at the leaf surface, and

Γ = CO_2 compensation point of photosynthesis (a value of 44 μmol mol^{-1} was used here (de Pury & Farquhar 1997)).

Consideration of the influence of low CO_2 concentrations on stomatal behaviour through the use of c_s - Γ, as opposed to c_s, was proposed by Leuning (1990). The model was fitted to the data and the predicted values of g_s compared with the measured values of g_s.

A/C_i curves

A was investigated for a range of intercellular CO_2 concentrations (C_i) and photosynthetic parameters were derived from the resultant A/C_i curves (Harley *et al.* 1992). Physiological measurements were made on three fully developed, but apparently non-senescing, leaves on each of two species at each of five levels in the canopy. These were made between 8 am and 12 midday. An artificial light source was used to give a PPFD which was assumed to be saturating (*ca* 1100 μmol m^{-2} s^{-1}). The relative humidity of the air entering the leaf cuvette was kept at about 80 % to improve the accuracy of the LCA-3 reading. No attempt was made to control leaf temperature. Full details of the method of A/C_i measurement and model fitting are given in Chapter Three. Model parameters of maximum rate of electron transport (J_{max}), maximum carboxylation velocity (V_{cmax}) and dark respiration during the day (R_d) were derived for each leaf and the average values compared between canopy levels using a one way analysis of variance and Tukey's Honestly Significant Difference Test (Fowler & Cohen 1990). The goodness of fit of modelled curves of A/C_i were tested using regressions of predicted versus observed values of A and

testing the product moment correlation coefficient for statistical significance (Fowler & Cohen 1990). The range of the coefficient of determination, r^2, is given for fitted curves.

Foliar nitrogen concentration

Foliar concentrations were investigated on both a leaf area and a leaf mass basis. Upon completion of gas exchange measurement, leaves were collected and discs of known area (7.86×10^{-5} m^2) oven-dried at 80 °C to constant mass before nitrogen analysis. The discs were then finely ground on a centrifugal grinder (Retsch, Glen Creston, Stanmore, UK). The samples (0.1 g) were acid/peroxide-digested (adapted from Grimshaw *et al.* 1989), diluted with water and analysed for nitrogen content colorimetrically, using a flow injection analyser (Flow Solution 3000, Perstorp Analytical, Oregon, USA, Appendix 4). Values of foliar nitrogen concentrations were averaged for each level in the canopy and then compared between levels. In addition, nitrogen concentrations of the individual leaves were compared with the derived J_{max} and V_{cmax} values for the same leaf. The relationships between foliar N concentration and J_{max}, V_{cmax} and SLA were compared with those reported by other authors. In the case of SLA, the inverse, a measure of leaf mass per unit area, was sometimes used to test for statistical significance using linear regression.

RESULTS

Considerable variation existed in the micro-environment between the top and bottom canopy levels (Fig. 4.1). The top level (24 m) showed the biggest range of values in PPFD and *D*. In this level g_s and *A* were maximal at the time of the first measurements of the day (about 7.30 am). Micro-environment, g_s and *A* varied little at ground level during the 24 hour measured time period. The maximum g_s recorded was 230 mmol m^{-2} s^{-1} at 24 m and the maximum rate of *A* was 7.1 μmol m^{-2} s^{-1} at 20 m, at about 7.30 am. The data show some evidence of stomatal recovery from closure in the late afternoon.

Even in this small data set some evidence of a correlation between both *A* and g_s and *A* and PPFD can be observed (Fig. 4.2, p. 157). The average vertical profile of PPFD is shown in Fig. 4.3 (p. 158) where a logarithmic decline with decreasing canopy height is evident. The increase in PPFD at 8 m of canopy height suggests a light gap at this point.

Modelled A/C_i curves fitted the observed A/C_i data very well. When predicted values of *A* were compared with observed values 90 % of the relationships had an r^2 of greater than 0.9, the best fit being 0.99 and the worst 0.68. A/C_i curves differed between the bottom and top of the canopy but intermediate levels were indistinguishable from one another (Table 4.2, p. 158 and Fig. 4.4, p. 159).

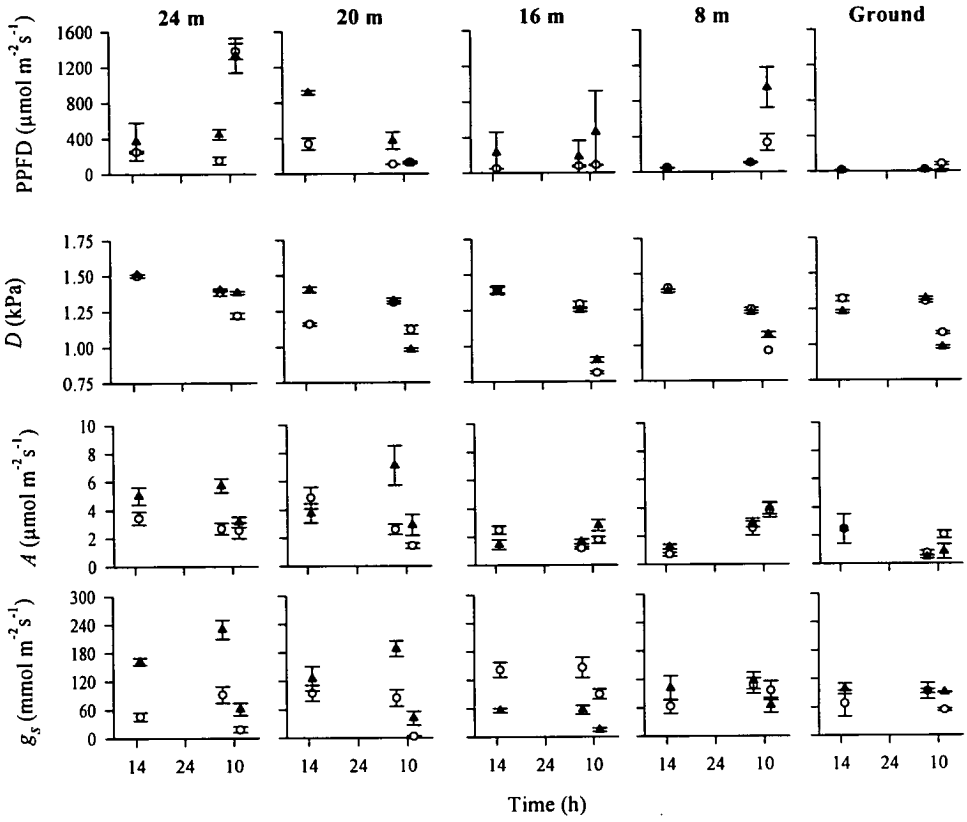


Figure 4.1 Daily variation in canopy gas exchange through a vertical profile of undisturbed rain forest at ZF2, Brazil. At least four leaves on each of two trees (shown as either an open or closed symbol) were measured at each of five heights in the canopy three times during a 24 hour cycle. Gas exchange was measured over two days therefore times are shown in order of collection. Data are shown for photosynthetic photon flux density (PPFD), vapour pressure deficit (D), rate of photosynthesis (A) and stomatal conductance (g_s) at the time of measurement. Mean values are shown plus or minus one standard error. Species used were as follows (first species is denoted by an open circle and the second by a closed triangle): 24 m: *Jacaranda copaia*, *Inga sp.*, 20 m: *Helicostylis sp.*, *Inga sp.*, 16 m: *Protium sp. Vochysia sp.*, 8 m: *Goupia glabra*, *Memora sp.*, Ground level: *Atolla otoleoides*, *Oenocarpus sp.*

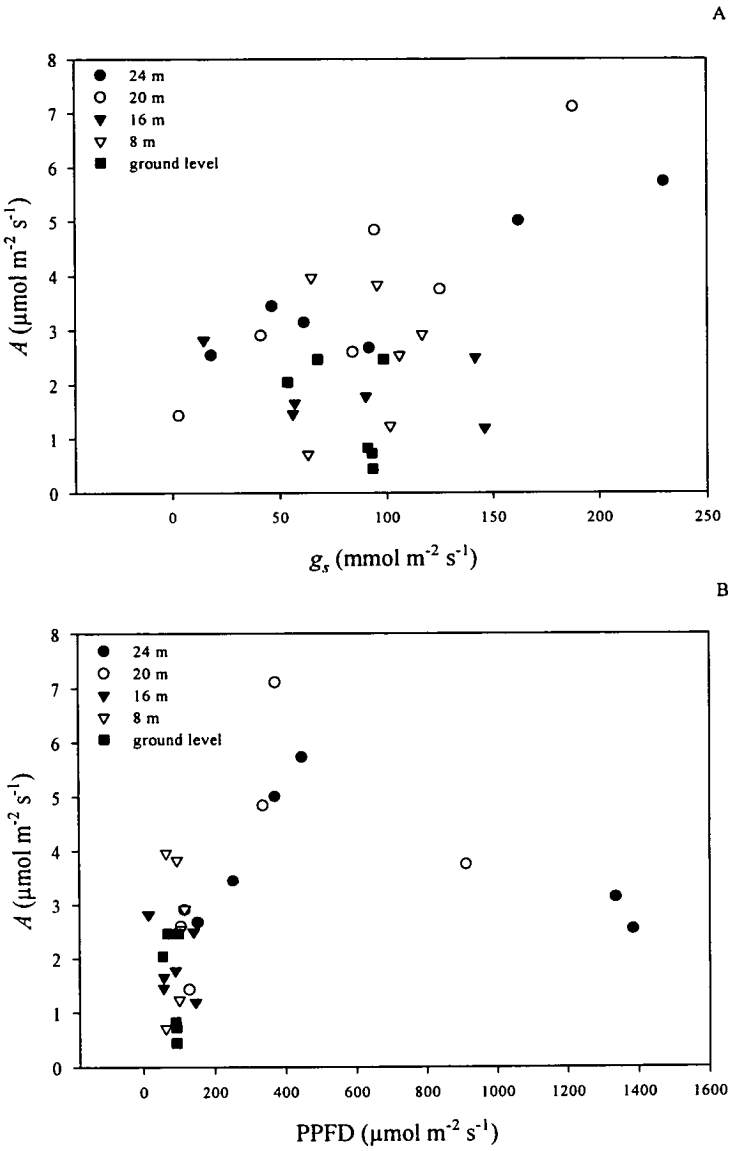


Figure 4.2 Plots of the relationships between stomatal conductance, g_s, instantaneous photosynthetic photon flux density, PPFD, and rate of photosynthesis, A, at each of the five canopy height levels measured at ZF2 in an undisturbed Brazilian rain forest. All five vertical levels are shown per graph, each level depicted by a different symbol. Values shown are means for each level - for standard errors see Fig. 4.1. A. Plot of the relationship between A and g_s. B. Plot of the relationship between A and PPFD.

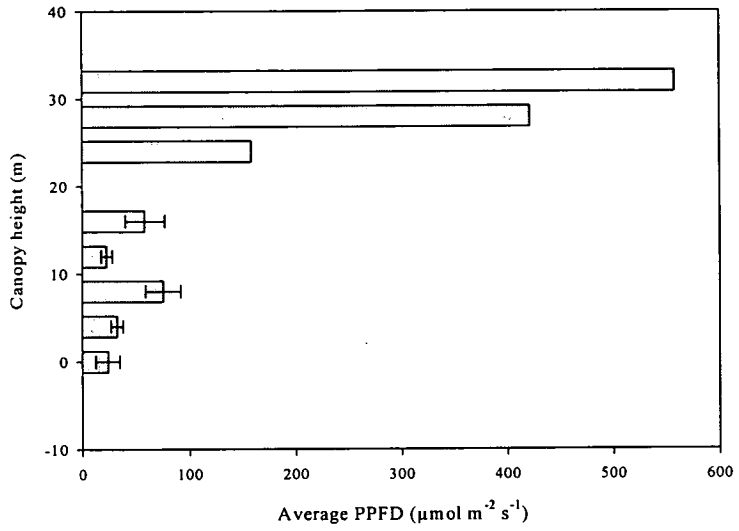


Figure 4.3 Average photosynthetic photon flux density (PPFD) through a vertical profile of rain forest in Brazil. PPFD was measured continuously in December 1995, by sensors fixed to horizontal poles attached to the ZF2 tower. One standard error of the mean is shown where there was more than one sensor at a given level.

Table 4.2 Average estimates of parameters of A/C_i curves fitted to data from each of five canopy levels in an undisturbed Brazilian rain forest. The fitted parameters shown for each level are J_{max} (maximum rate of electron transport), V_{cmax} (maximum carboxylation velocity) and R_d (rate of dark respiration in the day). Mean values for each level are shown with one standard error of the mean. n = 6 per level. Means with different superscripts within a column are significantly different ($p < 0.05$).

Fitted A/C_i parameter	J_{max} (μmol m^{-2} s^{-1})	V_{cmax} (μmol m^{-2} s^{-1})	R_d (μmol m^{-2} s^{-1})
Canopy height (m)			
24	94.17 ± 12.9 ^{a}	37.7 ± 5.9 ^{a}	-0.73 ± 0.19 ^{a}
20	64.33 ± 6.3 ^{b}	27.8 ± 2.3 ^{ab}	-0.65 ± 0.12 ^{a}
16	70 ± 3.6 ^{ab}	22.3 ± 1.9 ^{ab}	-0.62 ± 0.27 ^{a}
8	63.67 ± 4.4 ^{b}	26.3 ± 2.6 ^{ab}	-0.85 ± 0.26 ^{a}
Ground level	34.67 ± 5.3 ^{c}	17 ± 1.7 ^{b}	-0.2 ± 0.19 ^{a}

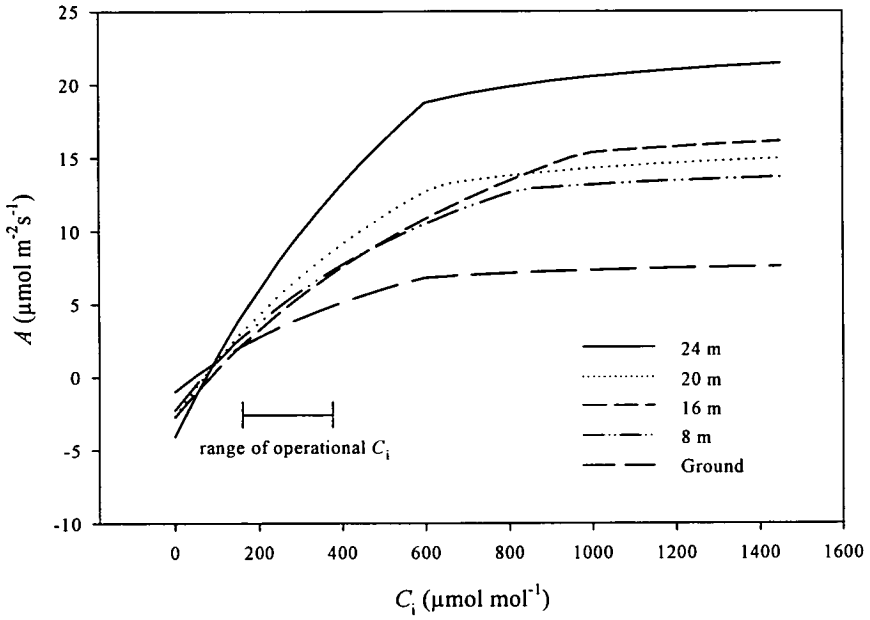


Figure 4.4 Modelled A/C_i curves for each of five canopy levels in an undisturbed Brazilian rain forest. Parameters for modelled curves are derived from the average parameters of all leaves measured at that height in the canopy. Also shown is the range of operational C_i for all leaves measured.

Of the derived A/C_i parameters, the most sensitive to change with canopy level was J_{max} (Fig. 4.5). The J_{max} value of the top canopy level was significantly different ($p < 0.05$) to that fitted to the lowest three levels. In general, there was a close agreement between species at the same level in the derived parameters, with the exception of the two species at level 24 m. Values of J_{max} and V_{cmax} derived for *Jacaranda copaia* were more similar to those values derived for species at levels 8, 16 and 20 m than they were to the values for *Inga sp.* at 24 m. All levels had a significantly different J_{max} to that of plants at ground level ($p < 0.05$, Table 4.2, p. 158). V_{cmax} differed significantly only between the top and bottom levels ($p < 0.05$, Table 4.2, p. 158). Fitted values of R_d did not vary significantly with canopy level ($p > 0.05$, Table 4.2, p. 158).

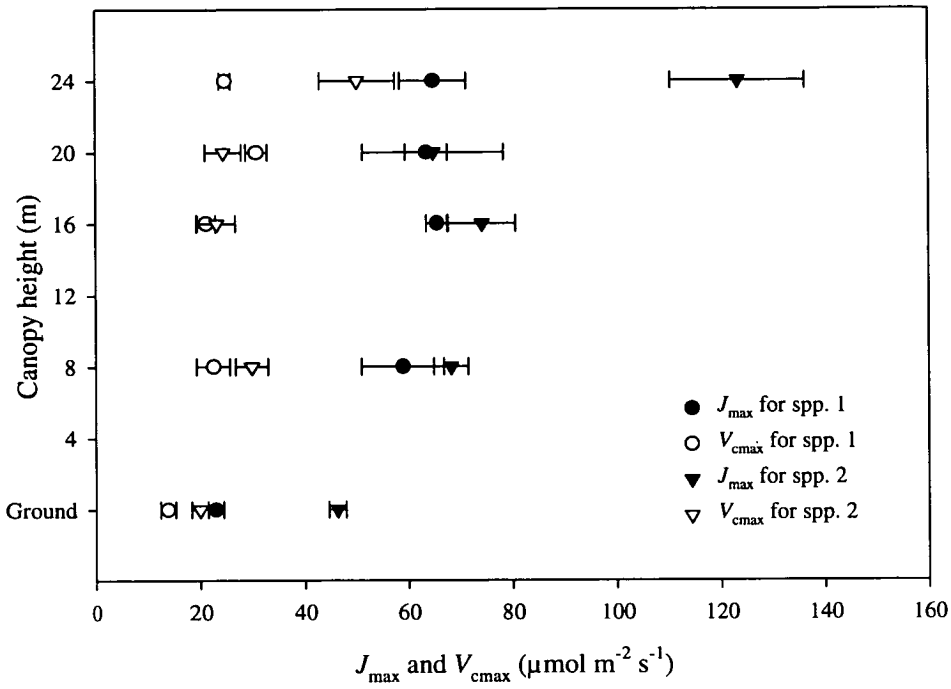


Figure 4.5 Relationship between J_{max} and V_{cmax} and canopy height at ZF2 in an undisturbed Brazilian rain forest. Average values for all leaves measured per tree are shown for every canopy level plus or minus one standard error. Species 1 (spp. 1) at each canopy height was: 24 m: *Jacaranda copaia*, 20 m: *Inga sp.*, 16 m: *Protium sp.*, 8 m: *Memora sp.*, Ground level: *Atolla otoleoides*. Species 2 (spp. 2) at each canopy height was: 24 m: *Inga sp.*, 20 m: *Helicostylis sp.*, 16 m: *Vochysia sp.*, 8 m: *Goupia glabra*, Ground level: *Oenocarpus sp.*

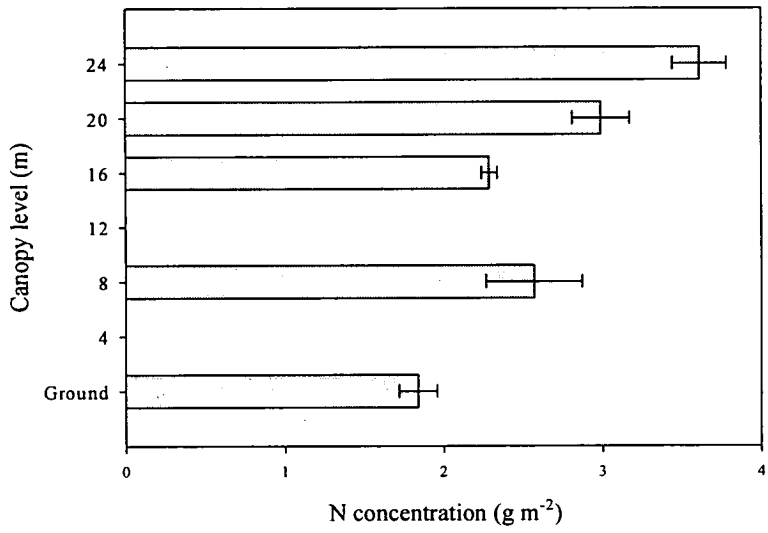


Figure 4.6 Foliar nitrogen concentration expressed on a leaf area basis for each of five canopy levels at ZF2 within a Brazilian rain forest. Mean estimates of all leaves measured are given plus or minus one standard error.

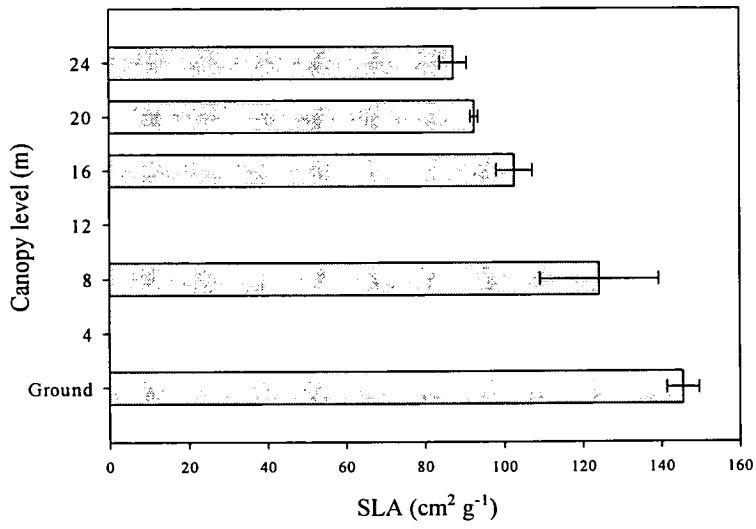


Figure 4.7 Specific leaf areas for each of five vertical strata at ZF2 in an undisturbed Brazilian rain forest. Values are shown as averages of all leaves measured per level plus or minus one standard error. The height of the canopy was approximately 30 m but leaves were only accessible up to 24 m at which point the trees were considered to be emergent.

Foliar nitrogen (N) concentration increased significantly ($p < 0.05$) with canopy height when expressed on a leaf area basis (Fig. 4.6) but not when expressed as a % of total leaf mass (Table 4.3). Specific leaf area (SLA) significantly decreased ($p < 0.05$) with canopy height (Table 4.3, Fig. 4.7). There was no relationship between SLA and N when expressed on a mass basis ($r^2 = 0.03$, $p > 0.05$) but SLA was significantly correlated with leaf N when expressed on an area basis ($r^2 = 0.69$, $p < 0.05$, Fig. 4.8).

Table 4.3 Foliar nitrogen concentrations (g m^{-2} and mg g^{-1}) from leaves of each of five canopy levels in an undisturbed Brazilian rain forest. Values of specific leaf area (SLA) are also shown for each level. Statistically significant differences ($p < 0.05$) are denoted by superscripts: [a] = significantly different to that of 16 m, [b] = significantly different to that of 8 m, [c] = significantly different to that of ground level. Mean values of all leaves measured at each level are shown with one standard error of the mean.

Canopy height (m)	Foliar N concentration (g m^{-2})	Foliar N concentration (mg g^{-1})	Specific leaf area (cm^2 g^{-1})
24 ($n = 21$)	3.61 ± 0.17^{a}	30.5 ± 0.06^{a}	87.3 ± 3.4^{a}
20 ($n = 13$)	2.99 ± 0.18^{b}	27.5 ± 0.16^{ab}	92.6 ± 1.0^{a}
16 ($n = 9$)	2.29 ± 0.05^{ab}	23.6 ± 0.13^{b}	102.6 ± 4.6^{ab}
8 ($n = 8$)	2.57 ± 0.30^{b}	29.3 ± 0.18^{ab}	124.2 ± 15.1^{bc}
Ground level ($n = 17$)	1.84 ± 0.12^{c}	25.9 ± 0.13^{ab}	145.5 ± 4.1^{c}

When expressed per unit area foliar N concentration was correlated with derived values of J_{max} (Fig. 4.9, p. 164, $r^2 = 0.49$, $p < 0.05$). However, when expressed on a mass basis, N concentration was not correlated with derived values of J_{max} (Fig. 4.10, p. 164, $r^2 = 0.01$, $p > 0.05$). The same thing was observed for derived values of V_{cmax} (Figures 4.11 & 4.12, p. 165).

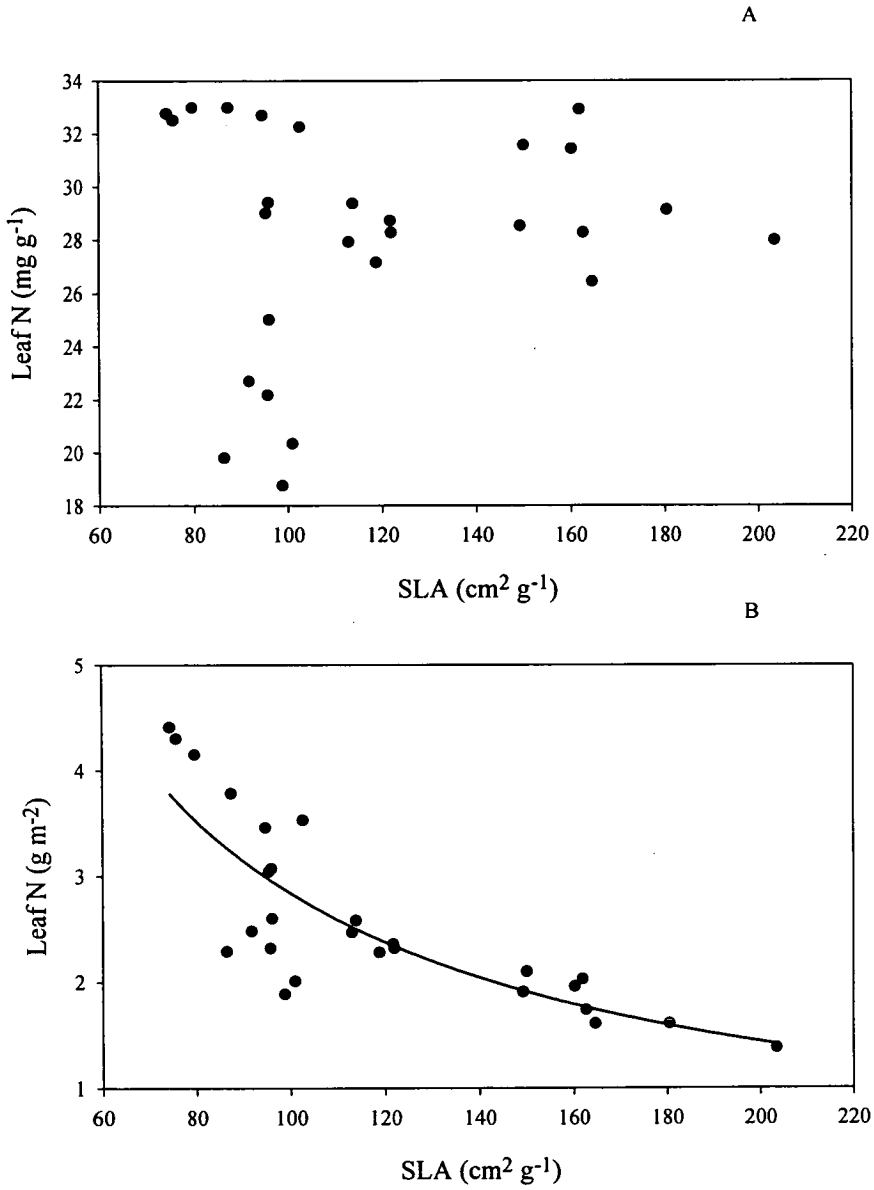


Figure 4.8 Relationship between specific leaf area (SLA) and leaf nitrogen concentration for an undisturbed Brazilian rain forest at ZF2. A. SLA vs leaf N on a mass basis (mg g^{-1}). B. SLA vs leaf N on an area basis (g m^{-2}).

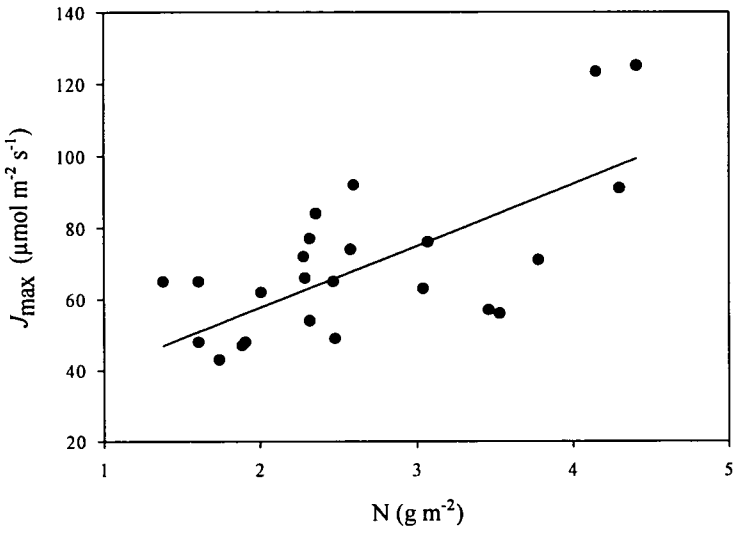


Figure 4.9 Relationship between J_{max} and N concentration when both are expressed on an area basis for corresponding leaves measured in a vertical profile of undisturbed Brazilian rain forest at ZF2. J_{max} values were fitted using the Farquhar *et al.* (1980) model (de Pury & Farquhar 1997). There was a significant correlation between J_{max} and N (J_{max} = 17.24N + 23.2 r^2 = 0.49, $p < 0.05$, n = 27).

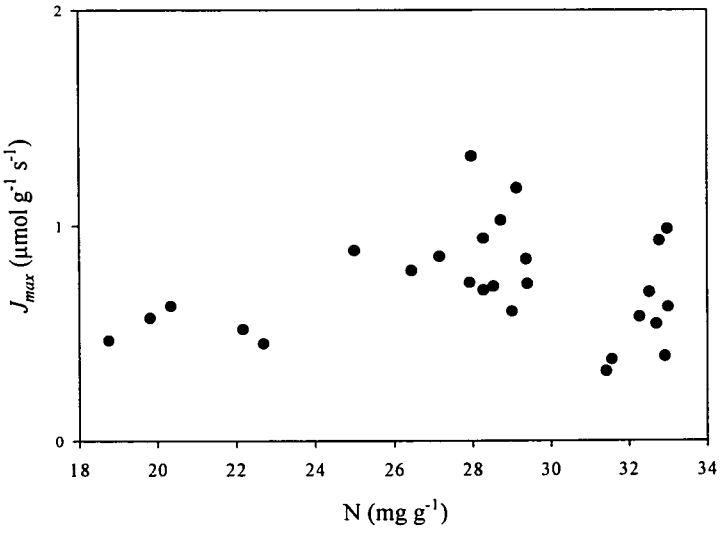


Figure 4.10 Relationship between J_{max} and N concentration when both are expressed on a mass basis for corresponding leaves measured in a vertical profile of undisturbed Brazilian rain forest at ZF2. J_{max} values were fitted using the Farquhar *et al.* (1980) model (de Pury & Farquhar 1997). There was no significant correlation between J_{max} and N. (J_{max} = 6.41N + 0.54 r^2 = 0.01, $p > 0.05$, n = 27)

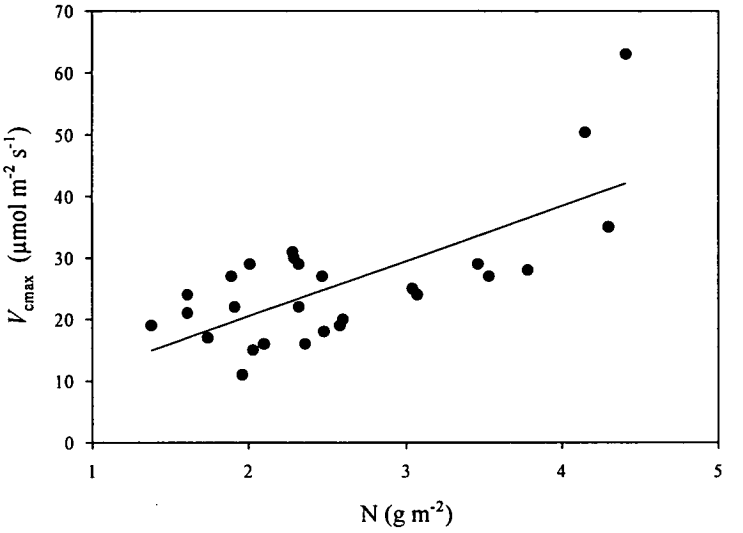


Figure 4.11 Relationship between V_{cmax} and N concentration when both are expressed on an area basis for corresponding leaves measured in a vertical profile of undisturbed Brazilian rain forest at ZF2. V_{cmax} values were fitted using the Farquhar *et al.* (1980) model (de Pury & Farquhar 1997). There was a significant correlation between V_{cmax} and N ($V_{cmax} = 8.96N + 2.59$ $r^2 = 0.51, p < 0.05, n = 27$).

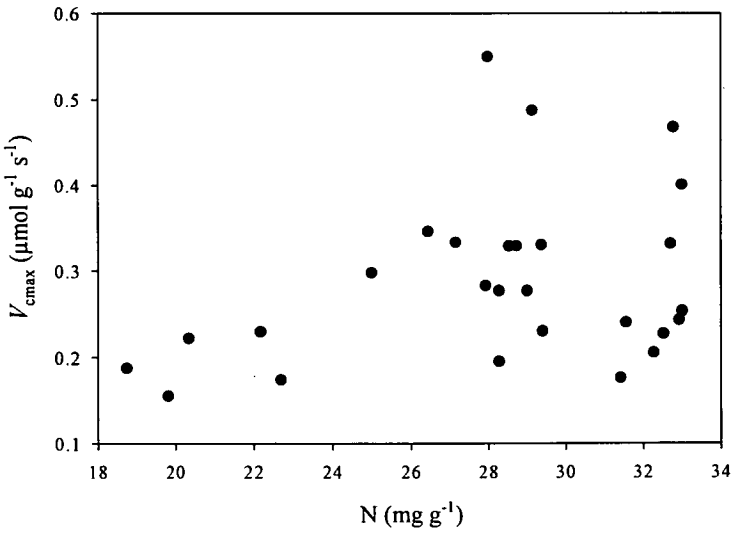


Figure 4.12 Relationship between V_{cmax} and N concentration when both are expressed on a mass basis for corresponding leaves measured in a vertical profile of undisturbed Brazilian rain forest at ZF2. V_{cmax} values were fitted using the Farquhar *et al.* (1980) model (de Pury & Farquhar 1997). There was no significant correlation between V_{cmax} and N. ($V_{cmax} = 7.52N + 0.076$ $r^2 = 0.11, p > 0.05, n = 27$)

Nitrogen use efficiency (when investigated on an area basis as J_{max} per unit of N) appeared lower than that of a previous study of Cameroon rain forest (Fig. 4.13).

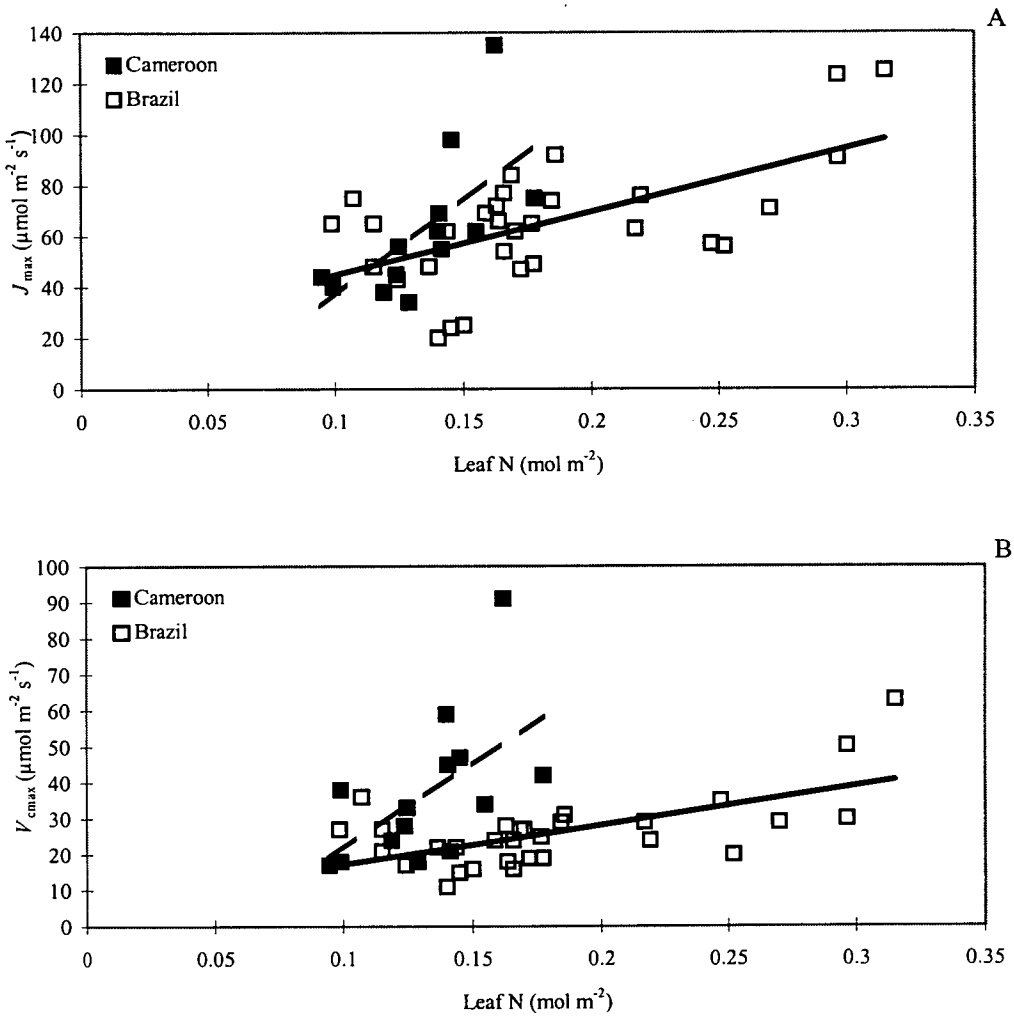


Figure 4.13 A comparison of the photosynthetic capacity of two types of rain forest. Results from the present study of an undisturbed forest at ZF2 in Brazil are compared with those of a previous study in Cameroon (Meir 1996). A. Linear regressions for the relationships between leaf N and J_{max} for the two studies (Cameroon: J_{max} = 760.2N - 39.4, r^2 = 0.46, n = 14. ZF2: J_{max} = 249.1N + 19.8, r^2 = 0.37, n = 30). B. Linear regressions for the relationships between leaf N and V_{cmax} for the two studies (Cameroon: V_{cmax} = 474.9N - 26.0, r^2 = 0.34, n = 14. ZF2: V_{cmax} = 109.2N + 6.24, r^2 = 0.38, n = 30). The relationship for leaves measured in Cameroon is shown as a dotted line and the relationship for leaves measured in Brazil is shown as a solid line.

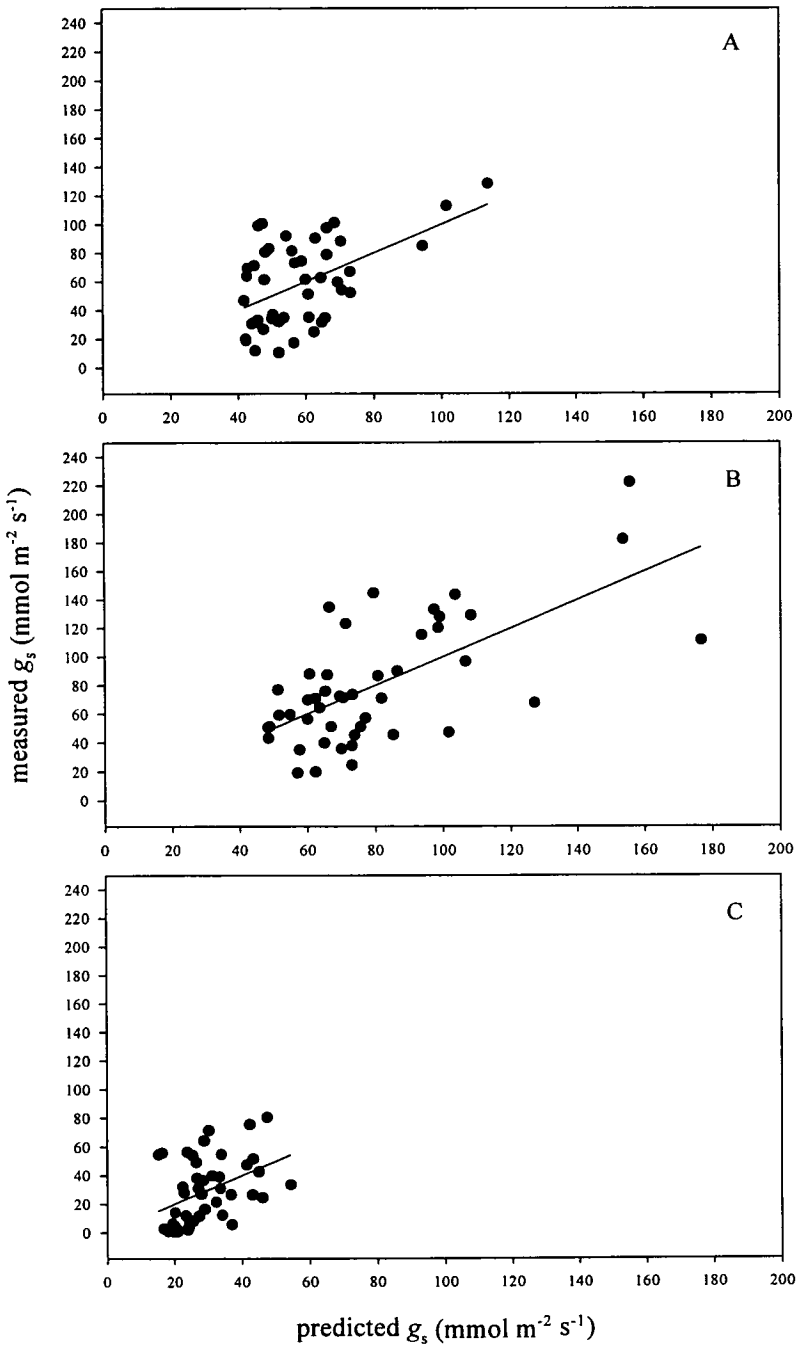


Figure 4.14 A test of the fit of the Ball *et al.* (1987) model of stomatal conductance on instantaneous measurements of stomatal conductance and photosynthesis at ZF2 in a Brazilian rain forest. The data have been combined for all canopy levels. A. Leaves measured at 2.45 pm. B. The same leaves measured the following morning at 7.45 am. C. The same leaves measured at 11.30 am. $n = 46$ for all plots. Straight lines represent the regression of predicted versus measured g_s and indicate the scatter of the data from this theoretical relationship.

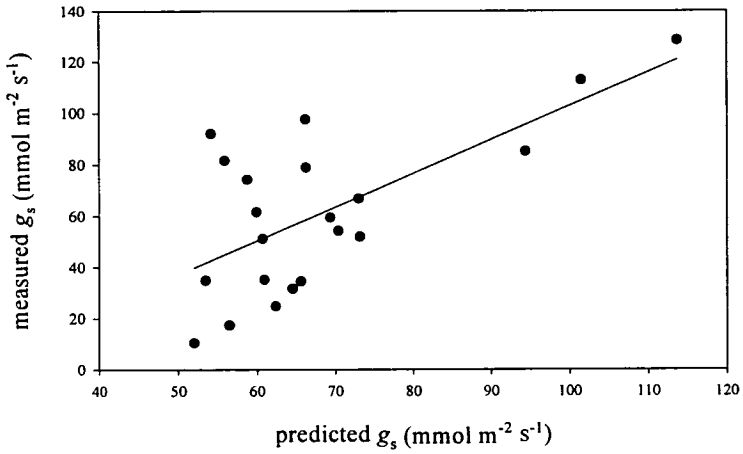


Figure 4.15 A test of the fit of the Ball *et al.* (1987) model of stomatal conductance on instantaneous measurements of stomatal conductance and photosynthesis at ZF2 in a Brazilian rain forest. The data shown are for one canopy level only (24 m) at 2.45 pm. $n = 21$.

The Ball *et al.* (1987) model of stomatal conductance is not a good fit of the data (Table 4.4, Fig. 4.14) when leaves are combined from all levels. There is significant scatter in the relationship when compared with the "collapse" of data to a single line which is predicted by the model, and observed in some other studies (*e.g.* Harley *et al.* 1992). The scatter is reduced, but not eliminated, when the model is fitted to data from one canopy level only (Table 4.4, Fig. 4.15). The level shown is the one where the model fit was best.

Table 4.4 A. Derived values of g_0 and k obtained through fitting the Ball *et al.* (1987) model to instantaneous measurements of stomatal conductance and photosynthesis through a vertical profile of the canopy of an undisturbed forest at ZF2 in Brazil. Data are combined for all levels of the canopy but the model was fitted anew to each of the three measurement times. $n = 46$ per measurement time. B. Derived values of g_0 and k obtained through fitting the Ball *et al.* (1987) model to leaves from one canopy level (24 m) only at one measurement time. $n = 21$

A. Measurement time	g_0	k	r^2
2.45 pm	40.2	21.8	0.27
7.45 am	45.1	38.6	0.43
11.30 am	14.1	20.7	0.17
B. 24 m at 2.45 pm	24.4	28.6	0.45

DISCUSSION

Daily canopy performance

Maximum recorded values of both g_s and A are lower than in earlier studies of Amazonian forest (McWilliam *et al.* 1996). However, the maximum values recorded here of g_s for the upper canopy and the understorey match almost exactly those previously recorded in *terra firme* rain forest at another site closer to Manaus (Roberts *et al.* 1990). The maximum values of A are also close to those reported in the same study. Although there is considerable scatter in the relationship, instantaneous measurements of A increase both with g_s and PPFD (Fig. 4.2, p. 157).

The low rates of maximum photosynthesis reported in this study could be attributed to the seasonality of the forest. Independent evidence suggests there may have been drought stress in this forest at the time of A/C_i measurement (Williams *et al.* 1997) and this may be responsible for the observed low rates of A and the large amount of scatter observed in Fig. 4.2, p. 157. The climate in central Amazonia is reported to be subject to large fluctuations in rainfall with accompanying soil water content changes (Hodnett *et al.* 1996). The low soil moisture content observed in the same forest one year earlier correlated strongly with depressions of ecosystem transpiration and net photosynthesis (Malhi *et al.* 1997). The drought was estimated to have ended in mid-November 1995 (Williams *et al.* 1997) and the measurements of the current study were completed in about mid-November 1996, at the end of the dry season and before any substantial increase in rainfall.

Light acclimation hypothesis

Photosynthetic capacity is generally well correlated with average light environment. The decline in PPFD with decreasing canopy height roughly matches the decline in J_{max} and V_{cmax} and foliar N concentration (Figures 4.4 - 4.6, pp. 159 - 160). In general, there were bigger differences between canopy levels than there were between species at a given canopy level (Fig. 4.5 p. 160), except for at 24 m. It is thought that the low values of J_{max} and V_{cmax} derived for *J. copaia* reflect drought stress which was thought to be occurring in the canopy at the time of the measurement. Because the *J. copaia* was a much smaller tree than the *Inga sp.* which was also measured at that canopy level, it is possible that the *Inga sp.* had a larger rooting system which was better able to reach a deeper water table. It is acknowledged that further investigation of the differences between species at each of the canopy levels investigated is required to ascertain that the simplification of the canopy, by level is valid for this particular stand. It is possible also that the variation in photosynthetic capacity within level 24 m was a result of differences in local, rather than mean, light environment. Data from hemispherical photographs of the light environment of individual leaves is currently being processed and should clarify whether or not this was a cause of the decreased photosynthetic capacity of this species.

Upon first inspection, the derived values of J_{max} and V_{cmax} seem a little low in comparison with those from the upper levels of some temperate forest types. For example, J_{max} for the uppermost measured level was 94 μmol m^{-2} s^{-1} while that measured in an English oak forest at a comparable height was 140 μmol m^{-2} s^{-1} (Kruijt, unpublished data). However, in a retrospective analysis of A/C_i curves Wullschleger (1993) gave the average value of J_{max} for tropical forest species as 107 μmol m^{-2} s^{-1} with the average V_{cmax} being 51 μmol m^{-2} s^{-1}. A huge range in fitted

values for tropical species was reported. J_{max} ranged from 30 to 222 μmol m^{-2} s^{-1} and V_{cmax} ranged from 9 to 126 μmol m^{-2} s^{-1} (n = 22). The values of the present study fall easily within these ranges.

When photosynthesis and N concentration were examined on an area basis the distribution of photosynthetic activity through the canopy appeared to be consistent with the predicted optimum (Field & Mooney 1986; Hirose & Werger 1987; Sellers *et al.* 1992), with the uppermost measured canopy layer showing a significantly higher capacity for light harvesting (higher J_{max}) than the other layers when expressed on an area basis (Fig. 4.9, p. 164 & Table 4.2, p. 158). However, when examined on a mass basis there is no relationship between J_{max} and N concentration (Fig. 4.10, p. 164). This is consistent with another study of photosynthetic capacity and foliar N concentrations in Amazonian *terra firme* forest. This study investigated the relationship of A_{max} with N and found a correlation only when expressed per unit area (Reich, Ellsworth & Uhl 1995).

The J_{max}:N and V_{cmax}:N relationships were considerably lower in this study than in a previous study of the vertical profile of photosynthetic capacity of a rain forest in Cameroon (Meir 1996). For example, the slope of the J_{max}:N relationship is three times steeper in Meir's study than in the present study, while the slope of the V_{cmax}:N is about four times steeper (Fig. 4.10, p. 164). This suggests that the forest in Cameroon has a higher N-use efficiency. Both forests are growing on relatively nutrient-poor ultisols or oxisols but it has been suggested that the leaf physiology in the forest of the Brazilian Amazon is more constrained by phosphorus limitation than that of the Cameroon site (Meir 1996) which may explain the lower photosynthetic capacity.

The increase in photosynthetic capacity per unit of leaf area with canopy height may be explained by the concomitant decrease in SLA (Fig. 4.7, p. 161). That is, leaves are thinner at the bottom of the canopy than at the top, hence the equivalent relationship of J_{max} to N concentration at both positions when expressed on a mass basis, assuming constant construction "costs" for leaves of different volumes. Alternatively, the decrease in SLA could be explained by a decrease in starch content.

The relationship between SLA and leaf N is the same as previously reported for a *terra firme* forest in Venezuela (Reich & Walters 1994). Both studies have found a significant inverse relationship between SLA and leaf N when expressed on an area basis but no relationship when leaf N is expressed on a mass basis. The other study concluded that the type of vegetation may differ in this relationship as there are other instances where a significant relationship does exist between SLA and specific leaf N. However, it was hypothesised that the increase in leaf N per unit area with leaf age or canopy height is actually a function of leaf mass increasing per unit area and that the nitrogen is itself not increasing per unit of mass, that is, the nitrogen is not becoming more concentrated in leaves, the leaves are merely accumulating more mass per unit area.

Many studies of photosynthetic acclimation to light have concluded that the primary mechanism is a reduction in leaf thickness with decreasing PPFD consequently increasing photosynthetic efficiency per unit area (Givnish 1988; Sims, Seemann & Luo 1997). Results of this study corroborate this hypothesis and suggest that light acclimation in this *terra firme* rain forest does occur through an increase in leaf thickness with increasing irradiance and a consequently higher photosynthetic capacity through increased N content per unit of leaf area (Evans 1996). The results of the present study support the hypothesis of photosynthetic capacity acclimating to the prevailing light environment (Kull & Jarvis 1995) rather than nitrogen

distribution *per se* acclimating to the prevailing light environment. Because leaves become thinner with decreasing canopy height, in addition to the concomitant decrease of nitrogen concentration, it is proposed that reallocation of nitrogen is only one of a range of responses operating to optimise photosynthetic capacity.

Canopy heterogeneity

Surprisingly, Rubisco activity which is characterised by V_{cmax}, differed significantly only between the uppermost and lowest levels of the canopy. Stoichiometry between V_{cmax} and J_{max} is to be expected, the ratio between them remaining relatively constant (Wullschleger 1993). In the present study the ratio between the two parameters (J_{max}/V_{cmax}) decreased from 2.5 to 2 between the uppermost and lowest level. The ratio may vary with temperature, however, in proportion to the temperature sensitivity of the components (de Pury & Farquhar 1997). This observed decrease in J_{max}/V_{cmax} ratio may be accounted for by variability amongst the leaves and the small sample size at each level. However, it may actually reflect a physiological shift in the emphasis of control with the decrease in PPFD. The errors associated with the derived values of R_d are too large to allow inferences about changes in dark respiration with canopy height to be drawn from these data.

Many modellers have used the Ball *et al.* (1987) model to predict the response of stomata and consequent photosynthetic response of forest canopies to environmental perturbations (*e.g.* Sellers *et al.* 1992; Harley & Baldocchi 1995; Leuning *et al.* 1995). Although this function has provided a good description of the interaction in some single-species studies (*e.g.* Lloyd 1991; Harley *et al.* 1992), data from the current study suggests that this model cannot adequately describe the complexity of the interaction in a multi-layered forest stand (Fig. 4.14, p. 167). There is so much scatter around the regression fitted to the modelled relationship that a Lohammer (1980)-type correction for stomatal response to humidity deficit rather than relative

humidity is unlikely to improve the fit significantly. Even for leaves from a single canopy level there is much scatter in the relationship (Fig. 4.14, p. 167). This observation calls into question the validity of scaling-up models which assume a Ball *et al.* (1987)- type response of stomata to changes in atmospheric humidity across a whole canopy without testing this assumption for the specific forest stand which is being modelled.

Terashima & Hikosaka (1995) reported the vertical distribution of photosynthetic capacity, on an area basis, to be slightly less efficient than the theoretical optimum, with intermediate levels showing greater similarity than expected. The current study did not pick up significant differences in photosynthetic capacity between the intermediate levels (20, 16 and 8 m). One possible explanation is the existence of canopy gaps near the tower (Fig. 4.1, p. 156) which interfere with a strictly vertical profile of decreasing photon flux density (de Pury & Farquhar 1997). For example, leaves measured on plants at the 20 m and 8 m levels experienced large sunflecks from canopy gaps at particular times of day. This is supported by data on the light profile where a canopy gap is visible at 8 m (Fig. 4.3, p. 158). At this point there is also an increase in photosynthetic capacity (Fig. 4.5, p. 160) and leaf N (Fig. 4.6, p. 161) which suggests that the canopy is acclimating to mean light environment.

In addition, the choice of species measured at each level influenced the observed pattern of photosynthesis. Plants measured at level 20 m had larger, thicker leaves and this confers a smaller boundary layer conductance and consequently higher g_s (Whitehead, Okali & Fasehun 1981; Grace 1983). These phenomena reflect the heterogeneity typical of the forest environment and care must be taken to account for such variability in quantitative descriptions. Further data is required on the light environment at the position of each of the individual leaves measured.

Implications for interaction with elevated atmospheric CO_2 concentration

The photosynthetic responses of plants subjected to elevated concentrations of atmospheric CO_2 typically involve reductions in V_{cmax} (or Rubisco activity) and J_{max} (Gunderson & Wullschleger 1994). However, as a result of the close coupling between V_{cmax}, J_{max} and R_d (de Pury & Farquhar 1997) plant response depends on other environmental variables in addition to CO_2 concentration, so that the response of photosynthesis to elevated CO_2 must depend upon the position of a leaf in the canopy, and its associated capacity to harvest light. Some empirical evidence suggests that those species most likely to show an acclimation response to elevated CO_2 concentrations are those with the ability to reapportion leaf N in response to low irradiation (Kubiske & Pregitzer 1996). However, the mechanisms of acclimation to each variable appear different and the latest research suggests that acclimation to low PPFD increases efficiency of use of the most limiting resource (PPFD) while acclimation to elevated CO_2 concentration depends on inherent capacity of the plant to utilise end products, namely carbohydrates, to avoid limitation by this now plentiful resource (Sims *et al.* 1997).

It is possible to use data from this chapter in a model of stand response to fluctuations in environmental driving variables. Because the parameters of V_{cmax} and J_{max} are sensitive to changes in both light and CO_2 concentration, it is possible to take the *in situ* values given for each canopy level and perturb them in the direction of expected change. In addition, having some idea of how the canopy responds as a whole to changes in D and PPFD allows the formation of a comprehensive picture of the interaction of these variables. The ideal tool for such a task is a model, such as MAESTRO (Wang & Jarvis 1990), and it is suggested that future efforts to predict photosynthetic response to elevated CO_2 concentration incorporate *in situ*

measurements of canopy physiology with experimental manipulation of the growth CO_2 concentration of as many of these canopy species as is possible.

CONCLUSIONS

1) Photosynthetic capacity increases with canopy height of this Brazilian forest site, the top level of the canopy having an electron transport capacity (J_{max}) three times that of the ground level. The maximum velocity of carboxylation (V_{cmax}) at the top of the canopy was approximately twice that of the ground level. There is some variation between species within a level and it is suggested that further investigation of species differences would help clarify the relative importance of species differences versus height of leaf insertion within a canopy.

2) The distribution of photosynthetic capacity correlated well with the distribution of mean PPFD. Even the gaps which alter a strictly logarithmic decline in PPFD density with increasing distance from the top of the canopy, could be correlated with photosynthetic capacity.

3) It is suggested that the chief mechanism of acclimation to the light environment is actually a decreasing leaf thickness with decreasing PPFD rather than an optimal allocation of nitrogen *per se*.

4) This type of *in situ* measurement of canopy physiology is very useful for integration into models predicting forest stand responses to elevated CO_2 concentration. Its chief value is that it is quantitative so simulations of forest response to perturbations in atmospheric CO_2 concentration could be achieved fairly easily. Additional data, including a thorough investigation of interspecific differences and stomatal responses to changes in PPFD through a vertical profile, would be required.

Chapter Five:
GENERAL DISCUSSION

INTRODUCTION

Despite the vast literature on responses of plants to elevated CO_2 concentration, there remain some gaping holes in our understanding. The responses of tropical species are under-investigated, despite their likely key role in the global carbon balance. Specifically, there exists little quantitative data on their photosynthetic response to elevated CO_2 concentration, particularly in conditions where the nutrient supply is thought to be limiting. The investigation of photosynthetic capacity of a tropical tree species after growth in an elevated CO_2 concentration, has been done only once previously using A/C_i curves (Gunderson & Wullschleger 1994). Furthermore, there has been no attempt made to "scale-up" from a single plant's response to the likely response of a tropical forest-stand to elevated CO_2 concentration. This study provides quantitative data on the photosynthetic and whole-plant response of one tropical tree species, *Cedrela odorata*, plus complementary data on the whole-plant

response of another species, *Schefflera macrostachya*, to elevated CO_2 concentration. In addition, the influence of CO_2 concentration on carbon assimilation has been quantified at a site of undisturbed tropical rain forest in Brazil, throughout the canopy. This type of data has not previously been published for tropical forests, despite being required for models of forest-stand response to elevated CO_2 concentration. In this chapter, a scheme is given for the integration of results from experiments on tropical tree species in an elevated concentration of CO_2 with *in situ* measurements of canopy photosynthesis. Methods of additional manipulation and standardisation across a range of scales are suggested.

It has been suggested that the modelling of plant responses to elevated CO_2 concentration revolves around two different goals (Reynolds *et al.* 1996). The first is the study of the effects of elevated CO_2 concentration on plant physiology and subsequent translation into growth. The second is the prediction of plant response to elevated CO_2 concentration within the context of larger scale, such as ecosystem and global, responses. Results from the present study contribute more to achieving the first goal but models arising from compilation of this kind of data will identify key attributes of plant response which should be incorporated into large scale CO_2 response models, necessary to achieve the second goal (Reynolds *et al.* 1996). The heterogeneity of species' responses is possibly the biggest problem with predicting whole forest response to elevated CO_2 concentration (Bazzaz & Miao 1993; Körner 1993; Mousseau *et al.* 1996) but much of this variability can be explained as a result of the use of differing methodology (Eamus & Jarvis 1989; Jarvis 1995). Closer analysis of the physiological parameters that respond to changes in atmospheric CO_2 concentration provides a mechanism for explaining the response of a given species and integrating the range in response through a canopy.

The modelling of basic physiological data to allow prediction of whole-forest response to elevated CO_2 concentration can be approached in a number of ways. Processes can be represented over a wide range of complexity with simple representations requiring fewer data but making large assumptions. The quantification of the rate and the extent of the limitations of photosynthesis, both in experiments involving elevated CO_2 concentration and *in situ*, is a fundamental step in integrating these data. This has been done for several models of varying complexity, all of which have used temperate tree species (Jarvis 1993; McMurtrie & Wang 1993). Other models explaining canopy function as a whole have been tested both in temperate and tropical forests (Williams *et al.* 1996; Williams *et al.* 1997) but have yet to include those leaf physiological parameters which are most sensitive to change in elevated CO_2 concentration, namely V_{cmax} and J_{max}. The data presented in this study could be directly incorporated into existing models of canopy function and the models modified to allow perturbation of these parameters with the predicted increase in atmospheric CO_2 concentration.

PHYSIOLOGICAL PARAMETERS OBTAINED THROUGH THE MEASUREMENT OF *IN SITU* CANOPY PHOTOSYNTHESIS

Using the Farquhar *et al.* (1980) model (discussed in Chapters Three and Four) to investigate vertical profiles of photosynthetic capacity of a Brazilian rain forest, it was shown that there was a significant increase in values of J_{max} and V_{cmax} between leaves near the ground and leaves within the uppermost layer of the canopy (Table 5.1). Only V_{cmax} showed evidence of a gradual increase between intermediate levels in the canopy with increasing canopy height. Further investigation of species differences for a given canopy height would also be useful.

This observed increase in photosynthetic capacity per unit of leaf area with canopy height could be largely explained by the concomitant increase in leaf mass area, LMA (Table 5.1), which effectively increases the nitrogen content per unit of leaf area (Evans 1996). The increase in photosynthetic efficiency per unit area via an apparent reduction in leaf thickness as photosynthetic photon flux density decreases, is a commonly reported phenomenon (Givnish 1988; Sims *et al.* 1997).

Additional information from the investigation of *in situ* photosynthesis is currently being processed and the exact light environment for each leaf measured will be characterised along with light response curves of the same leaves used to evaluate CO_2 response (Kruijt & Meir, unpublished data). In addition, we have values of $\delta^{13}C$ for both leaves and air at each level (Kruijt, Meir & Carswell, unpublished data). These measures will be able to tell us a lot about physiological functioning as well as the predominating environmental conditions (Medina *et al.* 1986).

Table 5.1 Parameters obtained *in situ* for a tropical rain forest canopy near Manaus, Brazil. J_{max}, V_{cmax} and R_d are all measured in μmol m^{-2} s^{-1}. LMA is given in g m^{-2} and foliar N concentration is given in mol m^{-2}.

Canopy height (m)	J_{max}	V_{cmax}	R_d	LMA	Foliar N concentration
24	94.2 ± 12.9	37.7 ± 5.9	-0.73 ± 0.19	118 ± 4	0.25 ± 0.06
20	64.3 ± 6.3	27.8 ± 2.3	-0.65 ± 0.12	108 ± 1	0.21 ± 0.07
16	70 ± 3.6	22.3 ± 1.9	-0.62 ± 0.27	99 ± 4	0.16 ± 0.08
8	63.7 ± 4.4	26.3 ± 2.6	-0.85 ± 0.26	87 ± 8	0.16 ± 0.09
ground	34.7 ± 5.3	17 ± 1.7	-0.2 ± 0.19	70 ± 2	0.14 ± 0.06

Diurnal change in the driving variables of photosynthesis is shown in Fig. 4.1 (p. 156). These data were collected over one 24 hour period. A more extensive data set would need to be collected before canopy models could be run.

PHYSIOLOGICAL PARAMETERS OF *C. ODORATA* OBTAINED THROUGH EXPERIMENTAL MANIPULATION

In 1995 it appears as if stimulation of photosynthesis in elevated CO_2 concentration persisted for the entire growing season whereas in 1996 there was evidence of down-regulation of this response. Root biomass of *C. odorata* plants grown in elevated CO_2 concentration in 1995 was significantly larger than in *C. odorata* grown in ambient CO_2 concentration, with a high rate of nutrient supply to the plants. Results from the two years' experiments differ widely and this is hypothesised to be a result of differences in the growing conditions.

Plant growth was much larger across all treatments in 1995 than 1996, presumably as a result of a higher total of sunshine hours as well as a lower vapour pressure deficit within the open-top chambers. 1995 was a very sunny year, so the plants received a continuously high PPFD, but were better protected from extremely high PPFD than in 1996, by white-washing of the glasshouse. It seems likely that the reduced growth of *C. odorata* in the 1996 experiment was further compounded by supplying nutrient at a rate intended for a much higher rate of growth.

The responses of *C. odorata* to elevated CO_2 concentration were investigated at both the leaf scale and the whole-plant scale. The process of CO_2 assimilation is discussed at the leaf scale, because there is the possibility for integration with canopy physiological measurements at this scale, if the canopy is considered as a group of leaves (Kull & Jarvis 1995), or even as one big leaf (Lloyd *et al.* 1995b). However, plant response to elevated CO_2 concentration will also be constrained by other features such as dry matter allocation, leaf 'costs' and life spans, developmental constraints, herbivore defence and canopy structure, all of which may be at least as

important as the response of leaf photosynthetic capacity (Körner 1991). Whole-plant results from the present study are summarised and discussed briefly in the context of predicting future responses to elevated CO_2 concentration. Suggestions are then given for scaling responses to the scale of forest-stands and, eventually, ecosystems.

CO_2 assimilation

Photosynthetic stimulation continued for the duration of the 1995 experiment, as indicated by the lack of significant difference in parameters fitted to A/C_i curves (described fully in Chapter Three) between CO_2 concentrations (Table 5.2). It also appeared as if mean net assimilation rate, NAR (defined in Chapter Two), for the experiment was stimulated whereas leaf area ratio, LAR, was decreased. This suggests that the increase in assimilation persisted for the duration of the experiment. Differences in final biomass were not significant but it is thought that the very high effect of nutrient supply rate reduced the statistical significance of the elevated CO_2 concentration effect.

Table 5.2 Physiological parameters of *C. odorata* grown in an elevated concentration of CO_2 compared with those of plants in an ambient concentration of CO_2. Only plants of high nutrient status are included. A1995 = plants grown in ambient CO_2 concentration in 1995. E1995 = plants grown in elevated CO_2 concentration in 1995. A1996 = plants grown in ambient CO_2 concentration in 1996. E1996 = plants grown in elevated CO_2 concentration in 1996. J_{max}, V_{cmax}, A_{max}, and R_d are all measured in μmol m^{-2} s^{-1}. These are the parameters which were derived from A/C_i curves using the Farquhar *et al.* (1980) model. Apparent quantum efficiency (AQE) is dimensionless and was estimated from linear regression of the initial slope of the light response curve. Values are separated for 1995 and 1996 as acclimation was thought to have occurred in 1996 only. R_d values quoted are those obtained from A/C_i curves rather than light response curves as they are directly comparable with those obtained from A/C_i curves of photosynthesis in the canopy.

Treatment	J_{max}	V_{cmax}	R_d	AQE	A_{max}
A1995	75 ± 12.4	31.2 ± 4.6	-0.2 ± 0.17	not measured	not measured
E1995	76.8 ± 3.9	27 ± 5.81	-0.42 ± 0.36	not measured	not measured
A1996	87.4 ± 9.0	38.1 ± 3.6	-0.36 ± 0.15	0.039 ± 0.004	10.33 ± 0.22
E1996	61.9 ± 10.3	23.3 ± 2.4	-0.09 ± 0.07	0.053 ± 0.007	23.60 ± 1.06

These parameters may be directly incorporated into existing models of canopy photosynthesis (Wang & Jarvis 1990; Woodward 1993) to investigate changes in canopy photosynthesis with a doubling in atmospheric CO_2 concentration. However, it is expected that the AQE and R_d values obtained from linear regression of the initial slope of the light response curve are more physiologically representative than those obtained from fitting the A/C_i curves which are shown here. This highlights a case for obtaining supplementary information before proceeding with the integration of the experimental data with *in situ* canopy response. Light response curves of plants grown in both ambient and elevated concentrations of CO_2 are required to improve the accuracy of the J_{max} and R_d estimates from the A/C_i curves (de Pury & Farquhar 1997).

The supplementary data which were used to investigate decreases in foliar nitrogen concentration with specific leaf area, possibly indicating the accumulation of carbon compounds in leaves (Farrar & Williams 1991), may also be used as a tool for the integration of measurements of leaf photosynthesis with canopy photosynthesis

(Table 5.3, p. 188). This is particularly useful for modelling experimental results within the range of photosynthetic activity found in the vertical profile of a forest (Kull & Jarvis 1995). The use of these measurements as a tool for "scaling-up" to canopy photosynthesis measurements are discussed later in this chapter.

The phenomenon of "acclimation"

In 1996, stimulation of photosynthesis did not persist for the entire growing season. This may be thought of as an acclimation response or a "phenotypic adjustment to a short term change in environment" (Amthor 1995). Although the term most commonly implies a down-regulation of photosynthesis with continued exposure to elevated CO_2 concentration, acclimation responses may also include up-regulation of photosynthesis (Ziska *et al.* 1991) and may also be observed in other physiological processes. For example, they may occur in stomata, as indicated by a decrease in the intercellular/atmospheric CO_2 concentration (C_i/C_a) ratio. So far, however, there has been no consistent change in this ratio except for under conditions of accompanying water stress (Sage 1994). Other reports have concluded that acclimation occurs as a change in plant form where the ability to form new sinks for carbohydrate precipitates a shift in the allocation pattern of the plant in favour of storage organs such as roots (Mousseau & Saugier 1992). Also, acclimation to light occurs in forest canopies where photosynthetic capacity decreases with canopy height and consequent light interception (Sellers *et al.* 1992; Terashima & Hikosaka 1995). In fact, this light acclimation model has formed the basis for predictions of the acclimation response of plants to elevated CO_2 concentration.

Measurements of whole-plant CO_2 uptake in the present study showed that plants grown in elevated CO_2 concentration had a significantly smaller CO_2 uptake than plants grown in ambient CO_2 concentration when both were measured at ambient CO_2 concentration (Table 3.5, p. 128). Some other studies of acclimation of tropical

tree species to elevated CO_2 concentration have also reported a down-regulation response so strong that the rates of photosynthesis in an ambient CO_2 concentration were equal to or higher than those in an elevated concentration of CO_2 (Oberbauer *et al.* 1985; Reekie & Bazzaz 1989). In a survey examining the acclimation response of tree species to elevated CO_2 concentration, using measurements of photosynthesis at a common measurement concentration of CO_2 for plants grown both at ambient and elevated concentrations, it was concluded that the trees did show an acclimation response on average, but that the variability of the response was high and that some net stimulation of photosynthesis still occurred (Gunderson & Wullschleger 1994).

A/C_i curves differed between plants in the two CO_2 concentration treatments, confirming a down-regulation of photosynthesis in twice-ambient CO_2 concentrations. The parameters of J_{max} and V_{cmax} derived from the measured A/C_i curves decreased significantly ($p < 0.05$) with growth in elevated CO_2 concentration (Table 5.2, p. 184). A decrease in V_{cmax}, in particular, is reported to reflect a decrease in the amount, activity or kinetic properties of Rubisco (Gunderson & Wullschleger 1994). This suggests that nitrogen was being allocated away from the Rubisco enzyme towards other more limiting processes in 1996. Derived values of J_{max} and V_{cmax} for plants grown in both ambient and elevated CO_2 concentrations and at a high rate of nutrient supply fall within the range of those measured at ambient conditions in tropical forest plants, but are lower than the reported averages (Wullschleger 1993). The only other published study of J_{max} and V_{cmax} values in a tropical tree species grown in elevated CO_2 concentration gives predicted values similar to those in the present study (Ziska *et al.* 1991; Gunderson & Wullschleger 1994). However, "up-regulation" was observed in their study to such an extent that J_{max} was increased from a derived value of 67 μmol m^{-2} s^{-1} to 94 μmol m^{-2} s^{-1}. V_{cmax} was increased by a proportionately smaller amount. Gunderson and Wullschleger (1994) concluded in their review that usually there is a small decrease in V_{cmax} with exposure to elevated

CO_2 concentration. In contrast another review, which uses the decrease in A for a given value of C_i as evidence of acclimation to elevated CO_2 concentration, reports acclimation in only a minority of species (Sage 1994). Values of J_{max} and V_{cmax} were not reported in this study.

The comparison of light response curves between treatments of the current study also implies a difference in maximum photosynthetic capacity. When the light response curves of plants grown in ambient CO_2 concentration and measured at elevated CO_2 concentration were compared with those from plants grown and measured in elevated CO_2 concentration, the ambient CO_2-grown plants showed a much larger response to elevated CO_2 concentration (Table 3.7, p. 133). Although some authors have suggested that acclimation occurs concurrently with an increase in resource-use efficiency (Drake *et al.* 1997) there was no evidence for an increase in light-use efficiency with acclimation as determined by evaluation of AQE in the current experiment (Fig. 3.11, p. 135).

What triggers acclimation?

As yet the mechanism of down-regulation of photosynthesis through acclimation remains unclear although there is mounting evidence for a chemical trigger of a substance such as sucrose or fructose which, after catabolism to glucose, feeds back on the nucleus to halt the expression of the Rubisco-producing gene (Sheen 1994; Koch 1996). This is consistent with the commonly observed build-up of sucrose and starch in leaves (Drake *et al.* 1997).

In addition, there is very strong evidence that source/sink relationships affect acclimation (Reynolds *et al.* 1996). If there is sink limitation, so that organs which are the usual destination of carbohydrates can not expand or grow, utilisation of the extra carbon assimilated cannot occur (Farrar & Williams 1991; Mousseau *et al.*

1996). This may then cause a build-up of carbohydrate in the source leaves which may lead to down-regulation. Certainly, acclimation has been reported in most experiments in which small pots were used and is generally absent from experiments in which large pots (> 10 dm^3) were used (Arp 1991; Sage 1994, reviewed by Drake *et al.* 1997). This is consistent with results from the study of *S. macrostachya* in this thesis, where the effect of small pot size is likely to have been significant. In *C. odorata*, the build-up of foliar carbohydrates was merely implied through a decrease in specific leaf area, SLA (Table 5.3). Despite strong evidence of acclimation from comparison of photosynthesis in elevated and ambient CO_2 concentrations the actual measured concentrations of leaf carbohydrates did not statistically differ between treatments in 1996 (Table 2.4, p. 90). How then could the source to sink ratio have been altered sufficiently to have induced acclimation in the 1996 experiment?

Table 5.3 Additional information gathered from experimental data which is required to standardise photosynthetic parameters between sites. Units for SLA are $cm^2\ g^{-1}$. Note that foliar P concentration is included with foliar N concentration as P is thought to be a more strongly limiting nutrient in tropical forests than N (Vitousek & Sanford 1986; Bowes 1991). Units for these nutrients are mg g^{-1}. Treatments are as follows: A1995 = plants grown at ambient CO_2 concentration in 1995. E1995 = plants grown at elevated CO_2 concentration in 1995. A1996 = plants grown at ambient CO_2 concentration in 1996. E1996 = plants grown at elevated CO_2 concentration in 1996. Mean values are given $\pm$ one standard error.

Treatment	SLA	Foliar N concentration	Foliar P concentration
A1995	303 ± 8	1.58 ± 0.06	0.23 ± 0.01
E1995	289 ± 12	1.44 ± 0.05	0.22 ± 0.01
A1996	242 ± 19	4.07 ± 0.15	0.33 ± 0.03
E1996	226 ± 10	3.51 ± 0.27	0.30 ± 0.03

Is nutrient deficiency responsible for acclimation?

Many authors have concluded that plant response to elevated CO_2 concentration is likely to be reduced by nutrient deficiency (reviewed by Ceulemans & Mousseau 1994). Experiments using small pots have been frequently criticised for their

restriction of root growth and consequent triggering of acclimation (Arp 1991; Thomas & Strain 1991). It is important to separate the effects of nutrient limitation from physical root restriction, both of which may occur in small pots and both of which may trigger acclimation (McConnaughay *et al.* 1993). If nitrogen is in such short supply that the ratio of C:N in a leaf rises dramatically, there is the potential for feedback by accumulated carbohydrates which leads to down-regulation of photosynthesis.

If nutrient deficiency alters the balance of the source to sink ratio in favour of root growth to encourage extra nutrient assimilation (Ceulemans & Mousseau 1994), yet there is a physical restriction on root growth, then acclimation may again be triggered by the inability to compensate for the extra carbon assimilation. In *C. odorata*, small pot size is extremely unlikely to have been a factor in acclimation as the pots were very large in comparison with the seedlings. There is some evidence, however, that pots may play a role in acclimation even without physical restriction of roots (McConnaughay *et al.* 1993), and this possibility cannot be entirely eliminated from this experiment. However, the impact of nutrient limitation could be assessed in its own right.

In the present study, *C. odorata* plants grown with high rates of nutrient application showed a larger absolute response in plant growth to elevated CO_2 concentration in 1995 than plants with a low rate of nutrient supply. Again in 1996, nutrient-limited plants were no more likely to show acclimation than their counterparts with a high rate of nutrient supply. In fact, there was a trend to a greater increase in growth with a low rate of nutrient supply (Fig. 2.8, p. 76). The stimulation of relative growth rate in elevated CO_2 concentration was significantly larger in plants grown with a low rate of nutrient supply, as opposed to a high rate of supply (Fig. 2.10, p. 78). In terms of the relative response, a large part of the literature corroborates the result of

the present study (reviewed by Lloyd & Farquhar 1996). Nutrient limitation was not responsible for acclimation in 1996.

Was a difference in growth conditions responsible for acclimation?

C. odorata plants grew much larger in 1995 than 1996. The difference in biomass between the two years can be largely explained by the more favourable growing conditions of the 1995 experiment. In 1995 the chambers in the Royal Botanic Garden of Edinburgh (RBGE) had a higher number of total sunshine hours per month along with higher temperatures and photosynthetic photon flux density (PPFD) than in 1996, at Edinburgh University (EU, Table 5.4). Also D was lower in the 1995 experiment. It is hypothesised that the consistently high values of D and during the light hours in particular during 1996, reduced g_s and that photosynthesis and consequent growth were also reduced. The smaller number of sunshine hours also would have reduced both the total photosynthesis and the growth of *C. odorata*. In addition, despite a mean decrease in PPFD in 1996, plants were exposed to a wider range of PPFD values (Fig. 2.4, p. 71 & Fig. 2.6, p. 73). It has been previously suggested that the growth of *C. odorata* may be depressed by high PPFD. PPFD values of both years, but particularly in 1996, were often higher than 400 $\mu mol\ m^{-2}\ s^{-1}$, and thus are likely to cause depression of photosynthesis (Ramos & Grace 1990). Although the mean PPFD was higher in the 1995 experiment than in 1996, excessively high PPFD values were guarded against by white-washing the greenhouse at the RBGE. This resulted in a proportionally shorter time of exposure to the very high PPFDs which may have been responsible for the 1996 depression. It is hypothesised that this depression of photosynthesis and growth in 1996 prevented storage tissues from utilising the extra photosynthate produced in elevated CO_2 concentrations and therefore, the carbohydrates accumulated in the leaf and triggered acclimation. In addition, it seems likely that once the plants began to grow at a rate

less than that predicted, the nutrient regime became inappropriately concentrated and further hampered growth.

It is, therefore, suggested that the driving variables of photosynthesis are included within any model attempting to predict future response of tropical plants to elevated CO_2 concentration. Data from the present study suggest that it is changes in these variables which ultimately determine whether or not the stimulation of CO_2 assimilation persists with extended exposure to elevated CO_2 concentration. A comparison of the driving variables of photosynthesis between the two years of experiments on *C. odorata* is given in Table 5.4.

Table 5.4 Mean driving variables of photosynthesis observed during the course of the experiment. Two values are given for g_s (ambient CO_2 concentration then elevated) in 1996.

Year	D (kPa)	g_s (mmol m^{-2} s^{-1})	PPFD (μmol m^{-2} s^{-1})	Temperature (°C)
1995	1.30 ± 0.03	not measured	285.8 ± 4.2	24.1 ± 0.04
1996	1.37 ± 0.01	(A) 152.8 ± 18.8 (E) 113.3 ± 17.4	211.4 ± 1.0	22.52 ± 0.02

A more detailed analysis of the driving variables of the system would be required to model canopy photosynthesis accurately using experimental parameters. Values of D should be obtained for all chambers and these should be correlated with measurements of photosynthesis to obtain a response curve in a similar fashion to the A/C_i curve. Response curves should also be provided for g_s in both ambient and elevated CO_2 conditions. Sunshine hours should also be investigated further. Photosynthesis may also vary with temperature, so, although A/C_i curves are standardised to a temperature of 25 °C (de Pury & Farquhar 1997), stand-scale predictions are more complicated. The amount of Rubisco required to sustain current

levels of photosynthesis present in leaves decreases as temperature rises (Woodrow 1994), so modelling of acclimation responses should consider this.

Completing the data set which we have already started would give us the ability to predict how photosynthesis of this stand of tropical forest might respond to increases in atmospheric CO_2 concentration. Ideally, the data set should be calibrated against a common species, such as *C. odorata*, for which we have impact data. Unfortunately timber extraction has made the presence of this tree very rare in the Brazilian forest today. However, future experiments could use an indicator species which is prominent at the study site to compare parameters obtained in ambient conditions.

From photosynthesis to whole-plant responses

This study gives estimates of changes in physiological parameters in conjunction with whole-plant growth response to elevated CO_2 concentration including below-ground responses and changes in allocation of biomass. These data are fundamental to our understanding of whole-plant response to elevated CO_2 concentration. If we know that V_{cmax} may decrease by 13 % (Table 5.2, p. 184) with exposure to elevated CO_2 concentration without a concomitant decrease in biomass, as was seen in 1995, we can surmise that nitrogen use is becoming more efficient (Drake *et al.* 1997).

No statistically significant changes were detected in plant morphology, as assessed by the measurement of the root to shoot ratio and the mean plant height. Neither were there any changes in nutrient allocation to tissues, canopy architecture and inferred developmental constraints. There were no differences in the amount of leaf material lost through senescence between treatments. There were no changes in leaf anatomy, such as an increase in the number of palisade layers (Mousseau & Enoch

1989; Thomas & Harvey 1983), which may have provided an alternative explanation to carbohydrate accumulation as the cause of the decrease in SLA.

Although much evidence suggests that below-ground responses to elevated CO_2 concentration are of utmost importance (Rogers *et al.* 1994; Arnone & Körner 1995; Lovelock *et al.* 1996), there was no significant evidence of an increase in the amount of carbon sequestered by either soil or roots in the current experiment. The large amount of variation in root response is partially responsible for this as there was a suggested net increase in root biomass. Lack of an adequate carbon signal in the growth medium surrounding the roots is likely to be a result of the small root biomass in the 1996 experiment, which would, therefore, have stimulated only a small population of microbes in the rhizosphere. It is also possible that the artificial growth medium in which the plants were grown reduced natural infection by associated mycorrhizal fungi. If there is truly no net influence on below-ground respiration (Table 3.5, p. 128), we can model below-ground responses in an elevated CO_2 concentration as matching those in ambient conditions. Further investigation of plants with larger root systems is desirable before building on this conclusion.

Table 5.5 Summary of changes in growth of *C. odorata* in elevated CO_2 concentration. Changes in plant growth were considered significant at the $p < 0.05$ level.

Plant response	1995	1996
total dry mass	suggested increase but not of statistical significance	no significant change
root dry mass	increased by 23 % in conditions of high N only	no significant change
stem dry mass	suggested increase but not of statistical significance	no significant change
leaf dry mass	no significant change	no significant change
root:shoot ratio	no significant change	no significant change
plant height	no significant change	no significant change
leaf area	no significant change	no significant change
relative growth rate (RGR)	increased by 8 % in conditions of low N only	no significant change
net assimilation rate (NAR)	suggested increase in both high and low N but not of statistical significance	no significant change
specific leaf area (SLA)	no significant change	no significant change
leaf area ratio (LAR)	no significant change	no significant change
stomatal density	no significant change	no significant change
nutrient content of organs	no significant change	no significant change
foliar carbohydrate and chlorophyll concentrations	no significant change	no significant change

Supplementary observations from a short study of another tropical tree species, Schefflera macrostachya

S. macrostachya plants showed no significant increase in plant height or mass with exposure to an elevated concentration of CO_2. Again there appeared to be a trend towards stimulation of growth but this was not significant because of the large amount of inter-plant variation within each CO_2 treatment (Figures 2.16 & 2.17, pp. 86 - 87). This variation in *S. macrostachya* plants may be accounted for by large differences in size of the one-year-old seedlings before the CO_2 concentration treatments commenced.

Root restriction is more likely to have affected this species. Unlike *C. odorata* plants, which were grown in very large pots for their small root size, it is possible

that the lack of statistical significance in the net increase in biomass could be attributed to restrictive pot size. Although the plants showed no evidence of pot binding at the conclusion of the experiment, the roots did occupy a large portion of the total pot volume. Additionally, no attempt was made to avoid nutrient limitation.

Further investigation of some of the tree species which occur at the rain forest site would confirm the whole-plant response, and parameters for these plants could then be used in forest-stand response models directly. It is suggested that the use of a model of whole-plant gas exchange would reduce the amount of direct measurement of gas exchange, and would provide the kind of parameters necessary for integration in forest stand models. Although time constraints prohibited the incorporation of the data of the present study into such a model, it is proposed that the model of Lloyd *et al.* (1995a) is ideal for this purpose. The whole-plant gas exchange measurements reported in this study could be used to validate the estimates of whole-plant gas exchange, modelled on the basis of leaf photosynthesis and stomatal conductance measurements.

From single plant scale to a forest stand

The scaling of the response of one plant species to elevated CO_2 concentration to the response of a forest stand requires resolution of two questions. The first is how should photosynthesis data be scaled from the leaf to the canopy? The second is how should the added complexity of below-ground responses to elevated CO_2 concentration be dealt with, including root respiration and nutrient cycling dynamics?

Given an empirical knowledge of how photosynthesis changes in the canopy with nitrogen content and mean light environment we can correct photosynthesis for these

variations and then modify the photosynthetic parameters for a change in CO_2 concentration. Even temperature change scenarios are possible although the interaction of respiration with temperature increase makes the situation a little more complex.

The obvious choice of model for this purpose is MAESTRO (Wang & Jarvis 1990) which is a generalised forest canopy model which uses a non-random radiative transfer model to predict the heterogeneity in the light environment from crown structure in both the vertical and horizontal dimensions (Jarvis 1993), and uses PPFD as the driving variable for photosynthetic predictions. Further information in addition to the data set detailed above would be required, such as the distribution of leaf area density through the canopy, data on leaf angles, parameters of stomatal response functions, and more detailed information on the site and stand structure. The model may then be easily parameterised for a change in elevated CO_2 concentration through variation in J_{max} and V_{cmax} and g_s, where appropriate. However, it should be noted that all such models cannot be validated for conditions other than ambient so there will always remain an element of uncertainty (Jarvis 1993), the exact value of which cannot be quantified.

A simpler approach, using the driving variables of canopy photosynthesis which were investigated by the current experiments is given by Woodward (1993). This may be described as one of the "big leaf" models. In this approach leaf energy balance equations are applied to the whole canopy. In addition to the variables listed above net input of radiation would be required plus detailed g_s estimates and leaf area index (LAI). It is acknowledged by the author that much uncertainty exists in estimating the impact of leaf response to environmental change on larger-scale ecosystem processes.

Another "big leaf" model such as that of Kull and Jarvis (1995) may be used to predict canopy photosynthesis simply by using PPFD and foliar N contents. This gives results with surprising similarity to the more complex radiative transfer models such as MAESTRO (Wang & Jarvis 1990).

Although below-ground responses to elevated CO_2 concentration are still not clear (Rogers *et al.* 1994), they must be included in stand-scale models of response. Photosynthetic models need to be integrated with information about the plant-soil continuum in order to predict stand response accurately (McMurtrie *et al.* 1992). A knowledge of nutrient dynamics is vital in this respect (Johnson & Ball 1996). These are themselves subject to uncertainty in the direction of change with elevated CO_2 concentration because C:N ratios of litter might change and thus affect decomposition rates and subsequent nutrient cycling (Berntson & Bazzaz 1997; Paterson *et al.* 1997). However, since many tropical forest types are relatively N-poor (Vitousek & Sanford 1986), and we know that at least the species of tropical tree studied here will increase its N-use efficiency, we would predict an overall growth increase in tropical forest with elevated CO_2 concentration with acclimation actually contributing to this stimulation as long as PPFD and vapour pressure deficit did not constrain growth. For *C. odorata* in the present study there were no changes in patterns of biomass allocation between roots and shoots (Table 5.5, p. 194) and no discernible decrease in root respiration with elevated CO_2 concentration (Table 3.5, p. 128). This is unlikely to be consistent among all species however (Amthor 1995) and models should allow for a change in these parameters at the stand scale.

Starting with the forest stand

A classical method of growth evaluation is to look at current net primary productivity (N_p), a commonly used measure of community productivity (Roberts *et al.* 1993):

$$N_p = \Delta M + L$$

where:

ΔM = change in biomass, and

L = material lost from system whether through death, decomposition, grazing, shedding or root exudation.

This has traditionally been measured by evaluating the biomass increment of trees and collecting litter that falls from the trees. Perturbations to the system as a whole can be analysed through measurement of biomass change although the effects of a single stimulus such as an increased input of atmospheric CO_2 cannot be easily separated from other possible stimuli (Phillips *et al.* 1998). Recent technological developments suggest the increased use of satellite data to estimate this type of increase in plant growth (Myneni *et al.* 1997), although much work remains to perfect the interpretation of the satellite images.

The traditional method is extremely labour intensive, requires measurement over a long period of time, usually neglects below-ground productivity (Grace *et al.* 1997) and cannot adequately address the problem of forest heterogeneity (Jarvis & Dewar 1993).

An alternative approach is that of "top-down" modelling where the starting point is the net CO_2 flux for the entire ecosystem. "Top-down" models can be contrasted with "bottom-up" models such as that described above or MAESTRO (Wang & Jarvis 1990) which employ information and relationships from below the scale of focus and mechanistically link these elements together (Reynolds *et al.* 1996). This top-down modelling is now possible with the development of eddy covariance

techniques. The theory is that we can measure the net ecosystem flux of carbon (F_c) and then by making measurements of the components of this flux, determine the portion attributed to the phytomass (Grace *et al.* 1997). The equation becomes:

$$F_c = G_p - R_p - R_h$$

where

G_p = gross primary productivity,

R_p = plant respiration, and

R_h = heterotrophic respiration.

The value of G_p is quantifiable through application of a model which uses climatological data and the physiological parameters derived using the Farquhar *et al.* (1980) model as well as those from other stomatal response models to explain the carbon and water fluxes measured by eddy covariance (Lloyd *et al.* 1995b).

It then becomes possible to estimate N_p provided that R_p and R_h can be accurately quantified:

$$N_p = G_p - R_p$$

In practice, this partitioning can be very difficult as soil measurements of respiration generally include a component of both with roots and microbes each contributing to soil CO_2 efflux. However, the use of theoretical respiration rates have so-far provided good estimates (Grace *et al.* 1997).

Flux measurement is an ideal tool to validate stand models which may have been built up from a physiological knowledge of their components, as was the case for an

Amazonian data set (Williams *et al.* 1997). Once such models have been validated, photosynthetic parameters and ensuing estimates of growth may be exchanged for those values measured in experimental manipulations of CO_2 concentration, leading to good estimates of the likely outcome of changes in the atmospheric composition.

Forest stand to ecosystem and integration with GCMs

Scaling-up from a stand for which flux measurements have been used to validate a physiological model of the process, to a whole forested region is a major exercise in itself (Jarvis & Dewar 1993). The heterogeneity of the land cover must be taken into account and the feedbacks between a large land mass and the atmosphere become increasingly important. The inclusion of predictions of ecosystem response can be approached in many ways which are reviewed elsewhere (Reynolds *et al.* 1996). Models requiring the least information are those which deal with the use of ecosystem resources by the components in the ecosystem (Field *et al.* 1992). Other models become increasingly complicated in their consideration of the interactions between species, space and time components of an ecosystem, but also output more detailed predictions (Reynolds *et al.* 1996). Perhaps the best way to progress is to evaluate each of the major global ecosystems separately, using ecosystem fluxes integrated with models of physiological processes, and to investigate the effects of increasing the concentration of CO_2 and temperature on each of these systems before making global predictions (*e.g.* Wang & Polglase 1995). These authors predicted that tundra and boreal forest ecosystems will become net carbon sources with an increased temperature and CO_2 concentration, while the tropical rain forest will continue to accumulate carbon.

The big step in the integration of physiological models with GCMs is to bridge the problem of scales (Jarvis & Dewar 1993). Currently GCMs operate on time scales of

years whereas most physiological models are used to describe a diurnal cycle. In addition, GCMs describe areas of 100-1000 km whereas heterogeneity of land use and forest type require consideration over much smaller areas. Much work remains to integrate this information obtained from widely divergent methods, but models are improving all the time enabling predictions of increasing accuracy.

An alternative approach to predicting elevated CO_2 concentration effects

Whilst a site that is naturally elevated in CO_2 concentration can give some confirmation of what has actually happened with exposure to elevated CO_2 concentration (Hättenschwiler *et al.* 1997), free air CO_2 enrichment experiments (FACE, Jäger & Weigel 1993; Wall & Kimball 1993; Johnson & Ball 1996) are possibly the best experimental solution. If the relationship between low PPFD and photosynthesis is resolved, we can make use of existing conditions in forests to confirm predictions of future growth responses to elevated CO_2 concentrations. For example, plants growing within 1 m of the forest floor of *terra firme* rain forest continually photosynthesise at CO_2 concentrations which are 200 μmol mol^{-1} above ambient (Medina *et al.* 1986). Isotope studies using $\delta^{13}C$ help to confirm the degree of carbon cycling within a particular ecosystem (Martinelli *et al.* 1991; van der Merwe & Medina 1991) and also explain physiological processes (Francey & Farquhar 1982; Medina *et al.* 1986; Berry 1988). Once we are sure of the role that low PPFD is playing in this ground layer of the canopy we can search for the physiological responses to the elevated CO_2 concentration as well.

CONCISE SUMMARY OF SUGGESTIONS FOR FUTURE RESEARCH

1) Application of elevated CO_2 concentration response parameters (V_{cmax}, J_{max} and stomatal responses, biomass changes *etc*) to a validated model to enable a "best guess" for the response of the stand of Brazilian forest which was studied to elevated CO_2 concentration.

2) Collection of supplementary data to enable scaling-up of physiological parameters quantified by this experiment to stand-scale and beyond. Light response functions are currently being analysed for the *in situ* study of Brazilian rain forest. In addition, more detailed investigation of driving variables of photosynthesis such as D and g_s are necessary for the scaling-up process.

3) Validation of a model of canopy photosynthesis for current atmospheric conditions using eddy covariance data which is currently available for the same site.

4) Further investigation of stomatal and light response functions to elevated CO_2 concentration in *C. odorata*.

5) Experimental manipulation of atmospheric CO_2 concentration for tropical tree species *in situ*. It is suggested that a constant concentration of CO_2 would be comparatively easy to maintain at an experiment in the field whether through the use of open-top chambers or the newer free air CO_2 enrichment (FACE) technology (Ellsworth *et al.* 1995) when compared with the difficulties of maintaining glasshouse experiments at tropical humidity, temperature and average PPFD in a non-tropical country.

6) These experiments should use plants grown in the soil to eliminate all pot complications whether nutrient, root restriction, mycorrhizal or other factors.

7) Longer-term studies using plants grown to greater maturity are recommended.

Concluding remarks

Clearly, modelling is the way forward with respect to predicting forest response to elevated CO_2 concentration. This study suggests ways to use basic physiological data to predict rain forest response to elevated CO_2 concentration. Factors such as interspecific interaction need also to be assessed and these data are possibly even more widely variable (Zangerl & Bazzaz 1984; Stirling *et al.* 1997). Eddy covariance techniques have a lot to offer but basic physiological data are always required to validate assumptions made about individual components. There is still scope for a great deal more data collection at the single plant scale to give better, more widely applicable parameters and to enhance understanding of the processes.

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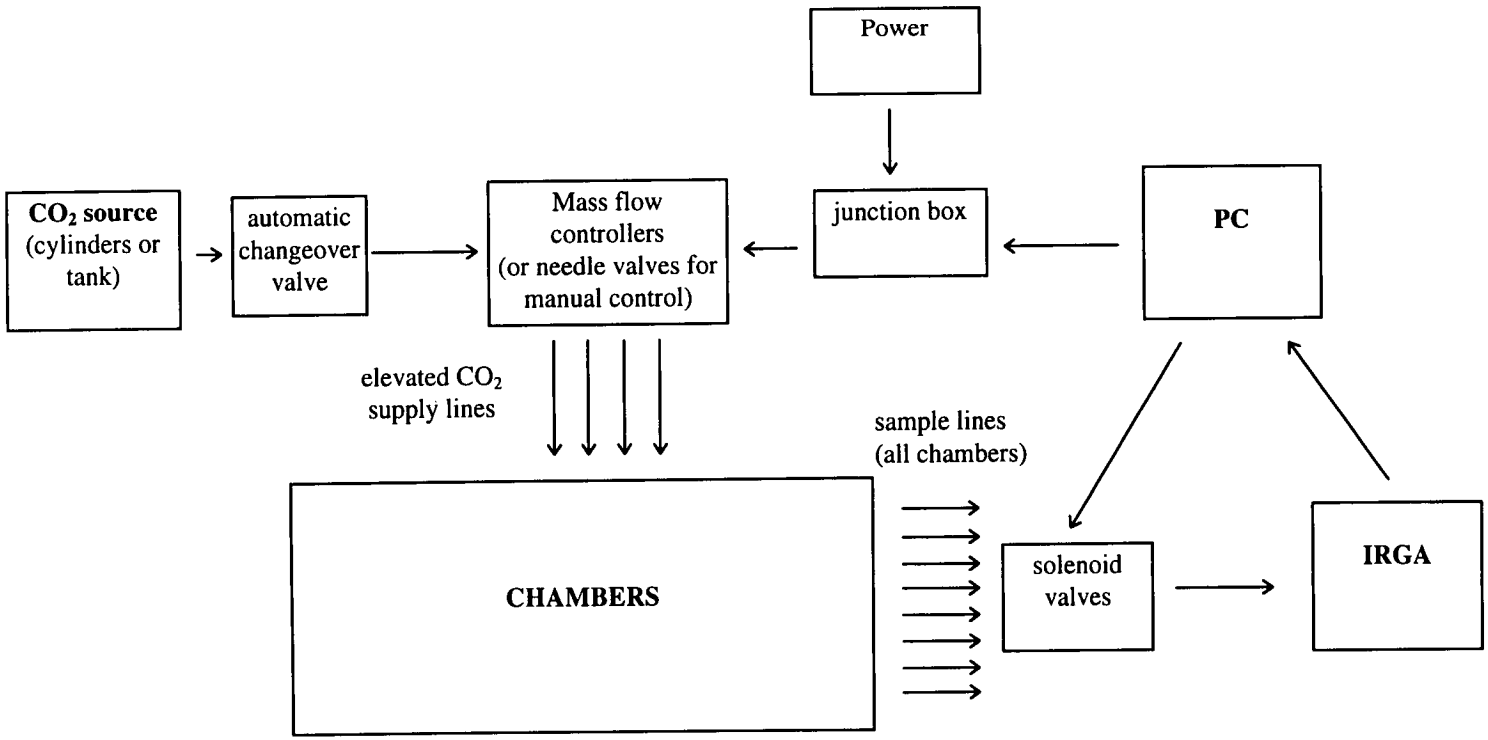


APPENDIX 1 CHAMBER SET-UP

Schematic representation of control system used to supply/monitor CO_2 in both years. Note however that in 1996 the PC was stolen from the glasshouse complete with interface cards so a temporary control mechanism was used for the remainder of the growing season. This involved the use of a Cambell CR10 data logger to monitor the voltages output by the infrared gas analyser (IRGA). Subsequent control of the CO_2 being supplied to the chambers was achieved manually with the use of needle valves.

APPENDIX 2 PHOTOGRAPHS OF EXPERIMENT AND FIELD SITE

Top: 1996 experiment showing *C. odorata* (smaller plants with pinnately compound leaves) and *S. macrostachya* plants (larger with palmately compound leaves) within an open top chamber.

Middle left: Whole-plant gas exchange chamber (Barton 1997) used in the glasshouse in 1996 to measure whole-plant CO_2 flux. The chamber is divided into two sections, the upper measuring above-ground flux and the lower measuring below-ground flux. Pipes supplying air to the chamber are visible on the front of the box with the Vaisala temperature/humidity probe in the top-right hand corner. Chamber opens on the opposite side to that shown with a removable panel.

Middle right: ZF2 tower near Manaus, Brazil. Site of measurement of vertical profile of canopy photosynthesis. Leaves were measured at each of the platform levels (4 vertical metres apart). The tower was 42 m tall with an estimated canopy height of approximately 30 m.

Bottom: 1995 experiment in the Royal Botanic Gardens of Edinburgh. The open top chambers were fed with air from within the glasshouse. This was drawn in by the fans in the wooden boxes on the left of the picture and redistributed between two chambers per box. In the case of the chambers at elevated CO_2 concentration, pure CO_2 was injected into the ducts between the fan box and the chamber. Air was removed from the chambers to outside the glasshouse by use of a fan at the end of the overhead duct.

APPENDIX 3 COMPOSITION OF INGESTAD SOLUTIONS

Table A3.1 Nutrient recipes:
a) stock solution used for *C. odorata* in 1995 and the first half of 1996
b) "Ingestad" (Ingestad & Lund 1986) stock solution used after intermediate harvest 3 (72 days) of 1996

Chemical	a) *C. odorata* solution ($g\ dm^{-3}$)	b) Ingestad solution ($g\ dm^{-3}$)
Solution 1		
K_2SO_4	49.0497	48.9700
K_2HPO_4	17.8120	33.6200
KH_2PO_4	17.4700	30.8900
KNO_3	63.0000	49.2400
NH_4NO_3	141.4160	221.6000
Solution 2		
$Ca(NO_3)_2$	262.3300	41.3390
$Mg(NO_3)_2$	106.0470	89.7690
$Fe(NO_3)_3$	5.0586	5.0504
$Mn(NO_3)_2$	1.8303	1.8271
H_3BO_3	1.1459	1.1440
$Zn(NO_3)_2$	0.2740	0.2733
$CuCl_2$	0.0805	0.0805
Na_2MoO_4	0.0179	0.0176

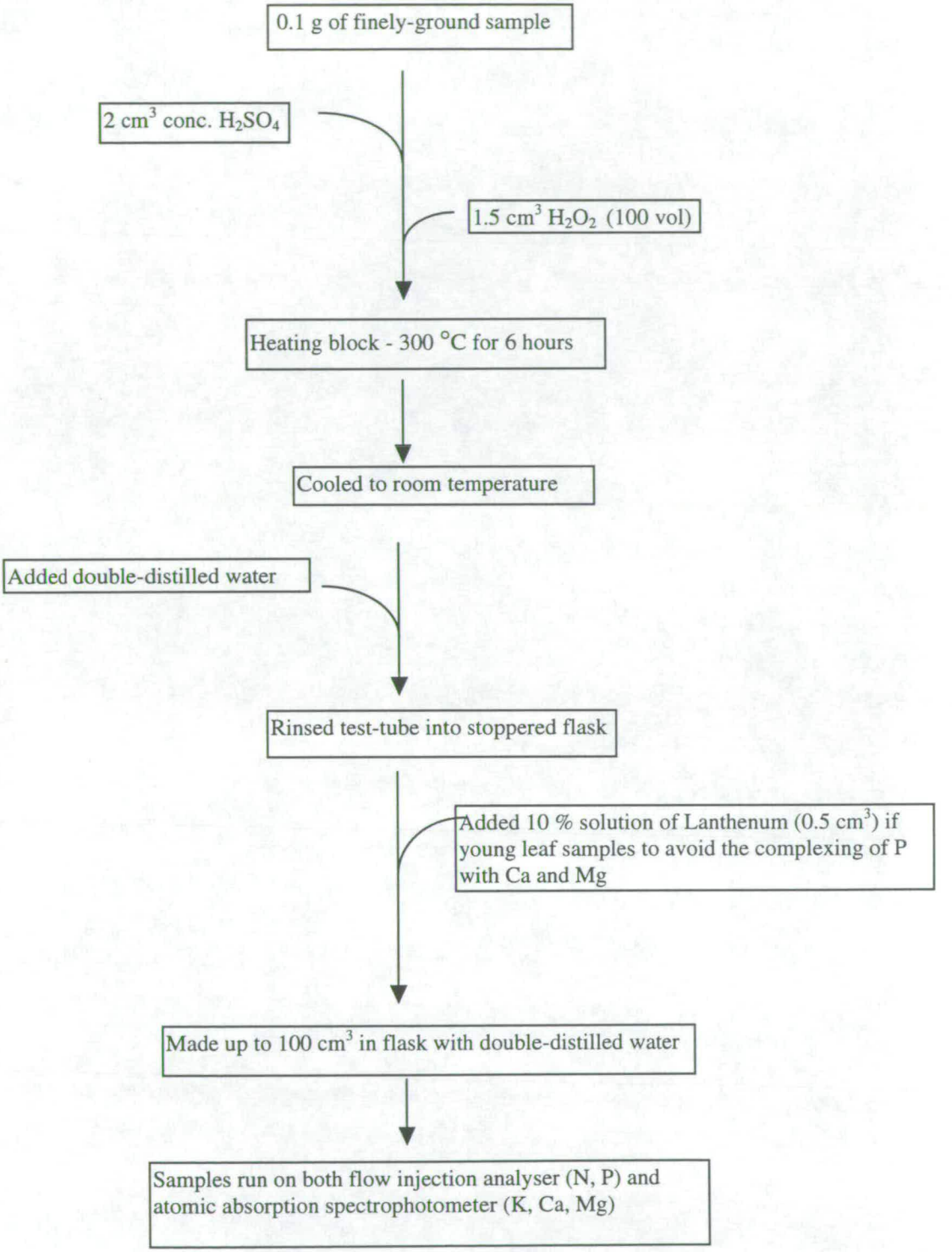
Notes: 1) These stock solutions were diluted by a factor of 30 000 and 40 230 000 for the first dose of high and low nutrient respectively in 1995. In 1996 the first dilutions were 30 000 and 97 000. For both rates of nutrient supply the dilution decreased exponentially to match the expected exponential growth in rate of uptake.

2) The table is divided into two solutions, 1 and 2. Each chemical was dissolved with like chemicals to avoid precipitation of certain combinations. The two solutions were mixed with water in equal quantities only at the time of application to the plant when the dilution was sufficiently large to avoid this problem.

Table A3.2 Total amount of each element added to a 1 dm^3 stock solution for both the *C. odorata* (after allowance for nutrient in the town water supply) and Ingestad birch (Ingestad & Lund 1986) nutrient solutions.

	Macronutrient					
Element	**N**	**P**	**K**	**S**	**Ca**	**Mg**
***C. odorata* (g dm^{-3})**	101.7	7.143	59.39	9.022	44.51	10.05
***C. odorata* (mol dm^{-3})**	7.259	0.231	1.519	0.281	1.111	0.414
Ingestad solution (g dm^{-3})	99.84	13.01	64.98	9.007	7.015	8.509
Ingestad solution (mol dm^{-3})	7.13	0.42	1.662	0.281	0.175	0.35

	Micronutrient						
Element	**Fe**	**Mn**	**B**	**Zn**	**Cu**	**Na**	**Mo**
***C. odorata* (g dm^{-3})**	0.699	0.401	0.200	0.060	0.03	0.003	0.007
***C. odorata* (mol dm^{-3})**	0.013	0.007	0.019	0.001	0.001	1×10^{-4}	7×10^{-5}
Ingestad solution (g dm^{-3})	0.698	0.4	0.2	0.06	0.03	0.003	0.007
Ingestad solution (mol dm^{-3})	0.013	0.007	0.019	9×10^{-4}	5×10^{-4}	1×10^{-4}	7×10^{-5}



APPENDIX 4 PROCEDURES USED FOR PHYTOCHEMICAL ANALYSIS

Figure A4.1 Procedure used for nutrient analysis beginning with dried sample. Stem, leaf and root samples were analysed for N, P, K, Ca, Mg concentration for all plants at final harvests of 1995 & 1996. In 1995 there were 56 *C. odorata* plants analysed (14 per CO_2 concentration and nutrient application rate treatment) and in 1996 there were 60 *C. odorata* plants (15 per treatment) and 24 *S. macrostachya* plants of which above and below ground samples were analysed for each of two CO_2 concentration treatments (12 plants per treatment).

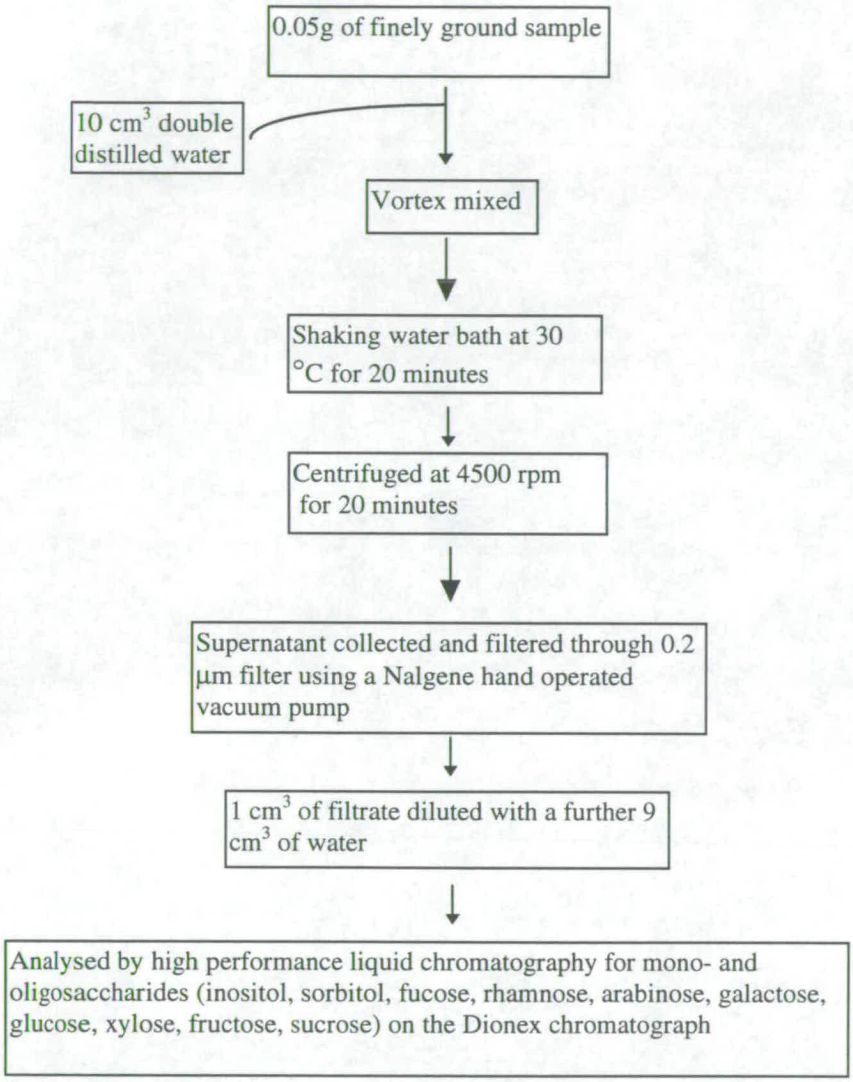


Figure A4.2 Procedure used for carbohydrate analysis of mono- and oligosaccharides beginning with freeze-dried sample. Leaf samples were analysed for concentration of inositol, sorbitol, fucose, rhamnose, arabinose, galactose, glucose, xylose, fructose and sucrose. In 1995 52 *C. odorata* plants were analysed and in 1996 44 *C. odorata* plants were analysed.

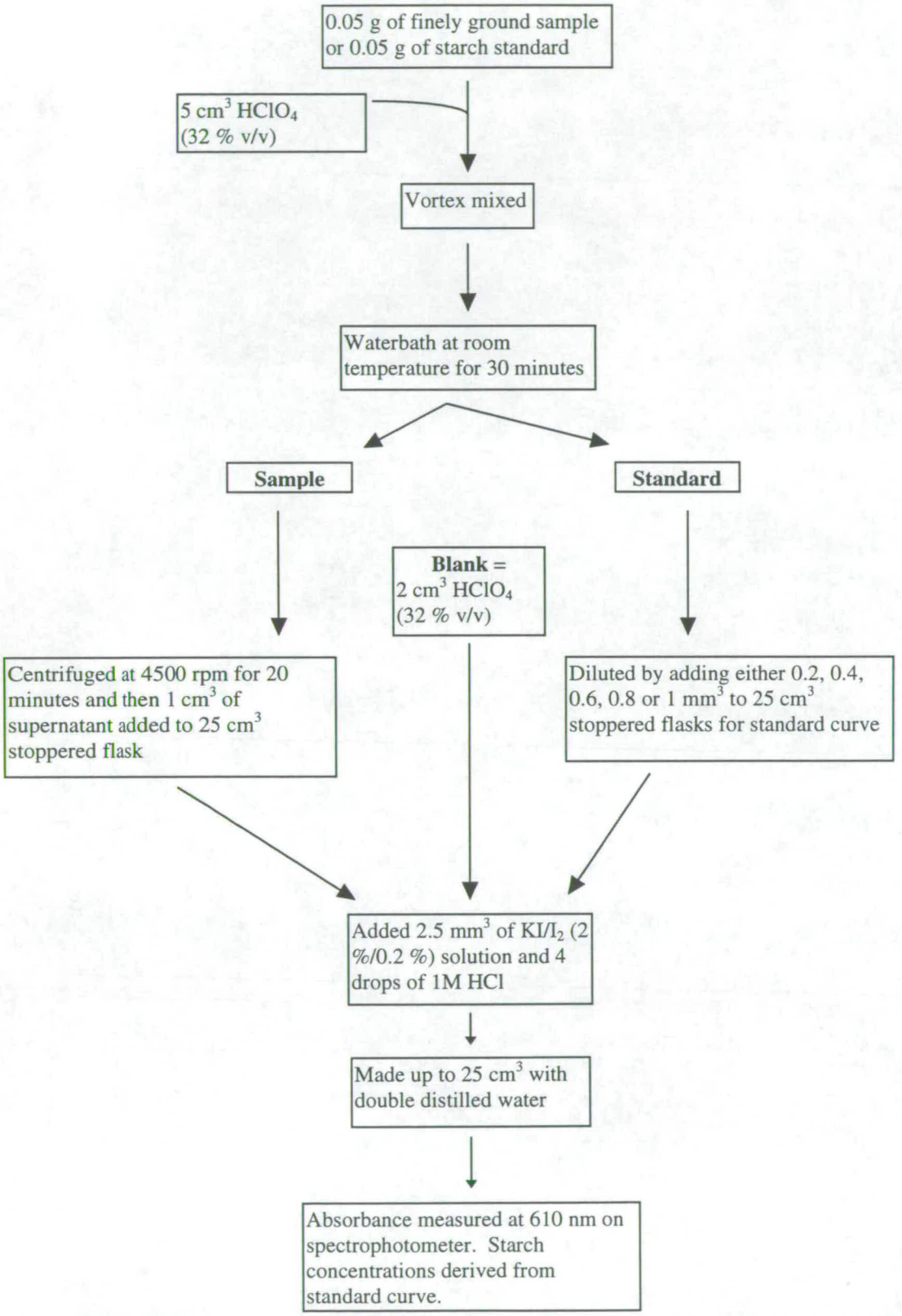
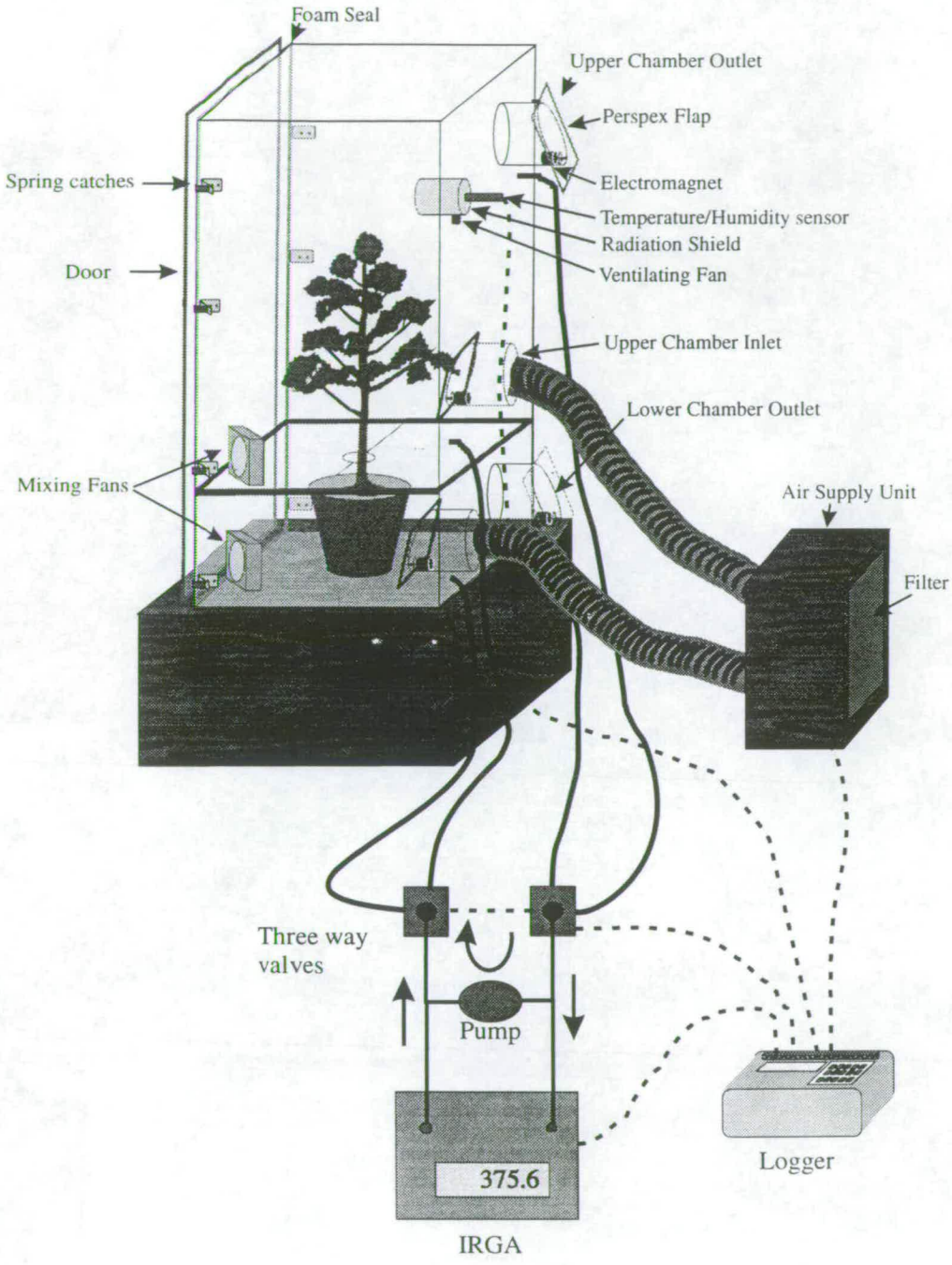


Figure A4.3 Procedure used for analysis of starch concentration beginning with freeze-dried leaf samples. In 1995 54 *C. odorata* plants were analysed and in 1996 40 *C. odorata* plants were analysed.

Whole tree CO_2 flux measurement system.



APPENDIX 5 DIAGRAM OF WHOLE-PLANT GAS EXCHANGE BOX.
The box is split into an upper and lower chamber at plant stem height, below the lowest branches. Leaf thermistors are not visible in this diagram. IRGA = infrared gas analyser. Diagram reprinted from Barton 1997.

APPENDIX 6 VARIABLES MEASURED IN EACH YEAR

List of all variables measured on *C. odorata* plants grown in ambient or elevated CO_2 concentration with high or low rate of nutrient application. Variables which differed significantly ($p < 0.05$) with CO_2 treatment in 1995 are marked with [a]. Note that these differences were only significant between plants of different nutrient regime. Those differing with CO_2 treatment after the Tukey's test are marked with [b]. Plants differing with CO_2 treatment in 1996 are marked with [c]. There were no differences which were still significant between plants of the same nutrient regime after the Tukey's test. All variables which differed significantly with rate of nutrient application are denoted by: [d] = $p <$ 0.05 in 1995, [e] = $p < 0.05$ in 1996.

Growth parameters:

Leaf area [de]
Total leaf dry mass [de]
Stem dry mass [ade]
Total shoot dry mass [de]
Root dry mass [abde]
Total plant dry mass [ade]
Root:shoot ratio [de]
Final plant height [de]
Final rachis length of longest leaf per plant [de]
Final leaf number [d]
Relative growth rate [ade]
Specific leaf area [d]
Leaf area ratio [de]
Net assimilation rate [de]
Stomatal density [e]

Leaf phytochemical measurements:

Chlorophyll *a* and *b* [de]
Starch content [d]
Total mono- and oligosaccharide content [ade]
Sucrose content

Nutrient content:

Leaf N [de], P [de], K [de], Ca [de], Mg [de]
Stem N [de], P [de], K [de], Ca [de], Mg [d]
Root N [de], P [de], K [de], Ca [d], Mg [de]

1996 additional variables measured were:

Growth parameters:

Final length of tagged internode [e]
Final rachis length measured on tagged leaf rather than longest leaf
Total number of internodes per plant at final harvest
Average internode length per plant at final harvest
Allometric relationship between rachis length and leaf area for all fully developed leaves

Leaf phytochemical measurements:

In situ chlorophyll determination using portable chlorophyll meter [e]