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Informal Report

MINERAL NUTRITION AND PLANT RESPONSES TO ELEVATED LEVELS OF ATMOSPHERIC CO_2

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August 1996

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MINERAL NUTRITION AND PLANT RESPONSES TO ELEVATED LEVELS OF ATMOSPHERIC CO_2

by

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University of Manchester

August 1996

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Mineral nutrition and plant responses to elevated levels of atmospheric CO_2

A dissertation submitted to the University of Manchester
for the degree of MSc in the Faculty of Technology

1995

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Declaration

I declare that no portion of the work referred to in this dissertation has been submitted in support of an application for another degree or qualification of this or any other university or other institution of learning.

Aneeta Ahluwalia, October 1995

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Abbreviations

A, A_{net}	Assimilation, net assimilation rate, net photosynthetic rate
ATP	Adenosine triphosphate
C_3	Photosynthetic pathway, 3-carbon initial product
C_4	Photosynthetic pathway, 4-carbon initial product
Ca	Ambient CO_2 concentration
Chl	Chlorophyll
Ci	Internal CO_2 partial pressure, intercellular CO_2 concentration
C/N	(Tissue) carbon/nitrogen ratio
dlh	Depth length height
DMSO	Dimethyl sulfoxide
d.w.	Dry weight
F_0	Constant yield fluorescence
F_v	Variable fluorescence
NADPH	Reduced form of nicotinamide-adenine dinucleotide phosphate
NAR	Net Assimilation Rate
NEP	Net Ecosystem Productivity
NO_x	Nitrogen oxides
NPP	Net Primary Productivity
NUE	Nutrient Use Efficiency
PAR	Photosynthetically Active Radiation
Pi	Inorganic phosphate
PiRC	Phosphate Regeneration Capacity
PPFD,PFD	(Photosynthetic) Photon Flux Density
PSII	Photosystem II
RGR	Relative Growth Rate
RH	Relative Humidity
Rubisco	Ribulose biphosphate carboxylase/oxygenase
RuBP,RubP	Ribulose biphosphate
SOM	Soil Organic Matter
T_{air}	Air temperature
$V_{c,max}$	Maximum Rubisco activity
VPD	Vapour Pressure Deficit

Introduction

The atmospheric concentration of CO_2, a radiatively-active ("greenhouse") gas, is increasing [Keeling et al 1989, 1995]. This increase is considered a post-industrial phenomenon [Gammon et al 1985] attributable to increasing rates of fossil fuel combustion and changing land use practices, particularly deforestation [Houghton 1989, Tans et al 1990]. Climate changes resulting from such elevated atmospheric CO_2 levels, in addition to the direct effects of increased CO_2, are expected to modify the productivity of forests and alter species distributions [Pastor and Post 1988, Kramer and Sionit 1987, Eamus and Jarvis 1989].

Elevated levels of CO_2 have been shown, in some cases, to lead to enhanced growth rates in plants, particularly those with C_3 metabolism [Bazzaz 1990, Drake and Leadley 1991] - indicating that plant growth is CO_2-limited in these situations. Since the major process underlying growth is CO_2 assimilation via photosynthesis in leaves, plant growth represents a potential for sequestering atmospheric carbon into biomass, but this potential could be hampered by plant carbon sink size. Carbon sinks are utilisation sites for assimilated carbon, enabling carbon assimilation to proceed without potential inhibition from the accumulation of assimilate (photosynthate). Plant growth provides new sinks for assimilated carbon which permits greater uptake of atmospheric carbon dioxide. However, sinks are, on the whole, reduced in size by stress events [Mooney et al 1991] due to the adverse effects of stress on photosynthetic rates and therefore growth.

Nutrient stress is widespread in natural ecosystems and often limits plant growth because the major mineral elements controlling plant growth are rarely available in nature in the correct amounts that plants require. In particular, nitrogen and phosphorus frequently limit growth in managed and natural ecosystems [Conroy 1992]. Moreover, about 20% of the earth's land mass contains nutrient levels low enough to cause some stress to plants [Dudal 1978]. Such stresses have been proposed to reduce the potential growth response of plants to elevated atmospheric concentrations of CO_2 [Strain and Cure 1985], particularly since the greatest absolute increase in productivity is expected to occur when soil availability of nitrogen and phosphorus is high [Conroy 1992]. However, elevated CO_2 may be able to alleviate nutrient stress. Forest trees growing on infertile soils would need to be able to enhance their uptake of the limiting nutrient in order to achieve long-term growth enhancements or to reduce their demand for such limiting nutrients [Norby et al 1986ab].

Productivity of many forest ecosystems is significantly controlled by nitrogen supply [Chapin et al 1987, Kramer and Kozlowski 1979], and it has been proposed that the supply of nitrogen and/or phosphorus controls the rate and amount of carbon storage in the available biotic sinks and that anthropogenic accelerations of nutrient cycling could lead to enhanced net carbon storage in aquatic and forest sinks [Kramer 1981, Peterson and Melillo 1985, Eamus and Jarvis 1989]. Thus, enhanced photosynthate supply may result from elevated CO_2, stimulating root growth and carbon exudation to the soil, and from modifications to plant nutrition as a result of expanded soil volume being explored and from increased mycorrhizal colonisations [Norby et al 1986ab]. More rapid leaf growth may decrease leaf longevity and accelerate litterfall nitrogen returns to the soil [Zak et al 1993]. Such modifications could accelerate plant-soil nutrient cycles which could permit increased nutrient-use efficiency (NUE) in addition to increased plant productivity [Vitousek 1982]. On the other hand, it is also possible that nutrient limitations could constrain plant responses to elevated CO_2 resulting in homeostatic adjustments [Mooney et al 1991, Oechel et al 1994]. In the following chapters, some of the literature on plant responses to increasing levels of atmospheric CO_2 and to inadequate nutrient supply rates is reviewed, and with this background, the potential for nutrient-limited plants to respond to increasing CO_2 is addressed. Conclusions from the literature review are then tested experimentally by means of a case study exploring carbon-nitrogen interactions in seedlings of loblolly pine, a commercial forest tree species important in the southeastern United States [Baker and Langdon 1990].

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One: Plant mineral nutrition

Mineral nutrition has been proposed to constrain the potential growth response of plants to elevated levels of atmospheric CO_2 [Strain and Cure 1985]. Both seasonally and geographically, a substantial amount of plant growth may occur on soils that are nutrient limited [Sinclair 1992]. Nutrient limitation of individual plants and monospecific stands can be observed when inputs of the limiting nutrient results in increased growth [Chapin et al 1986]. Since atmospheric CO_2 drives plant growth, nutrient limitations may substantially constrain plant growth responses to elevated CO_2 and the opportunity for the increasing levels of atmospheric carbon to be sequestered into biomass via growth enhancements may not be realised. Studies of plant responses to elevated CO_2 at varying nutrient states are therefore needed to determine whether mineral nutrition will constrain plant growth and to what extent. It is important to know how nutrient availability interacts with plant growth and how multiple-resource limitations may complicate such interactions. These issues are discussed briefly in the following chapter to provide a background to the relationship between elevated CO_2 and nutrients in plants. This chapter is intended to highlight some important and relevant aspects of mineral nutrition and to stress the widespread nature of nutrient limitations to plant growth.

Nutrient availability and plant growth

A nutrient limitation is the extent to which plant productivity is decreased as a result of an inadequate nutrient supply rate [Chapin et al 1986]. Thus a plant is considered to be nutrient limited when nutrient additions result in higher productivity. The rate at which nutrients are supplied to plants is determined by the rate of flow of nutrients to plant-available soil pools from pools where the nutrients were previously not available. Supply rates vary from site to site due to differences in many factors, including decomposition and mineral weathering rates, amongst sites [Chapin et al 1986]. Adequate plant growth depends on the flow of nutrients from the bulk soil medium to root surfaces at a rate adequate to maintain nutrients available whilst balancing nutrient uptake across all nutrients [Ingestad and Lund 1979]. Under low nutrient supply, most species have reduced tissue nutrient concentrations, and some may substantially remobilise nutrients from senescing leaves [Flanagan and Van Cleve 1983]. Litter quality (nutrient/carbon ratio) is thus reduced in nutrient-limited ecosystems [Vitousek 1982]. In addition, plants grown on low nutrient sites often have reduced maximum potential growth rates when compared to plants grown on fertile sites and their incremental response to nutrient inputs may also be reduced [Chapin 1980].

To isolate the effects of a nutrient limitation on plant growth and to correctly attribute the observed responses to the single factor, all the environmental factors governing plant growth need to be controlled, including photon flux density, total nutrition, CO_2 concentration, water vapour deficit and ambient temperature [Linder and McDonald 1994] and kept optimal. Specifically, optimal nutrition is where access to nutrients is free and without excess uptake; and it occurs when relative uptake rates equal (or marginally exceed) relative growth rates (RGR) [Ingestad and Agren 1992].

The critical nutrient concentration, the foliar concentration of nutrient necessary for sustaining the optimal (maximal) growth rate of the plant, can be derived from empirical growth response curves on the assumption that the nutrient under question is the only limiting factor to growth; it is a parameter relatively independent of the growth environment unlike nutrient uptake [Conroy 1992]. The critical nutrient concentration may be altered under elevated CO_2 - less of one nutrient may be required to sustain optimal growth and/or more of another. In a CO_2 enrichment experiment on wheat at a range of nitrogen treatments, the critical nitrogen concentration was observed to be reduced under elevated CO_2 [Hocking and Meyer 1991], whilst the critical phosphorus concentration was observed to actually increase under elevated CO_2 in a study of *Pinus radiata* and *P. Caribea* [Conroy et al 1990]. Furthermore, the utilisation site of the nutrient is considered to determine the potential gain in biomass [Sinclair and Horie 1989]. For example, nitrogen may be allocated to producing additional leaf area or to increasing the nitrogen content of current leaf area.

Nutrient supply control over growth rate has been observed in many tree species [Ingestad and Lund 1979, McDonald et al 1991] with the greatest increases in growth rate with increased nutrient supply being observed at low nutrient concentrations. A similar balance of resources - energy, water, nutrients - is required by most plants to maintain growth at the optimal level [Chapin et al 1987]. For optimal growth, mineral nutrients must be supplied to trees in species-specific ratios [Ingestad and Lund 1979]. Yet, nutrients are rarely supplied in nature in the sufficient amounts and ratios for optimal plant growth and thus nutrient stresses predominate and can determine the distributions and physiological adaptations of plants [Chapin et al 1987]. Both the total amount of nutrient available and the concentration of nutrient in the soil solution (nutrient supply rate) are considered important variables in determining whether a nutrient is limiting to plant growth, though the net influx of nutrient is dependent on the plant's need for the nutrient rather than on the concentration in the rooting medium. In natural systems, differences in species composition, plant developmental stage, and moisture supply can sometimes uncouple low nutrient supply rates from nutrient limitation [Pearcy et al 1991].

The initial nutrient availability in a system may be established from the supply rate to, or concentration in, the soil or nutrient solution. However, availability will become modified [Linder and McDonald 1994] from leaching losses, mineralization gains, or uptake by plants for facilitating growth and incorporation into plant tissue, thus increasing nutrient demand and resulting in potential depletion of nutrient reserves as the plant grows bigger [Linder and McDonald 1994]. Accelerated growth depletes reserves more rapidly, hence possible accelerated growth under elevated CO_2 may result in nutrient reserves becoming depleted more quickly and perhaps to the extent where growth rate is negatively affected.

Issues regarding nutrient supply rate, plant relative growth rate, etc. have been investigated theoretically and in studies involving small plants or seedlings only [for example, Ingestad 1979]. The following question can be raised: Is this applicable to field-grown mature vegetation, which account for the majority of the earth's vegetation? Mature plants face many other constraints: for instance, increased growth and/or leaf area can only be observed during the growing season, which in many regions can be quite short. However, since a significant amount of the earth's surface contains nutrient levels low enough to cause some stress to plants [Dudal 1978], particularly forest trees which frequently grow on infertile habitats [Norby et al 1986a], nutrient stress is still an important potential constraint on plant responses to increasing levels of CO_2. Furthermore, carbon-nutrient interactions, particularly in forest species, are an important aspect of the global carbon cycle, especially in the face of potential climate change and rising atmospheric [CO_2], and thus remain an important area for further investigation.

Single limiting factors to plant growth: singly-limiting nutrients

While carrying out single-nutrient manipulation experiments on potted plants, Justus von Liebig (1841) concluded that nutrients could limit plants from attaining their growth potential - that nutrients were growth-limiting factors. This led to the formulation of what is now referred to as Liebig's Law (or the 'law of the minimum') which states that when the availability of one resource necessary for plant growth is least abundant relative to plant requirements, it would be the single limiting factor to greater plant growth. To explore the potential for plants to respond to changes in the atmosphere and climate, it is necessary therefore to know which factors limit plant growth in the present environment. The concept of singly-limiting factors could thus be utilised to determine those factors which may limit plant growth responses to the increasing levels of anthropogenically released CO_2 in the atmosphere and, furthermore, to determine whether <u>nutrient availability</u> specifically will limit plant growth responses to elevated CO_2 [Sinclair 1992].

Growth enhancements represent carbon sequestering potential, possibly slowing the atmospheric accumulation of CO_2. Thus, nutrient limitation to plant growth response is an important consideration when evaluating biosphere feedbacks on the atmospheric CO_2 reservoir. According to Liebig's Law, if a single nutrient element is limiting plant growth, then growth enhancements may not be realised under elevated CO_2 since the limiting nutrient will prevent plant response [Sinclair 1992]. However, there may perhaps be a mechanism through which elevated atmospheric CO_2 levels may alleviate or overcome the growth constraints imposed by limiting nutrient supply, thus contrasting the concept of singly-limiting factors to growth. In this case, CO_2 could be simultaneously (with nutrient supply) limiting growth, for plant growth to be enhanced with increased CO_2 in addition to increased nutrient supply. Environmental resources could interact: simultaneous growth limitations may occur, or an enhanced supply of one resource may either increase the supply or decrease the demand (increased "nutrient use efficiency") for another [Norby et al 1986a]. Nutrient use efficiency (NUE), is defined as the mass of organic matter lost from a plant or permanently stored within the plant per unit nutrient lost or permanently stored, in a mature perennial [Vitousek 1982]. So, increased NUE may be observed under elevated CO_2, facilitating plant growth even under nutrient limitation, and may occur via internal nutrient reallocations or changes in metabolic requirements. On the other hand, increased nutrient uptake (and, perhaps, increased nutrient supply) may occur under elevated CO_2 to alleviate nutrient constraints, possibly via enhanced root and/or mycorrhizal growth, or via enhancements in soil and rhizosphere microbial activity [Norby et al 1986a].

Enhanced growth responses have been seen under elevated CO_2 compared to ambient controls and at varying nutrient concentrations ranging from deficient to adequate to excessive at both CO_2 treatments [Conroy 1992, Norby et al 1986ab]. Simultaneous limitations to growth may have been occurring, with enhanced levels of one growth limiting factor (CO_2 or mineral nutrition) alleviating stress imposed by another. This would question the applicability of Liebig's law since enhanced response should only have been seen when one factor (the single growth limiting factor) was increased, and suggests the potential for stress-alleviating plant internal re-allocations in the face of multiple stresses or limitations [Bloom et al 1985].

Simultaneously-limiting factors

Plants in natural environments often encounter multiple stresses [Chapin et al 1987] and these factors may not interact additively. As discussed previously, the single limiting factor concept may be oversimplistic. There are many studies [for example, El Kohen and Mousseau 1994, Conroy et al 1992, Norby et al 1986a, Lauenroth et al 1978] where enhanced plant growth occurs when more than one controlled factor is individually increased, suggesting a compensatory response to multiple resource limitations [Chapin et al 1987] since enhanced growth can be achieved when any one of the multiply-limiting factors is increased. Growth increases were observed when single factors were increased in isolation and further increases occurred when all controlled factors were increased simultaneously, indicating that there had been some simultaneous limitation of growth occurring amongst these growth-determining factors, with a contribution to enhanced growth from each factor. A proposed mechanism for simultaneously-occurring growth limitations [Bloom et al 1985] takes a cost-benefit analysis approach to explain the acquisition and regulation of resources by plants, arguing that plants optimise their expenditure on each of various resource acquisition activities in order to achieve optimal growth over a range of environmental conditions and to respond to varying demands on their sinks.

Biomass is considered as the gain for plants and the expenditure is considered to be the carbon allocated to resource acquisition in order to achieve the biomass increment, being utilised in growth and physiological functioning of leaves, stems and roots to obtain these resources [Sinclair 1992]. This implies that biomass accumulation is the "goal" of plants under the rationale that larger plants are more competitive and have greater resources for reproductive fitness [Pearcy et al 1987]. Also is implied that plants are continually trading off 'luxury' acquisition of some readily available resources in order to avoid excessive expenditure on other less readily-available resources which would be required to maintain productivity at the level demanded by the 'luxurious' acquisitions [Sinclair 1992, Chapin et al 1987]. Hence greater plant growth may be constantly compromised by the need to avoid the excessive costs entailed in meeting such growth. However, response to enhanced levels of one resource may result in physiological adjustments leading to changed patterns in acquisition and utilisation of all resources, with the consequence of achieving enhanced growth [Chapin et al 1987]. Thus elevated CO_2 levels may facilitate developmental and physiological adjustments that result in the alleviation of potential nutrient stress. For example, the enhanced levels of carbon assimilated may be allocated to root growth in preference to shoot growth, enabling enhanced exploration of the soil medium for nutrients and possibly alleviating potential nutrient stress. As discussed earlier, nutrient demand may be reduced or increased nutrient uptake may occur in order to compensate for prevailing nutrient stress [Norby et al 1986a]. In addition, there is a tendency toward increased nutrient-use efficiency in forests low in nitrogen. The underlying mechanism for this may be an increase in nutrient use efficiency in active leaves and/or increased reabsorption of nutrients from leaves to stems prior to leaf abscission [Vitousek 1982]. Lower leaf nitrogen contents in trees from low nutrient sites may be a result of higher rates of carbon fixation per unit nitrogen or from increased duration of carbon fixation per unit nitrogen or from lower nitrogen uptake per unit biomass [Vitousek 1982].

Cost-benefit type analogies portray plants as efficient, forward-seeing enterprises. Yet plants can be seen to grow into resource limitations which could perhaps have been prevented through slowed growth strategy [Chapin 1980]. For example, plant growth can be seen under conditions of increasing resource scarcity. It is important to know how plants regulate and allocate the resources they acquire: are plants efficient at allocating their resources, particularly when facing environmental stress? The most limiting resource is thought to often have the least incremental gain per cost [Sinclair 1992] and thus substantial photosynthate should be allocated to the acquisition of such a resource. In conditions where mineral resources are the scarcest resource (and hence are growth-limiting), root growth would be expected to be favoured over stem and leaf growth, thus root/shoot ratios are often used in studies as a broad indicator of carbon allocation, particularly in response to nutrient stress.

Multiply-limiting nutrients

Nutrients are required by plants in specific ratios [Olson 1950] and growth will adapt to the nutritional resources of a site [Ingestad 1982]. Mineral nutrients may be simultaneously limiting to growth [Dudal 1978, Chapin and Shaver 1985]. Unfortunately, due to the experimental complexity of conducting such studies, the majority of experimental work to date has examined single-nutrient enhancements rather than nutrient ratio [Ingestad 1982] or multiple-nutrient enhancements [for example, Conroy et al 1992]. Thus I will focus on single-nutrient limitations and carbon-nutrient balance approaches to analysing plant growth and plant responses to CO_2. It is necessary to investigate how nutrient availability and CO_2 interact, particularly to determine whether elevated CO_2 may be able to overcome the constraints to plant response imposed by a nutrient limitation, as suggested by experiments where both mineral nutrition and CO_2 were varied [Conroy 1992, Norby et al 1986ab, Patterson and Flint 1982].

Plant nutrient and carbon balance

Nutrients and carbon can be considered in balance with respect to each other in the sense that nutrient uptake regulates increases in biomass, and carbon assimilation supplies the structure into which nutrients are incorporated [Sinclair 1992]. Plants may allocate resources to balance the relative uptake of nutrients and carbon as a function of the relative availability of these resources [Bloom et al 1985]. Models of plant carbon/nutrient balance suggest plants will respond to nutrient limitations by allocating more resources to nutrient uptake or by utilising the nutrients more efficiently in growth [Coleman et al 1993]. Plants impact on the nutrient pool by exploiting more soil volume through changed fine root production, mycorrhizal associations and by altering rhizosphere chemistry, and in the longer term, through organic litter production [Norby et al 1984, Clarkson 1985]- all requiring significant expenditure of carbon stocks. Nutrients can be leached, or are fixed into biomass and later lost as detritus or through herbivory.

The major nutrients need to be available at an early stage in plant development, otherwise biomass production is immediately restricted by an insufficient supply of nutrients. As biomass production continues, the ratio of dry organic/inorganic matter increases as the rate of biomass accumulation exceeds the rate of nutrient uptake. A controlled relative nutrient uptake rate (uptake rate per amount of nutrient in plant) can result in stable plant nutrient concentrations and stable relative growth rates [Ingestad and Agren 1992], linking nutrient and carbon fluxes into and within the plant and, the amount of nutrient in the plant and the growth rate.

Carbon may accumulate as a result of enhanced assimilation rates under elevated CO_2 [Dahlman 1985]. Nutrient uptake rates may correspondingly remain unchanged, increase marginally or increase in proportion to carbon uptake, and the consequence for plant internal nutrient concentrations would be significant decreases, small decreases or no change [Overdieck 1993]. In a study of the effects of elevated CO_2 on the mineral content of woody and herbaceous species [Overdieck 1993], tissue nutrient concentrations, in general, decreased with elevated CO_2, with the greatest decreases in nitrogen concentration, though phosphorus concentration did not decrease and absolute amounts of nutrients in the whole vegetation were observed to increase. Plants may undergo internal physiological adaptations to improve nutrient use efficiency in order to ensure adequate nutrients are available to support increased growth under elevated CO_2 [O'Neill et al 1987]. Reduced tissue nitrogen concentrations may occur as a result of increased nutrient use efficiency, thus nitrogen availability may not constrain plant response to elevated CO_2. The reduced nitrogen concentrations observed under elevated CO_2 [Wong 1979, Norby et al 1986a, Overdieck 1993] could lead to substantially increased herbivory [Fajer et al 1989], in addition to reduced litter decomposition rates - potentially limiting ecosystem nutrient cycling [Bazzaz and Fajer 1992]. This would be a negative feedback on plant response to CO_2. Comparing plants at a common size though, rather than a common age, to neglect the effect of accelerated development under elevated CO_2, indicates that plant nitrogen concentration may not be modified by increased CO_2 supply [Coleman et al 1993].

Potentially limiting nutrients: Nitrogen

The amount of nitrogen available to plants varies substantially across natural ecosystems, from 0.09 to 22.8 g/m^2.yr, from polar desert to rich tropical rainforest, respectively [Dowding et al 1981, Vitousek 1984]. Nitrogen enters ecosystems as NH_4^+, NO_3^-, and organic nitrogen in solution in precipitation, as gaseous and aerosol nitrogen to the forest canopy, and by microbial ammonia fixation (**Table 1**). It is the mineral nutrient required in the largest quantities by plants and may often be growth-limiting in both agricultural and natural environments [Chapin et al 1987].

Table 1: Annual ecosystem nitrogen inputs *Source: Pearcy et al 1991*

Annual nitrogen inputs to ecosystems *(most common ranges)* :		
Precipitation (unpolluted regions)	1-5	kg/ha
Precipitation (nitric acid rain)	10-40	kg/ha
Symbiotic nitrogen-fixation dominated ecosystems	100	kg/ha
Inorganic-nitrogen by-product of SOM microbial oxidation	2-5 % of gross soil organic nitrogen released	
Microbial immobilisation of nitrogen that is inorganic byproduct of SOM microbial oxidation	50-99% of gross soil organic nitrogen released	
Total nitrogen content of most ecosystems within	1500-15 000	kg/ha
Bound as organic detritus and soil humus until available through microbial decomposition (mineralization).	>98% of total ecosystem nitrogen content	
Annual nitrogen uptake in most nonagricultural systems	within 20-200	kg/ha

SOM soil organic matter

Nitrogen is absorbed from the soil medium into the plant, translocated as inorganic nitrogen within the plant, and then converted to organic forms, that are assimilated [Chapin et al 1987]. Absorption surfaces on roots require a large carbon input for growth and maintenance, in order to continually explore rooting volume for locations where nutrients haven't been depleted. The site of nitrogen assimilation determines the carbon cost in nitrogen translocation around the plant and assimilation occurs in a variety of ways, with mycorrhizae having the least cost and nitrate reduction and atmospheric nitrogen fixation having the most [Chapin et al 1987].

Nitrogen availability impacts primarily on the rate of growth of leaf area, a variable which can quickly change with changes in availability, thus plants can stabilise their internal physiological status by adjusting growth according to the rate of supply of nitrogen [Ingestad and Lund 1979]. Hence, a close relationship between nitrogen availability, nitrogen uptake, and plant growth rate is often observed. Increased additions of nitrogen have been observed to increase leaf size, number of shoots and number of needles in fascicles in various tree species [Linder and Rook 1984].

Nitrogen stress

Plant growth rate is linearly related to the plant nitrogen content at a wide range of nutrient states [Ingestad 1982] as are leaf photosynthetic rates [Evans 1989]. Around 75% of the nitrogen in a C_3 plant leaf is invested in chloroplasts, the majority in photosynthesis [Chapin et al 1987]. Nitrogen is an important component of chlorophyll so severe nitrogen deficiency will limit leaf chlorophyll and result in leaf chlorosis. When a nitrogen stress is imposed on tree seedlings, characteristic deficiency symptoms may develop, including leaf chloroses and rapid root elongation with reduced branching. If the stress is constant (that is, supply rate is relative to uptake rate), then above-ground symptoms will disappear as the plant stabilises growth rate and internal nutrient status at a sub-optimal nitrogen status. Below-ground symptoms may remain until eventual stabilisation of root growth [Ingestad and Lund 1979]. Under conditions of strong nitrogen limitation, about 50-70% of plant biomass is allocated below-ground [Zak et al 1993]. Rates of increase in leaf area and dry weight rapidly decrease at sub-optimal nitrogen supply, with this decreased leaf growth rate leading to reduced photosynthesis (leaf area productivity) and overall growth rate.

Potentially limiting nutrients: Phosphorus

Phosphorus is often in limited supply for plant growth with a large proportion of phosphorus being involved in reactions with other soil minerals leading to precipitation in unavailable forms [Pearcy et al 1991]. Phosphorus inputs to ecosystems are typically much smaller than nitrogen inputs, and arise mainly from atmospheric and weathering processes (**Table 2**). It is an element which is not readily available in acidic soils and so represents an important nutrient limitation to plant growth in ecosystems found on acid, nutrient-poor substrates [Clarkson 1985]. The tropics and subtropics, in particular, include vast areas of land with soils with high phosphorus fixation capacity but low levels of plant available phosphorus [Strain and Cure 1985]. The soil solution concentration of phosphorus in such regions is particularly low, usually around a few μeq/l.

Table 2: Annual ecosystem phosphorus inputs *Source: Pearcy et al 1991*

Annual phosphorus inputs to ecosystems *(most common ranges)* **:**		
Atmospheric	0.1-0.5	kg/ha
Weathering	0.1-0.5	kg/ha
Annual P uptake	2-15	kg/ha

Phosphorus availability is controlled by direct precipitation with Fe and Al in acid soils, while in arid soils most phosphorus is held on the surface of $CaCO_3$ or precipitated as calcium phosphate. Plant growth depends on the rapid root uptake of phosphorus released from dead organic matter in the biogeochemical cycle [Wood et al 1984]. Organic acids can influence the availability of phosphorus in the upper soil layers via weathering. Following its release, phosphorus may be more available in the presence of organic acids, such as oxalate, which also remove Fe and Ca from the soil solution by means of chelation and precipitation [Pearcy et al 1991]. The production and release of oxalic acid by mycorrhizal fungi underlies their importance in maintaining and supplying phosphorus to plants [Clarkson 1985].

Rhizosphere populations and CO_2

Plant production controls the extent of carbon input to the soil [Zak et al 1993]. Rhizosphere-located changes in microbial populations may thus occur under elevated CO_2 with the potential to enhance the response of trees to elevated CO_2 levels [Norby et al 1986a] since products of photosynthesis are substrates for these often carbon-limited [Smith and Paul 1990] microbial populations - these populations have the ability to modify nutrients such that plant nutrient uptake is enhanced [Wood et al 1984]. Greatest rates of growth are expected to occur in the presence of both mycorrhizal fungi and symbiotic nitrogen-fixing bacteria, with a concomitant decrease in root/shoot ratios.

Furthermore, symbiotic associations between fungi and higher plants are found in most ecosystems, are important for plant nutrition, and have several forms. In temperate regions, many trees are infected by ectotrophic mycorrhizae which form a hyphal sheath around the active fine roots and extend additional hyphae into the surrounding soil [France and Reid 1983]. Alternatively, plants may be infected by endotrophic mycorrhizae in which the fungal hyphae actually penetrate root cortex cells. Due to their large surface area and efficient absorption capacity, mycorrhizal fungi are able to obtain soil nutrients and transfer these to the higher plant root, while depending upon the host plant for supplies of carbohydrate [Clarkson 1985]. Mycorrhizal fungi are most important in the transfer of soil nutrients with low soil diffusion rates and are considered important in plant phosphorus acquisition but are also known to facilitate the absorption of nitrogen and other nutrients [Clarkson 1985]. However, mycorrhizae consume a fraction of fixed carbon of the host plant and represent a drain on NPP that might otherwise be allocated to growth.

High soil levels of nitrogen and phosphorus decreased sucrose content of short roots in a study of the mycorrhizal infection response of loblolly pine roots to varying site fertility [Marx et al 1977], site fertility is inversely correlated to carbohydrate content. In conditions of nutrient deficiency, plant growth usually slows whereas photosynthesis continues at relatively high rates, and the content of soluble carbohydrate in the plant increases. Increased levels of carbohydrate are also expected as a result of enhanced photosynthetic rates under elevated CO_2 [Hollinger 1987, Williams et al 1986]. High concentrations of carbohydrate in roots of loblolly pine have been observed to stimulate mycorrhizal infections [Marx et al 1977]. Thus, internal plant allocation of carbohydrates to roots may result in increased nutrient uptake by mycorrhizae and an alleviation of nutrient deficiencies. However, elevated CO_2 was not observed to increase ectomycorrhizal colonisation population density in white oak seedlings grown in nutrient-poor forest soil, compared to ambient grown controls [Norby et al 1986ab]. In addition, mycorrhizal infection did not affect CO_2 response or photosynthate sink strength of loblolly pine seedlings after long-term exposure to elevated CO_2 and different levels of phosphorus supply [Lewis et al 1994a], though this may have been due to growth cycle mycorrhizal senescence [Lewis et al 1994b]. Further studies on the interaction of mycorrhizae, nutrient supply and elevated CO_2 are necessary: genotypes and species need to be compared under elevated CO_2, and at varying nutrient states, for differences in mycorrhizal colonization rates, particularly in terms of seasonal and long-term responses in the field [Lewis et al 1994a].

Essential cation and micronutrient availability

Nitrogen and phosphorus frequently limit growth in managed and natural systems [Conroy 1992], hence many investigations of CO_2-nutrient responses have concentrated on these mineral nutrients. Yet, other nutrients may be limiting: nutrient deficiencies may be localised and determined by the soil chemistry and geology of the underlying bedrock. Micronutrients may be available in abundance in natural ecosystems, though base cation limitations are common [Barnard et al 1990]. Cations can be available in substantial amounts as unweathered minerals, on cation exchange sites and in organic matter. However, availability of several base cations may covary in acid, podzolic soils with low base cation status [Wilmot et al 1995].

Which nutrients limit growth in the present environment and to what degree?

The direct evaluation of nutrient availability to plants is difficult and several indices of nutrient availability in natural ecosystems exist [Chapin et al 1986]. Nutrient-addition experiments in the field have been used most often to determine the extent of nutrient limitations to plant systems [Linder and McDonald 1994]. Three plant processes are highlighted as being potentially nutrient limited: the growth of current populations; net primary production NPP (gross primary production or photosynthesis less respiration of producers); and net ecosystem production NEP (NPP less heterotrophic respiration). In addition to the species composition, the degree of nutrient limitation of these processes is also dependent upon the extent to which nutrients are sufficient to attain the maximum potential growth rate of the component species [Chapin et al 1986]. However, there are great problems in establishing the degree of nutrient limitation of either NPP or NEP, particularly since species present in a low nutrient site may respond marginally to large inputs of a nutrient, while other species requiring high levels of nutrients could have responded more if present [Chapin 1980]. Therefore it is only applicable to discuss the presence or absence of limitation by particular nutrients and not the degree to which limitations are present, since the degree of nutrient limitation is very difficult to establish. However, all biomes include strongly nutrient-limited ecosystems - tundra, some northern deciduous forests, tropical forests, boreal and montane coniferous forests and chaparral [Strain and Cure 1985].

Nitrogen limitation to net primary production in terrestrial ecosystems is usually seen as the most likely mineral nutrient limitation in these ecosystems [Chapin et al 1987, Vitousek 1982]. It is not easily possible to measure nitrogen availability in forest soils at present

[Vitousek 1982], though suggested indices of nitrogen availability exist [see review Keeney 1980]. There is usually only a small pool of instantaneously available soil nitrogen. This pool is rapidly turned over by inputs of organic nitrogen compounds microbially broken down and by losses from leaching and/or plant and microbial uptake [Robertson and Vitousek 1981]. However, additions of nitrogen alone has been observed to increase productivity in temperate [Aber et al 1989, Lea et al 1980] and boreal forests, temperate grasslands, and arctic and alpine tundra, though other nutrients may also limit, sometimes co-limiting with nitrogen. With increased water supply to water-limited systems, nitrogen limitations often become apparent [Peterson and Melillo 1985]. Nitrogen is a major limiting factor to forest growth in eastern North America [Pastor and Post 1988]. Phosphorus limitation to growth is important in ecosystems found on acid, nutrient-poor substrates, possibly encompassing large areas in the tropics, especially in the Amazon Basin and the South African grasslands [Owen-Smith 1982]. In North America, phosphorus can be limiting in the south eastern coastal plains and Piedmont regions, or nitrogen and phosphorus may co-limit. Other macronutrients such as potassium, calcium and/or magnesium can be limiting in certain forested areas, particularly montane regions in north eastern North America [Wilmot et al 1995] and in polluted areas of central Europe [Schulze et al 1989]. Thus nutrient limitations to growth are widespread, occurring in many ecosystems. Since nutrient limitations are proposed to constrain the potential for plants to respond to increasing atmospheric CO_2 [Strain and Cure 1985], there needs to be a spatial consideration of the impacts of increasing CO_2 on nutrient-limited plants. Nutrient-limited plants may be expected to exhibit differential responses to increasing atmospheric CO_2 according to the extent and nature of the nutrient limitation, and whether other factors are co-limiting. Furthermore, as a result of prevalent nutrient limitations, possible modifications in nutrient uptake from the soil, remobilisation in the plant and utilisation efficiency under elevated CO_2 will impact nutrient cycling in natural systems [Conroy 1992] and potentially feedback on plant growth rates.

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Two: The stress/sink hypothesis and CO_2-nutrient interactions

- Plant responses to elevated CO_2: is mineral nutrition a controlling factor?
- The stress-sink hypothesis
- Plant carbon balance
- Plant carbon partitioning and allocation under CO_2
- Plant carbon partitioning and allocation under nutrient stress
- Photosynthesis
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  - Downregulation of photosynthesis and nutrient supply
  - Role of nitrogen and phosphorus in limitation of photosynthesis
- Will nutrient stresses affect plant responses to rising CO_2 levels?
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- References

Two: The stress-sink hypothesis and nutrient-CO_2 interactions

Plant responses to elevated CO_2: is mineral nutrition a controlling factor?

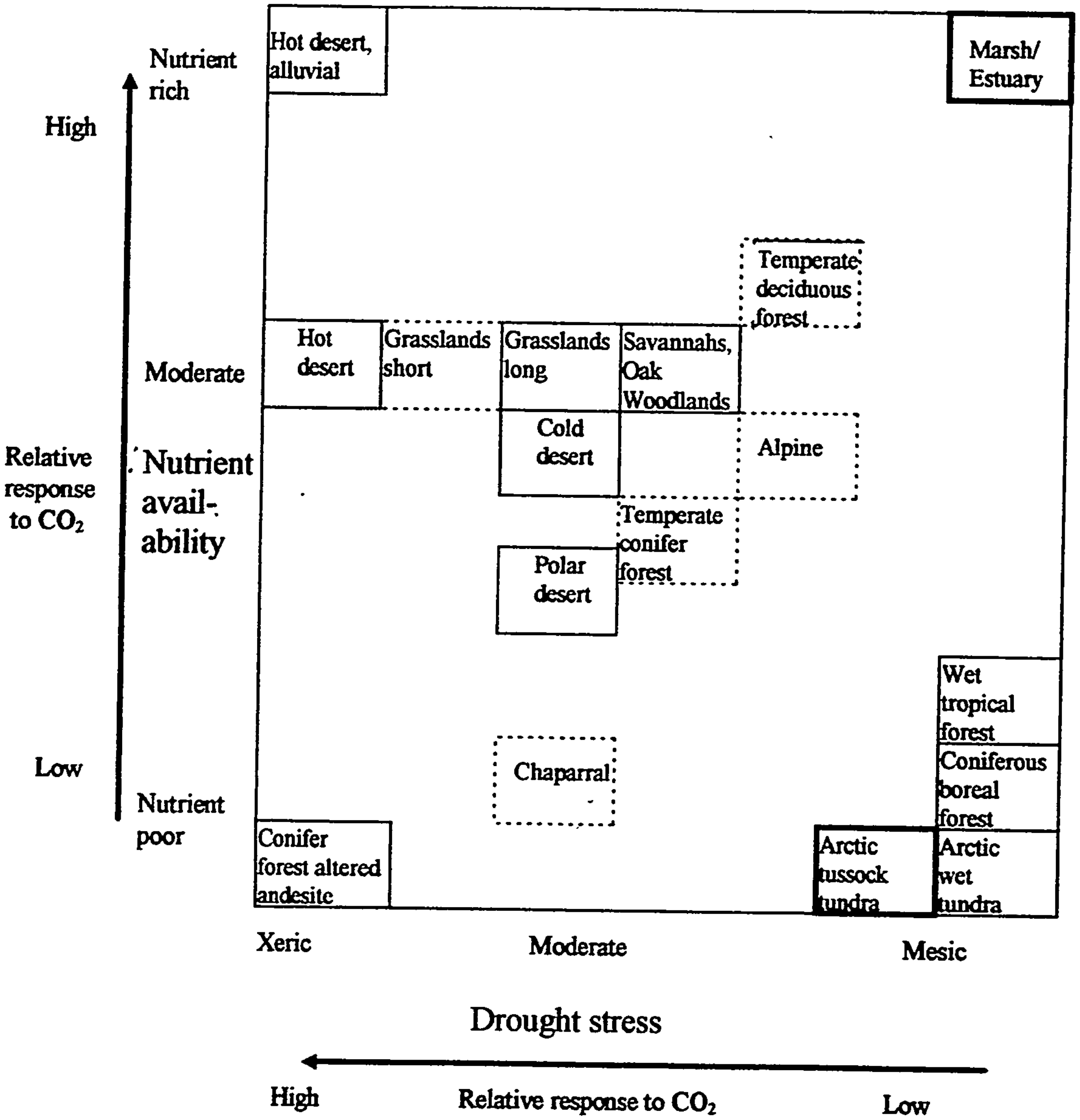
Two long-term CO_2 enrichment studies on herbaceous species - an open-top chamber tidal salt marsh study at Rhode River, Chesapeake Bay [Tissue and Oechel 1987] and a greenhouse-based study on Alaskan arctic tundra ~~at Toolik Lake~~ [Curtis, Drake and Whigham 1989] - produced apparently conflicting results regarding seasonal observations of variations in photosynthetic rates under elevated CO_2. The dominant species in the floristically diverse tundra ecosystem was the C_3 species *Eriophorum vaginatum* (cotton grass), whilst the salt marsh ecosystem contained the higher plants *Scirpus olneyi* (C_3) and *Spartina patens* (C_4), mainly in monospecific stands. Downregulation of photosynthetic rates under elevated CO_2 (the decline in photosynthetic rate after an initial enhancement or stimulation relative to ambient grown controls, see later discussion of downregulation) was observed in the arctic tundra experiment [Tissue and Oechel 1987, Grulke et al 1990, Oechel et al 1994], in addition to a decline in ecosystem metabolism to a level similar to that prior to when exposure to elevated CO_2 had occurred. In contrast, photosynthetic rates were observed to be enhanced under elevated CO_2 (25-50% compared to ambient-grown controls) in the Chesapeake Bay experiment [Curtis, Drake and Whigham 1989, Drake and Leadley 1991] and maintained at an enhanced level for over three years, with no evidence that this enhancement would not be sustained [Drake and Leadley 1991]. Such apparently contrasting observations of trends in photosynthetic rates under elevated CO_2 led to an attempt to generalise possible ecosystem responses to CO_2 [Mooney et al 1991] into an ecological framework relating apparent response to CO_2 in terms of nutrient and water supplies to the ecosystem under observation to suggest why the afore-mentioned long-term experiments may have produced such differing results (**Figure 1**). In the cold arctic tundra environment nutrients are relatively unavailable, whilst in the temperate saltmarsh environment, nutrients are highly abundant. Thus it was hypothesised that site fertility may control whether photosynthetic rates can be maintained at an enhanced level for a sustained period of time under elevated CO_2. That is, nutrients may be constraining the response of the nutrient-poor tundra (and similar ecosystems) to elevated CO_2. If valid, this hypothesis has important implications for future responses of terrestrial vegetation to CO_2 since many portions of the world's land-surface are characterised by low-nutrient substrates, as discussed in the previous chapter. However, temperature and/or light may also have been constraining the response of the tussock tundra system [Grulke et al 1990].

The stress-sink hypothesis

Stress may be defined as any factor which reduces plant growth and reproduction below the potential of the genotype [Osmond et al 1987]: the major stresses affecting plants include nutrient excesses and deficits, temperature extremes, water availability, pathogens and pests, and air pollutants such as ozone, NO_x, and SO_2. Thus, plant growth occurs under a wide range of stresses.

It has been hypothesised that nutrient stresses may constrain plant responses to elevated CO_2 as a result of reduced potential sink capacity for the increased photosynthate supply likely under elevated CO_2 [Mooney et al 1991]. Plant growth creates new sinks for carbon by

Figure 1: Hypothesised ecosystem response framework. Response of ecosystems to elevated CO_2 with regard to prevailing nutrient and water availability.
Field-studied ecosystems shown with heavy lines, part-studied in controlled environment systems in broken outlines, remaining mostly unstudied.
Source: Mooney et al 1991.



providing locations for the utilisation of newly acquired assimilate (photosynthate). Strong responses to CO_2 enrichment are seen in plants with active photosynthate sinks [Ackerson et al 1984, Downton et al 1987]. However carbon sinks are, on the whole, reduced in size by stress events [Mooney et al 1991] from the adverse effects of stress on photosynthetic rates and thereby growth. Furthermore, plant sink size (or strength) can modify the stress response via reallocation of fixed carbon [Chapin et al 1987], though carbon sinks will be reduced if stored carbon is utilised to avoid reduced growth or injury brought on by stress events.

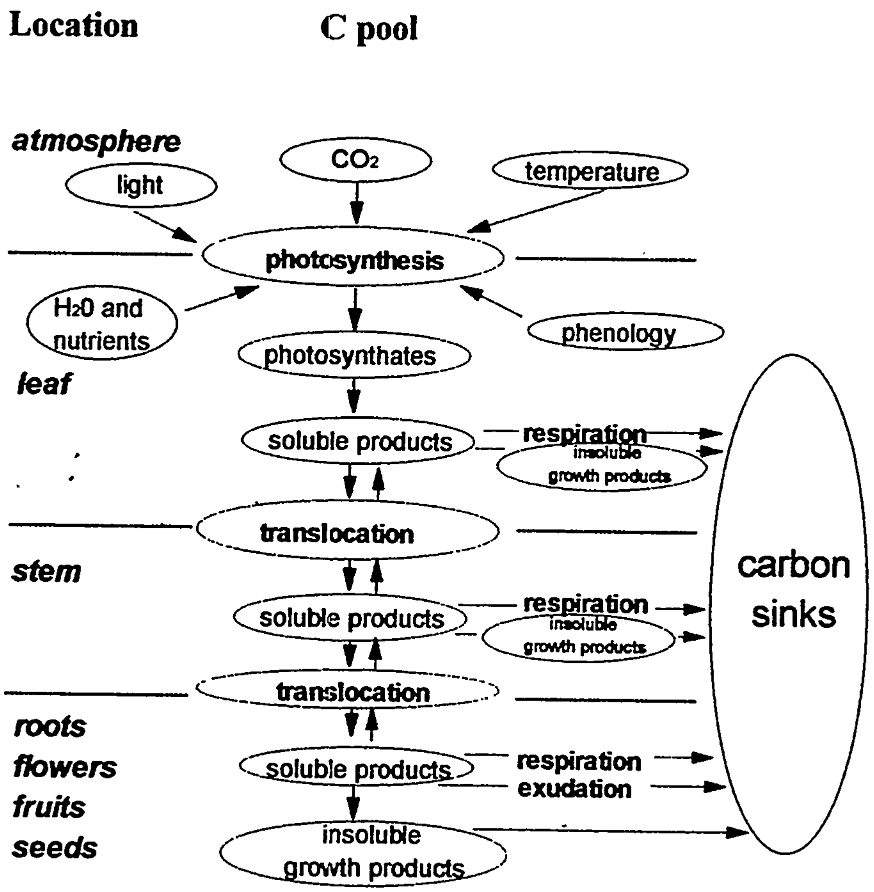
Stress-sink analyses therefore provide a useful investigative approach when exploring stress interactions in plants, particularly when increasing concentrations of atmospheric CO_2 could potentially increase sink size and thus modify plant responses to stress. Since nutrient stresses prevail [Dudal 1978] particularly on sites where forest species grow [Norby et al 1986a, Figure 1], the response of nutrient-limited trees to elevated CO_2 is particularly relevant to the response of natural vegetation to the increasing levels of atmospheric CO_2. To explore whole-tree responses under elevated CO_2, tree responses need to be studied at a variety of levels to fully observe CO_2-nutrient stress interactions, including phenology, life-cycle events (e.g. reproduction, senescence), and physiological processes (e.g. photosynthesis, transpiration, respiration, carbon allocation, nutrient uptake and nutrient allocation). It remains to be seen whether nutrient stresses will constrain the response of forest species to elevated CO_2, as proposed by Mooney et al (1991), due to reduced sink capacity for photosynthate or if the potentially stimulated supply of photosynthate under elevated CO_2 could be allocated to stress alleviation activities to maximise plant performance, as discussed in the previous chapter.

Plant carbon balance

Reduced carbon or photosynthate sink capacity in the face of nutrient stress may thus constrain plant responses to elevated CO_2 or, conversely, elevated stress may alleviate constraints imposed by prevalent stresses, perhaps through internal reallocations of carbon. It it is necessary, therefore, to know how and where the potentially increased supply of photosynthate under elevated CO_2 could be utilised (**Figure 2**). In particular, there is some indication that growth under elevated CO_2 changes the carbon sink-source balance of plants, from observations of carbohydrate accumulations in "source" leaves and of differences in plant responses to elevated CO_2 across species, developmental stages and environmental conditions [Stitt 1991]. In addition, long-term plant responses to elevated CO_2 may be controlled by the plant sink-source balance [Stitt 1991]. Since forests and their soils hold over 90% of the biosphere's carbon [Houghton et al 1990], evaluation of the distribution of carbon between sources and sinks in response to elevated CO_2 is especially relevant in forest species. In addition, plant source-sink balance may constrain or facilitate biosphere feedbacks on the atmospheric CO_2 reservoir.

How is carbon utilised in plants? Plants use carbon to obtain energy for growth and maintenance of plant tissues and organs, for protection against pests and pathogens, for reproduction/life extension and for storage reserves for unfavourable periods [Laurence et al 1994]. Carbon enters plants via photosynthetic assimilation and is imported to sinks as photosynthate or soluble products of photosynthate. Partitioning and allocation of carbon is discussed in the next section. Respiration, storage and new structure synthesis constitute the

Figure 2: Plant carbon fluxes
Source: Oechel & Strain 1985



major fluxes of carbon within sinks (**Figure 2**). In cost-benefit analysis terminology, photosynthetic import of carbon would be considered as the gain or benefit to the plant - carbon is beneficial to plants in that it confers greater competitiveness. Carbon leaves plants via respiration, leakage and export (seed and fruit dissemination, root exudations, herbivory, senescence, organ abscission, etc.) and these would be considered as losses to the plant

Plant carbon gain is derived from the CO_2 uptake rate per unit of photosynthetic surface area multiplied by overall plant photosynthetic surface area, then subtracting any losses, for example, those from respiration and herbivory [Pearcy et al 1987]. It is a dynamic variable since leaf photosynthetic capacity depends on leaf age, canopy structure, etc. Canopy architecture influences the distribution of limited resources amongst leaves of different age and position in the canopy to maximise whole-plant carbon gain [Field 1983]. Photosynthetic capacity is low during initial leaf expansion, with the leaf importing much carbon for construction and rate of respiration is high. Around full leaf expansion is when the maximum photosynthetic rate is usually achieved. Then photosynthetic capacity declines during leaf senescence as nitrogen becomes remobilised and is exported prior to abscission. Leaf cumulative carbon gains must exceed the carbon costs of leaf construction, maintenance and protection for a leaf to be of benefit to the plant. Environmental constraints are important in determining the long-term carbon gain of a leaf, delaying the time for a leaf to produce a net profit [Pearcy et al 1987]. Repair mechanisms necessary to compensate for stress effects also increase maintenance costs, adding further delay for net leaf profit.

Plant carbon partitioning and allocation under elevated CO_2

Resource availability and plant ability to utilise these resources for carbon gain and productivity are strongly linked to size, survival, reproductive output and other parameters at the population level [Bazzaz et al 1987]. Under elevated CO_2, there is a potentially enhanced supply of carbon as photosynthate to the plant, particularly in C_3 species, and it is important to determine how plants will utilise this resource particularly in response to prevailing stress. Thus, knowledge of plant carbon allocation and partitioning patterns is necessary to evaluate the response of plants to changed atmospheric CO_2 levels in terms of carbon sources and sinks, such an analysis may be an invaluable basis for establishing how plants allocate and partition their carbon assimilation inputs in response to concurrent stresses. Will plants be able to overcome stress constraints on carbon sink capacity? Plants may show adaptive behaviour when growing under stress conditions, via tolerance or avoidance mechanisms [Osmond et al 1987]. High metabolic activity under mild stress and reduced activity under severe stress are facilitated by tolerance mechanisms, whereas avoidance mechanisms enable autotrophic activity to be reduced and dormancy to be initiated in response to extreme stress [Osmond et al 1987]. Plants may therefore be able to utilise tolerance and avoidance mechanisms to alleviate stress constraints and this behaviour may be stimulated by an enhanced photosynthate supply under elevated CO_2 via changed patterns of carbon allocation and partitioning.

Carbon allocation is defined [c.f. Dickson and Isebrands 1993] as the flow of carbon between organs, usually from a source (mature leaf) to a sink (growing point) **(Figure 2)**, indicated by changes in the dry mass of the particular plant organ that is operating as a sink. In contrast, carbon partitioning is defined as the flow of carbon within organs, indicated by changes in the chemical form of the carbon within the plant organs, for example, changes in foliar or root starch or sugars. Carbon partitioning influences the efficiency of photosynthate utilisation and the degree to which it has been productively invested [Farrar and Williams 1991]. Carbon is partitioned to storage when supply exceeds demand enabling carbon to be partitioned away from storage to alleviate reduced growth or injury in the face of stress. If storage is sufficiently large, these stores can be used for growth when demand exceeds supply [Laurence et al 1994].

Partitioning and allocation of carbon amongst species is influenced by timing, duration, form, rate of growth, and capacity for storage [Laurence et al 1994]. There is considered to be a significant genetic determinant of plant carbon allocation and sensitivity to stress as well as a modified response by plants when faced by multiple stresses [Temple et al 1993, Mooney and Winner 1991]. Species have been proposed to fall into categories according to carbon-storage growth type in order to lineate their responses to stress [Laurence et al 1994]. According to this scheme, faster growing species are proposed to have a higher capacity to allocate carbon to compensate for stress due to their high assimilation rates, high stomatal conductances and short leaf longevity.

---------- increasing capacity to allocate C to compensate for stress ---------->

slow growing **fast growing**

red spruce ponderosa pine loblolly pine aspen radish

stress tolerant **more sensitive to stress**

Such a functional classification of plant behaviour may provide a useful insight into examining impacts of elevated CO_2 and interacting stresses on plant carbon balance [Reich et al 1992].

Plant carbon partitioning and allocation under nutrient stress

As discussed above, carbon allocation can be modified by environmental factors [Mooney and Winner 1991], thus plants may be able to adapt to some losses (and gains) imposed by environmental stresses [Laurence et al 1994]. The partitioning of dry matter in forest trees between roots and shoots is affected by mineral nutrition, as well as several other factors. Under increasing levels of nutrient limitation (decreasing internal nutrient concentrations), there may be increased carbon partitioning to the roots [Luxmoore et al 1986b, Norby et al 1987]. Photosynthate partitioning to root sinks under elevated CO_2 may therefore depend on the nutrient status of the plant [Farrar and Williams 1991]. High nitrogen levels can significantly lower root/shoot ratios [Linder and Rook 1984], though they may remain unchanged [Eamus and Jarvis 1989]. However, when phosphorus, potassium, iron or manganese were increasingly limiting in birch seedlings [Ingestad 1979] and in field grown trees [Linder and Axelsson 1982, Linder and McDonald 1994], carbon partitioning to the roots was observed to actually decrease. Thus some nutrient stresses may be actually reducing the size of root sinks, in favour of photosynthate utilisation elsewhere. Under conditions of nutrient stress, therefore, carbon partitioning is altered and may not always be increased to roots to favour enhanced nutrient acquisition at this site. Instead, photosynthate may be used to reallocate mineral nutrient resources from one plant site or process to another.

Nitrogen limitation to agricultural and natural systems is the most common nutrient limitation in these systems [Chapin et al 1987]. Nitrogen and carbon can be seen to be closely linked in terms of their acquisition and allocation, and nitrogen stress can impact on both the assimilation and allocation of photosynthate. Photosynthates are acquired in amounts proportional to the amount of nitrogen used to construct leaf area and biochemical photosynthesis constituents since photosynthates provide energy and structure for mineral acquisition and assimilation, particularly to acquire and synthesise the large quantities of nitrogen necessary in plant proteins and nucleotides [Sinclair 1990]. Thus photosynthate production may change under conditions of nutrient stress since there would be reduced biochemical constituents and diluted leaf/nutrient ratios, though nutrient stress may be alleviated if more photosynthate is available to provide energy and structure for acquiring and assimilating nutrients - substantial photosynthate or carbon reserves may be allocated towards acquiring nitrogen [Chapin et al 1987]. Regardless of where the photosynthate is utilised, it is clear that plants may be able to allocate and partition carbon resources under elevated CO_2 in order to maximise resource use and gain to overcome reduced sink capacity imposed by nutrient stress. Such behaviour would enable the plant to be more productive and confer greater competitivity under conditions of nutrient limitation but would require enhanced levels of photosynthate to be made available, and sustained at this level, under elevated CO_2.

Photosynthesis

Leaf level photosynthetic rates are likely to be enhanced under elevated CO_2 - with potential changes in physiology, morphology and growth occurring as a result [Gunderson and Wullschleger 1994] - as photorespiration is suppressed and carboxylation stimulated from an increased supply of CO_2 to ribulose biphosphate carboxylase/oxygenase (Rubisco), the central enzyme to photosynthesis [Strain and Cure 1985]. If this enhancement occurs on a substantial global scale, then the effects of elevated releases of CO_2 could be partially alleviated by the enhanced productivity of natural vegetation. Productivity can be generally calculated by [Hall et al 1993]:

Pn: Net productivity = Q: quantity of incident light
or net biomass gain x B: proportion of light intercepted by photosynthetic organs
x E: efficiency of photosynthetic conversion of intercepted light into biomass
- R: respiratory losses of biomass

Productivity in natural plant systems is an important measure of total energy or carbon inputs into those systems and it will be critical to evaluate if photosynthetic enhancements as a result of climate change will scale up to stand and ecosystem level increases in productivity. Respiratory biomass losses (R) occur from the maintenance of existing tissues and growth of new tissues and limit productivity. The light interception efficiency of the system (B) depends on size, structure and spectral properties of the plant canopy. Many inorganic fertilisers such as NH_4NO_3 improve yield through leaf area and duration changes (improved B), whilst many stress factors act oppositely. Energy conversion efficiency (E), another component of plant productivity, is directly determined by the efficiency of light utilisation by the photosynthetic pathway, is affected by the environment and can be improved by enhanced levels of CO_2 as a result of reduced photorespiration. It is uncertain whether E could be improved under optimal conditions since it is usually close to the theoretical maximum for plants but under sub-optimal conditions E may be enhanced by elevated CO_2 as a result of suppressed photorespiration and stimulated carboxylation. Many environmental stresses decrease E, at least in the short term.

Photosynthesis, a component of E, may be controlled by a number of internal variables, principally: the structure of the leaf and its chlorophyll content; the accumulation within the chloroplasts of the products of photosynthesis; the influence of enzymes; and, the presence of minute amounts of mineral constituents. A number of external variables also control photosynthesis, principally: the quality and quantity of incident light on the leaves; ambient temperature; the CO_2 concentration in the surrounding atmosphere and in leaf pores; the O_2 concentration in the surrounding atmosphere and in leaf pores; and, water and nutrient availabilities [Linder and McDonald 1994]. Some of these occur as instantaneous conditions affecting photosynthesis, such as sunflecks, and others, such as water and nutrient supply, are longer-term or chronic conditions that influence photosynthesis. Furthermore, leaf photosynthetic capacity of most species is very plastic, strongly depending on resource availability [Pearcy et al 1987].

In intact leaves, photosynthesis is usually limited by one of three general processes: the capacity of Rubisco to consume RubP in CO_2 fixation; the capacity of thylakoid reactions to supply ATP and NADPH for RubP regeneration; and, the capacity of starch and sucrose synthesis to utilise triose phosphates and subsequently regenerate Pi for photophosphorylation (PiRC) [Sage 1994].

Curves of net photosynthesis or CO_2 assimilation (A) against intercellular CO_2 concentration (Ci) - A/Ci curves - can help elucidate the active mechanism limiting photosynthesis [Stitt 1991, Harley et al 1992] since well-supported equations describe how photosynthesis is regulated by parameters (such as those mentioned above) whose level of control is shown by the shape of the A/Ci curve [Farquhar et al 1980, Harley and Sharkey 1991]. Photosynthetic rates respond to elevated CO_2 in the short term according to which partial process or enzyme is in control at that time [Stitt 1991] and therefore may vary widely between individuals, and as a result of the plant's history and the prevailing environmental conditions. Photosynthetic capacity may be adjusted to match the most limiting environmental resource [Mooney and Gulmon 1982]. Moreover, photosynthetic rates are often seen to <u>adjust</u> or <u>acclimate</u> (biochemically and physiologically) to an increased supply of CO_2 [Gunderson and Wullschleger 1994], an observation that is termed photosynthetic acclimation. Photosynthetic acclimation appears to be a reversible process [Sasek, DeLucia and Strain 1985].

Photosynthetic acclimation

Photosynthetic acclimation describes the long term adjustments in the biochemistry and physiology of a plant's photosynthetic apparatus that may be seen in plants grown under enhanced atmospheric CO_2 concentrations when compared to ambient grown control plants. Upon measurement of photosynthesis at similar internal CO_2 concentrations (to exclude stomatal conductance differences), photosynthetic acclimation may be upward [Arp and Drake 1991, Bazzaz 1990, Conroy 1989] or downward [Delucia et al 1985, Tissue and Oechel 1987] in terms of changed (light-saturated) photosynthetic rates. Downward acclimation (often referred to as homeostasis or downregulation) is the decline in photosynthetic rate under elevated CO_2 after an initial enhancement or stimulation relative to ambient grown controls. Downward acclimation of photosynthesis to the rate of assimilation of ambient grown plants would be considered "complete" photosynthetic acclimation as any difference in assimilation rates between the elevated treatment and the ambient <u>control</u> has completely disappeared. Since forests are responsible for greater than 70% of terrestrial carbon fixation [Waring and Schlesinger 1985], the onset of downward photosynthetic acclimation in these species under enhanced CO_2 would represent a substantial loss of potential for sequestering the anthropogenically enhanced concentrations of atmospheric CO_2. However, in a number of tree studies [Norby and O'Neill 1989, Petterson and McDonald 1992, Gunderson et al 1993], enhanced leaf photosynthetic rates have been observed over months or years of exposure to elevated CO_2. Such sustained upward acclimation in forest trees would represent a significant sink for the anthropogenic increase in atmospheric CO_2, particularly amongst woody perennials due to their continual physiological adjustment and feedback potential, high leaf longevities and substantial potential for carbon storage [Gunderson and Wullschleger 1994].

Unfortunately, many studies have shown downward photosynthetic acclimation in woody perennials under enhanced CO_2 [Fetcher et al 1988, Grulke et al 1993, Mousseau 1993, see review by Gunderson and Wullschleger 1994]. The mechanism behind this acclimation response has been suggested to involve the reallocation of resources (especially nitrogen) away from photosynthesis in order to optimise the utilisation of all resources [Sage et al 1989]. Thus downward acclimation may be a positive plant response to maximise the efficiency of resource use and optimise growth under elevated CO_2. Reduced sink size may be influencing this acclimation behaviour through sucrose or starch accumulations [Azcon-Bieto 1983, DeLucia et al 1985, Ehret and Joliffe 1985] reflecting endproduct inhibition, altered CO_2 diffusion route to the chloroplast [Neales and Incoll 1968], and possibly Rubisco downregulation [Wong 1979]. An analysis of A/Ci curves generated from studies of trees grown at elevated CO_2 [Gunderson and Wullschleger 1994] shows $V_{c,max}$ to be often decreased in elevated-grown plants, suggesting that Rubisco content, activity or kinetics has decreased, though this may not be apparent under more rigorous experimentation. Furthermore, there is no indication of a single predominant factor controlling the acclimation response of photosynthesis in trees exposed to elevated CO_2 [Gunderson and Wullschleger 1994]. It is necessary, however, to know how photosynthesis will respond to elevated CO_2 in field-grown vegetation, since the optimal conditions of nutrition [Petterson and McDonald 1992], light, temperature and other environmental factors used in the majority of studies, do not reflect the constraints under which most plants in natural environments grow.

Downregulation of photosynthesis and sink strength

Leaf photosynthetic capacity has been observed to decrease upon experimental removal of plant carbon sinks [Gifford and Evans 1981]. Therefore, an insufficient sink capacity (or strength) for the enhanced levels of assimilate generated under elevated CO_2 may be the cause of declines in C_3 photosynthetic rates observed in some studies [Arp 1991]. The concentration and duration of elevated CO_2 exposure may be important in determining whether photosynthetic acclimation will occur [Arp 1991] since these will affect the amount of carbon supplied to the plant for distribution to available sinks. Rooting volume [Arp 1991, Thomas and Strain 1991] and developmental sinks may affect photosynthetic acclimation, though increased nutrient supply in larger pots, concomitant with increased root sink capacity, may be minimalising the effects of pot volume [McConnaughay et al 1993, Coleman et al 1993]. Root restrictions may not necessarily result in downregulation of photosynthesis, rates may increase or remain unchanged [Arp 1991]. Carbohydrate accumulations may directly inhibit photosynthetic rates or lead to a feedback of carbohydrate synthesis [Stitt

1991]. If carbohydrate levels feedback to reduce amounts of photosynthetic components, then other components such as nitrogen may be remobilised and used for sink growth [Stitt 1991]. Perennial plants with large storage organs may not become sink-limited at all [Arp and Drake 1991].

Downregulation of photosynthesis and nutrient supply

Cost-benefit analogising suggests that photosynthetic capacity may be adjusted to the most limiting environmental resource [Mooney and Gulmon 1982]. Under the assumption that leaf photosynthetic capacities are achieved such that they are the highest possible under the prevailing resource limitation(s), poor nutrient availability should lead to reduced photosynthetic capacities compared to plants at adequate supply [Pearcy et al 1987].

Nutrient use efficiency is improved under elevated CO_2 if photosynthetic rates are stimulated because Rubisco is more efficient due to lower photorespiration and reduced diffusional resistance. Plants may undergo internal physiological adaptations to improve nutrient use efficiency by reallocating nutrients to support increased growth under elevated CO_2 [O'Neill et al 1987]. Utilisation and allocation of a resource such as nitrogen may be optimised to support the same amount of carbon fixation in the elevated concentrations of CO_2 [Gunderson and Wullschleger 1994]. Thus, photosynthetic acclimation may result in optimised plant performance under elevated CO_2 rather than a physiological dysfunction in response to stress. If the plant undergoes "complete" acclimation of photosynthetic rate, however, down to the photosynthetic rates of ambient grown controls, then enhanced CO_2

may not result in changed productivity or carbon sequestering and the potential for feedback on the anthropogenic rate of accumulation of CO_2 in the atmosphere will not be realised.

Role of nitrogen and phosphorus in the limitations to photosynthesis

All the mineral nutrients essential for plant growth have been observed to affect photosynthetic rates, some are directly involved in primary photosynthetic processes whilst others facilitate growth and metabolism, indirectly impacting photosynthesis or photosynthate use [Linder and Rook 1984]. The photosynthetic capacity of a leaf is a function of the amount of biochemical machinery that is present in the leaf, and the photosynthetic performance of the leaf is the expression of that capacity within the current environmental constraints [Smith and Hinckley 1995]. Leaf photosynthetic capacity is related to leaf nitrogen content primarily because the majority of leaf nitrogen is found in Calvin cycle and thylakoid proteins, and within species there are strong linear relationships between nitrogen and Rubisco (soluble protein), and nitrogen and chlorophyll [Evans 1989].

Leaf photosynthetic capacity is generally higher in plant species in resource-rich environments compared to those growing under resource limitations [Pearcy et al 1987]. Increased photosynthetic performance should often - but not always - result from increased nutrient supply, depending on nutrient supply regime (timing, form, content), tree health, and climatic and environmental factors (stand density, moisture, temperature, etc.) [Linder and Rook 1984]. When tree growth is stimulated by mineral nutrient fertilisation, a substantial - if not major - part of the response is a result of increased total leaf area of the photosynthetic surface, photosynthetic production may also be affected by nutrient fertilisation through changes in leaf morphology and anatomy and of the tree crown [Linder and Rook 1984].

Elevated CO_2 primarily stimulates photoreduction and suppresses photorespiration, thus modified nutrient requirements are likely under elevated CO_2 [Conroy et al 1992]. At prevailing CO_2 levels, photosynthetic rate and leaf Rubisco content are positively correlated with leaf nitrogen (and phosphorus) [Evans 1989, Jacob and Lawlor 1992]; low nitrogen nutrition reduces photosynthetic rate and capacity of plants grown under elevated CO_2 more than plants grown at ambient CO_2 [Wong 1979]. The relative photosynthetic response of *Salix x dasyclados* to CO_2 was increased with increased nutrient supply [Silvola and Ahlholm 1992], however an increased photosynthetic response to CO_2 in a study of *Liriodendron tulipfera* seedlings in native soils grown with/without further nutrient additions, was only seen in two of three observations made [Norby and O'Neill 1991]. This suggests that the timing of measurements of photosynthetic response may highlight the temporal fluctuations that occur in rates of photosynthesis.

If elevated CO_2 reduces Rubisco levels (a possible mechanism for downregulation of photosynthesis), then the plant's requirement for nitrogen - and possibly phosphorus - should also decrease unless the nutrient is reallocated to other processes to optimise plant performance under elevated CO_2 [Bloom et al 1985], as discussed in the previous chapter. Leaf phosphorus requirement, however, may increase under elevated CO_2 due to the enhanced flux of carbon through the photoreductive cycle potentially raising the Pi flux into the chloroplast [Conroy et al 1990]. This may explain the enhanced uptake of phosphorus by

Eucalyptus grandis seedlings at elevated CO_2 relative to ambient controls, whilst nitrogen uptake was not stimulated [Conroy et al 1992]..

Will nutrient stresses affect plant responses to rising CO_2 levels?

What is a response to elevated CO_2? Response is any detectable differences in plant physiology and behaviour, preferably positive for the plant, from measures made on ambient controls. The major response of interest is photosynthesis especially since this is the primary plant process to be directly affected by elevated CO_2. Some species may be inherently, or strategically, more able to respond to increases in CO_2 and thus less prone to acclimation of photosynthetic response, possibly as result of higher relative growth rates or earlier successional status, or larger seed sizes [Oechel and Strain 1985, Bazzaz and Miao 1993], though timing and comparability of photosynthesis measurements in studies are issues that need to be resolved in order to verify this hypothesis [Gunderson and Wullschleger 1994]. In addition, photosynthetic response may translate to a growth response which could mean larger plants with more CO_2 demand (with potential feedbacks on the atmospheric CO_2 reservoir) and/or more carbon resource for the plant to allocate or partition, for example, to stress alleviation or limited resource acquisition activities which could facilitate even greater growth. Enhanced stress alleviation activities under elevated CO_2 would have great global response implications since the majority of plants are subjected to some type of stress governing their photosynthetic and growth responses. It is necessary to integrate nutrient stress and elevated CO_2 effects on plants to evaluate the implications of increasing levels of atmospheric CO_2 on plants, a vast amount of which are growing under nutrient stress. Of course, other stresses may be constraining plant response in conjunction to nutrient stress [see earlier discussion of simultaneously limiting factors] and it is not practicable to discuss here the interactions of CO_2, nutrient stress and other stresses. However, it is important to keep in mind that there could be concurrent stresses complicating plant responses to elevated CO_2 when attempting to evaluate the potential for field-grown, mature vegetation to exhibit, and sustain, a response to elevated CO_2.

Many studies have generated information on plant responses to elevated CO_2 that involve changed nutrient cycling [Zak et al 1993, Coleman 1993]. This is crucial to the evaluation of forest responses to rising CO_2 levels since the majority of forests grow under nutrient limitations [Pastor and Post 1988]. Seedling morphology changes have been observed under elevated CO_2 including increased branching, fine/coarse root ratios, nodule mass on nitrogen-fixing species. Litter decomposition rates, litter quality and quantity, plant nutrient uptake rates and/or internal nutrient cycling have all been found to respond to elevated *CO_2 with important implications for long-term forest responses.* Foliar C/N ratios have been found to increase under elevated CO_2 with possible increased C/N ratios resulting in the litter as a consequence, potentially decreasing litter decomposition rates and thus immobilising the incorporated nutrients for longer periods of time. Negligible changes or decreases in litter lignin/N ratios have been found previously [Norby et al 1986a, Williams 1986], which may provide a better estimate of decay rate responses to elevated CO_2 than C/N ratios.

Higher nutrient supply rates to forest species under elevated CO_2 have resulted in significant growth enhancements [Patterson and Flint 1982, Goudriaan and de Ruiter 1983, Zangerl and Bazzaz 1984, Conroy et al 1986, Cure et al 1988, Bazzaz and Miao 1993], though nutrient and water limitations in natural forest stands may prevent elevated atmospheric CO_2 levels from increasing photosynthetic rates [Kramer 1981, Eamus and Jarvis 1989], and could be controlling the extent of acclimation of photosynthetic activity to elevated CO_2 [Sage et al 1989]. Growth enhancements under elevated CO_2 may result in depleted nutrient reserves in natural conditions as there is a limit to the total amounts of nutrients available [Sinclair 1992, Linder and McDonald 1991] and this would curtail long-term growth enhancements in response to elevated CO_2.

Mineral nutrient constraints on growth enhancements of plants in response to elevated CO_2 may also occur on fertile sites [Diaz et al 1993], in non-mycorrhizal species. The effects of doubling CO_2 concentration (350 and 700 μmol mol^{-1}) were investigated on seedlings from a productive tall grass community and nutrient-poor, acidic grassland community regenerated from natural seedbanks and grown in lab microcosms for three months. Leaf symptoms of the most common species of the productive community were associated with non-structural carbohydrate accumulation, reduced leaf nitrogen content and great increases in soil microbial carbon and nitrogen. Whilst there were lesser shifts in leaf nitrogen and carbon in the acidic grassland community but still great increases in soil microbial carbon and nitrogen. It was suggested that there may have been increased substrate release to the soil as a result of elevated CO_2 levels leading to mineral nutrient sequestration via enhanced growth of free-living soil microbial populations. Hence shifts in species composition of plants and microbial organisms may occur in communities containing mycorrhizal and non-mycorrhizal species, even on fertile sites. Thus elevated CO_2 may cause plants to become nutrient-limited even on fertile sites as a result of enhanced assimilate levels being released to soil microbes.

Could elevated CO_2 alleviate nutrient stress?

According to cost-benefit analogies of plant growth [Bloom et al 1985] described in the previous chapter, plant response to enhanced levels of one resource may result in physiological adjustments leading to changed patterns in acquisition and utilisation of all resources, with the consequence of achieving enhanced growth. Thus a mechanism has been proposed whereby elevated CO_2 levels may facilitate physiological adjustments that result in the alleviation of potential nutrient stress. For this to be realised, nutrient demand may be reduced - increased nutrient use efficiency - via internal nutrient reallocations or changes in metabolic requirements, or increased nutrient uptake may occur via enhanced root and/or mycorrhizal growth, or via enhancements in soil and rhizosphere microbial activity [Norby et al 1986ab]. Is there any evidence to substantiate that this activity occurs, and particularly in forest species, hence enforcing the possibility that enhanced carbon storage may occur under elevated CO_2 in these frequently nutrient-limited species?

Studies on trees grown in natural forest soils [Norby et al 1986ab, 1992, Norby and O'Neill 1989, 1991] indicate that even plants with low nutrient supply rates have substantial growth responses under elevated CO_2 levels. Overall dry weight increases and root/shoot ratio increases were observed at elevated CO_2 levels in *Pinus virginiana*, *Quercus alba* and *Liriodendron tulipfera* seedlings grown in pots containing low-nutrient soils [Luxmoore et al 1986a] and enhanced nutrient uptake did not occur concurrently with dry weight increases in all species. In a controlled-environment study of *Pinus ponderosa* seedlings at three CO_2 treatments and three nitrogen (as $(NH_4)SO_4$) treatments, a growth response was prevented at extreme nitrogen deficiency (no fertilizer additions) but facilitated at higher suboptimal levels of fertilization [Johnson et al 1995]. The growth increases occurred without any additional nitrogen uptake, though uptake rates of other nutrients followed growth increases generally, thus increased nitrogen use efficiency under elevated CO_2 seemed likely to have occurred.

One year old white oak seedlings grown for 40 weeks in nutrient-deficient forest soil at ambient and elevated CO_2 (362 and 690 μmol mol^{-1}) [Norby et al 1986a] exhibited severe nitrogen deficiency symptoms. However, elevated CO_2 treatment seedlings showed 85% growth enhancements compared to the ambient grown controls, despite the apparent nitrogen deficiency. The greatest biomass enhancement occurred below-ground, though nitrogen uptake *per se* did not increase. Nitrogen was observed to be partitioned more to metabolically active tissue, rather than storage or abscissed tissue under elevated CO_2 as compared to the ambient treatment, indicating greater nitrogen use efficiency, and this may have facilitated the growth response to CO_2 despite nutrient deficiency.

Populus grandidentata Michx. ramets were grown in open-top chambers for one field season in nutrient poor soil [Zak et al 1993] at two CO_2 levels (342 and 692 μmol mol^{-1}). Elevated-grown plants had higher photosynthetic rates, greater total and below-ground biomass, and greater carbon in below-ground biomass compared to ambient-grown plants. Thus, elevated CO_2 was suggested to have a positive feedback on soil carbon and nitrogen dynamics resulting in increased availability of nitrogen. If this result could be seen, and sustained, on a significantly larger scale, atmospheric carbon may be substantially sequestered into terrestrial biomass despite the widespread nitrogen limitations to growth.

In another field study using a natural forest soil and yellow-poplar trees (*Liriodendron tulipfera* L.) along with white oak trees (*Quercus alba* L.) [Gunderson, Norby and Wullschleger 1993], photosynthetic responses of seedlings exposed to two enriched CO_2 levels (ambient +150 μmol mol^{-1} and ambient +300 μmol mol^{-1}) in open top chambers (OTCs) were studied for three years and compared to responses of ambient-grown (350 μmol mol^{-1}) controls. The plants were not watered or fertilised, and hence were likely subject to water and nutrient limitations. Net photosynthesis was observed to be enhanced between 12-144% in the enriched CO_2 treatments compared to ambient controls over the duration of the investigation, with greatest enhancements observed in the higher enriched CO_2 treatment. Again, there was no observed decline in photosynthetic rates over this time period, despite declines in foliar nitrogen concentrations and chlorophyll content. A lack of sink limitation with respect to roots was proposed to be causing the differences in observed photosynthetic responses in the study as compared to responses observed in similar species grown in pots [Norby et al 1992]. So, while there is evidence supporting the ideas in Mooney et al (1991), many studies of nutrient-limited trees suggest that elevated CO_2 may still enhance long-term growth in woody plants and, furthermore, carbon storage may be enhanced such that the terrestrial biosphere exerts a significant feedback on the atmospheric carbon reservoir, perhaps with the consequence of slowed anthropogenic accumulations of CO_2.

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Three: Carbon-nitrogen interactions in seedlings of loblolly pine: A case study

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Abstract

Loblolly pine (*Pinus taeda*) seedlings were grown from seed for 20 weeks in controlled environment chambers at two CO_2 concentrations, 385 µmol mol^{-1} and 585 µmol mol^{-1}, and at two levels of nitrogen, 0.5 mmol and 5.0 mmol nitrogen as NH_4NO_3, with 9 replicate seedlings per treatment. This study was conducted to determine whether nitrogen supply rate controlled the response to elevated CO_2 or if elevated CO_2 could alleviate the stress imposed by low nitrogen supply rate. Gas exchange measurements were performed on individual fascicles from a sample of plants from each treatment. Chlorophyll *a* fluorescence fast kinetics, and chlorophyll *a* and *b* concentrations were measured prior to harvesting. Needle surface areas were calculated from needle diameter measurements and by coating needles with uniform monolayers of glass microbeads. Harvested seedlings were separated into above-ground (stem and foliage) and below-ground fractions, dried and weighed. Chlorophyll content and inorganic nitrogen level have been observed to be linearly related in both hardwoods and conifers [Linder and Rook 1984]. Chlorophyll *b* content increased with increased nitrogen, chlorophyll *a* was not significantly correlated to nitrogen, though. Chlorophyll *a* declined significantly with increased CO_2 and the effect of CO_2 on chlorophyll *b* was not significant. Photosynthetic response of loblolly pine seedlings was not significantly correlated with nitrogen treatment. However, at a common CO_2 concentration in air, net photosynthesis was significantly correlated with CO_2, A_{net} was reduced in elevated grown plants by 24%. Nitrogen had the strongest effect on total biomass accumulation. Elevated CO_2 facilitated a significant biomass response in high nitrogen plants, indicating simultaneous

limitations to growth and implying that high levels of nitrogen need to be made available to plants in order to enable them to fully respond to elevated CO_2. The largest (and hence fastest-growing) plants, the high-nitrogen elevated-grown plants had the lowest photosynthetic rates by the end of the experiment. This was suggested to be a result of enhanced growth stimulations in these plants compared to the other treatments early in the experiment, enabling high-nitrogen elevated-grown plants to have downregulated photosynthesis later on in the experiment yet still have ended up as the largest plants. A feedback of enhanced growth and/or carbohydrate accumulations diluting needle nitrogen levels was proposed to be causing the apparent downregulation in rates of photosynthesis. These results indicated that growth stimulations will be expected under elevated CO_2, despite nutrient limitations, but that these enhancements may not be observed in the long-term as photosynthetic rates may become downregulated. Thus, the potential for the terrestrial biosphere to feedback on the atmospheric CO_2 reservoir may not be fully realised.

Introduction

Atmospheric CO_2 concentrations are increasing [Keeling et al 1989, 1995], and elevated CO_2 levels have been observed to modify photosynthetic rates in C_3 plants [Strain and Cure 1985]. Variable short and long-term plant responses have been seen under elevated CO_2, ranging from stimulated to decreased photosynthetic rates in relation to ambient grown controls [Drake et al 1991, Tissue and Oechel 1987, Bazzaz 1990]. Enhanced leaf photosynthetic rates have been observed in forest species at elevated CO_2 [Fetcher et al 1988, Wullschleger et al 1992, Liu and Teskey 1995, Wang et al 1995]. If such a positive photosynthetic response is observed and sustained at (and above) the whole-plant level in forest species, enhanced growth rates could result [Gunderson and Wullschleger 1994], with important implications for long-term global carbon storage [Peterson and Melillo 1985]. However there are wide ranging nutrient limitations to the growth of forest species, particularly in terms of nitrogen availability [Eamus and Jarvis 1989, Kramer and Sionit 1987] and these may hinder forest species' response to elevated CO_2 [Kramer 1981].

Long term changes in photosynthetic rates under elevated CO_2 have been proposed to be controlled by plant nutrient balances [Strain and Cure 1985] and the size of plant sinks for the increased quantities of carbon that could be potentially assimilated [Mooney et al 1991]. In addition, mechanisms may exist by which woody plants may be able to acquire more nutrients from low nutrient soils under elevated CO_2 [Luxmoore 1986ab, Pearcy and Bjorkman 1983]. Nutrient demand may be reduced - increased nutrient use efficiency - via internal nutrient reallocations or changes in metabolic requirements (for example, decreased leaf enzymes being needed to fix a specific amount of CO_2). Or increased nutrient uptake may occur, possibly from changed photosynthate allocation, via enhanced root and/or mycorrhizal growth, or via enhancements in soil and rhizosphere microbial activity [Norby et al 1986].

It is necessary, therefore, to determine whether enhanced CO_2 levels will be unable to stimulate photosynthesis in forest species in the long-term due to nutrient limitations or whether enhanced levels will alleviate nutrient stresses via internal nutrient reallocations or improved nutrient-use efficiencies, for example. Sink limitations also need to be considered to determine if this is where potential growth constraints of plant responses to elevated CO_2 may occur [Mooney et al 1991]. Many studies have shown downward photosynthetic acclimation in woody perennials under enhanced CO_2 [Grulke et al 1993, Mousseau 1993, see review by Gunderson and Wullschleger 1994]. The mechanism behind the acclimation response has been suggested to involve the reallocation of resources (especially nitrogen) away from photosynthesis in order to optimise the utilisation of all resources [Sage et al 1989].

Studies of long term photosynthetic acclimations or adaptations have compared elevated and ambient CO_2 grown plants in a number of different ways: by comparing leaf photosynthesis at the respective growth concentrations, indicating changes under increasing CO_2 in terms of the plant's capacity to exploit the potential for enhanced assimilation; by comparing plants at the same CO_2 concentration, indicating acclimation response if photosynthetic rates differ significantly; and, by constructing and comparing A/Ci curves (change in net photosynthetic rate with changing intercellular CO_2 concentration) to elucidate the mechanism for observed responses. Well-supported equations describe how photosynthesis is regulated by photosynthetic parameters whose level of control is indicated by the shape of the A/Ci curve [Farquhar et al 1980, Harley and Sharkey 1991].

Photosynthetic rates were relatively unaffected in a study of two-month old loblolly pine (*Pinus taeda*) seedlings under elevated CO_2 [Tolley and Strain 1985], but were noticeably higher in 500 μmol mol^{-1} grown plants compared to ambient (350 μmol mol^{-1}) grown controls in a fourteen month long study of loblolly pine seedlings [Fetcher et al 1988]. Under elevated CO_2, nitrogen may be allocated towards growth-limiting processes and away from Rubisco. This could explain the decreases in total Rubisco activity and content, increases in Rubisco activation state and proportionate increases in nitrogen found in the light reaction components rather than Rubisco observed at elevated CO_2 in seedlings of loblolly pine from a two year open top chamber study [Tissue, Thomas and Strain 1993]. In addition, nitrogen was observed to significantly alter biomass accumulation, height and leaf area of aspen (*Populus tremuloides* Michx.) and white spruce (*Picea glauca* (Moench) Voss) seedlings grown for 100 days from germination in controlled environment rooms maintained at ambient (350 μmol mol^{-1}) and elevated (750 μmol mol^{-1}) CO_2, and at three levels of nitrogen (15.5 mM, 1.55 mM and 0.155 mM) [Brown and Higginbotham 1986]. Significant effects of nitrogen have been observed at elevated CO_2 and this provides a basis for evaluating CO_2-nitrogen interactions in terms of photosynthesis and biomass in seedlings of loblolly pine.

Coniferous species frequently grow under conditions of water and/or nutrient stress [Smith and Hinckley 1995]. Loblolly pine, in particular, is an important commercial forest species in the south-eastern United States, dominant on about 12 million hectares (30 million acres) [Baker and Langdon 1990] and is widely planted, as with pines in general. The effects of rising atmospheric CO_2 levels on this coniferous species are thus of primary importance from both a commercial and global carbon cycling standpoint.

This study was conducted to examine the photosynthetic response of loblolly pine seedlings to potential nitrogen stress at two growth CO_2 levels in terms of observed gas exchange, biomass accumulation, Chl *a,b* concentrations and Chl *a* fluorescence measurements. Gas exchange values were measured to elucidate short-term carbon gains and relative assimilation rates amongst the treatments. Chl *a* fluorescence kinetics were determined to explore potential growth limitations and efficiencies in terms of photosynthetic activity of PSII and Chl *a,b* concentrations were used to evaluate the mechanism for observed responses. Seedling dry weights (below-ground, above-ground - foliage and stems) were determined to follow long-term growth patterns in terms of carbon allocation and relative growth rates amongst the treatments, necessary for determining whether any observed increases in above-ground productivity arose as a result of photosynthetic gain or just from a redistribution of matter from the roots to the above-ground system.

Materials & Methods

Plant culture

Loblolly pine seedlings were grown from seed (Source: Arkansas, USA) in two controlled environment chambers (Conviron E15 plant growth chambers (Conviron, Winnipeg, Canada) (Appendix 2), dimensions 0.8 m by 2 m by 1.3 m (dlh), in a 2 x 2 factorial study, at ambient or elevated growth CO_2 concentrations (389 or 585 µmol mol^{-1} CO_2) and two levels of nitrogen supply (0.5 or 5.0 mM nitrogen as NH_4NO_3). The seeds were germinated from sand-filled trays supplied only with water and then transplanted into 0.15 m diameter by 0.15 m deep pots to either an ambient or elevated CO_2 chamber, prior to shedding their seedcoats. Seedlings were transplanted to each 2 x 2 CO_2 x N treatment as they germinated, resulting in nine seedlings per treatment (36 pots total), and any four seedlings germinating at one time were distributed evenly amongst the treatments, allowing four-wise comparisons to be made amongst treatments. The growth medium contained 3:1:1 parts by volume sand, vermiculite and field soil from a commercial loblolly pine plantation in North Carolina, USA (Enon silt loam), respectively and each pot contained 3.2 kg d.w. (2120 cm^3) of growth medium. Field soil served as mycorrhizal inoculum and presence of mycorrhizae was confirmed at the end of the study by visual inspection.

CO_2 levels were continuously monitored and maintained at 585 µmol mol^{-1} (average: 585.2 µmol mol^{-1}, sd: 20.6) in the elevated CO_2 chamber by means of a PP Systems EGM-1 and CIM-1 CO_2 monitoring system (PP Systems, Hitchin, Herts., England). Ambient chamber

CO_2 supply was monitored periodically and found to be 385 µmol mol^{-1} nominal. Both chambers were set for four cycles per 24-hr period:

Time /24-hr	Temp /°C	%RH	Air vapour pressure deficit /Pa	lamps	PPFD at plant height µmol/m^2/s
7:00	20.0	60	9.4	11	546
9:00	28.0	70	15.2	32	~1400
19:00	20.0	70	9.4	11	546
21:00	18.0	60	8.3	00	0

The nutrient treatments were based on a modified half-strength Hoagland's solution with values adjusted to correspond to those used in similar pine studies (Cumming 1993, Cumming and Brown 1994, Thomas 1994). A 1:10 ratio of nitrogen as ammonium nitrate was used for the two nitrogen treatments. Nutrients were supplied as 5.0 or 0.5 mM N, 1.0 mM P, 3.0 mM K, 2.00 mM Ca, 1.5 mM Mg, 3.1 mM S, 0.15 mM Fe and micronutrients. Pots were initially supplied 40 ml nutrient solution daily, then fertilisation rate was reduced to 40 ml every two days after 4 weeks. Pots were watered to saturation with deionised water every day that nutrients were not supplied, and watered regularly four times during the photoperiod on all days. In addition, iron and magnesium were supplemented as 20 ml each 0.75 mmol FeEDTA and 0.5 mmol $MgSO_4.7H_2O$ daily to compensate for potential deficiencies arising from the growth medium.

All measurements were made at 20 weeks unless otherwise stated.

Gas exchange

Gas exchange measurements were made on intact, individual fascicles (3 needles per fascicle) in the morning or early afternoon (prior to possible stomatal closure) using an open-differential type portable gas exchange system (PP Systems CIRAS 1, Hitchin, Herts., England) (Appendix 2) and a Parkinson-type cuvette. Self-shading was avoided by laying needles flat and holding any protruding foliage away from the cuvette. Adequate water supply was maintained during the measurement duration. Saturating light was supplied at >1200 μmol/m^2/s to the cuvette via an external light source (50 W halogen lamp). Chamber air temperature was kept at ambient 28°C and VPD was maintained at <1.4 Pa kPa^{-1}. Ambient CO_2 to the cuvette was varied between 80 and 1500 μmol mol^{-1} to determine the full A/Ci curve, from CO_2 compensation point to above-saturation. A selection of plants from each treatment was sampled (n=5 per treatment) and net photosynthesis and leaf conductance rates were calculated. Following measurements, needles were sectioned to the segment length in the leaf cuvette and surface areas of the segments were determined using needle diameter measurements and from coating and weighing needles coated with a uniform monolayer of 0.1 mm diameter glass microbeads, yielding surface area-based photosynthetic rates for area-based comparisons amongst treatments.

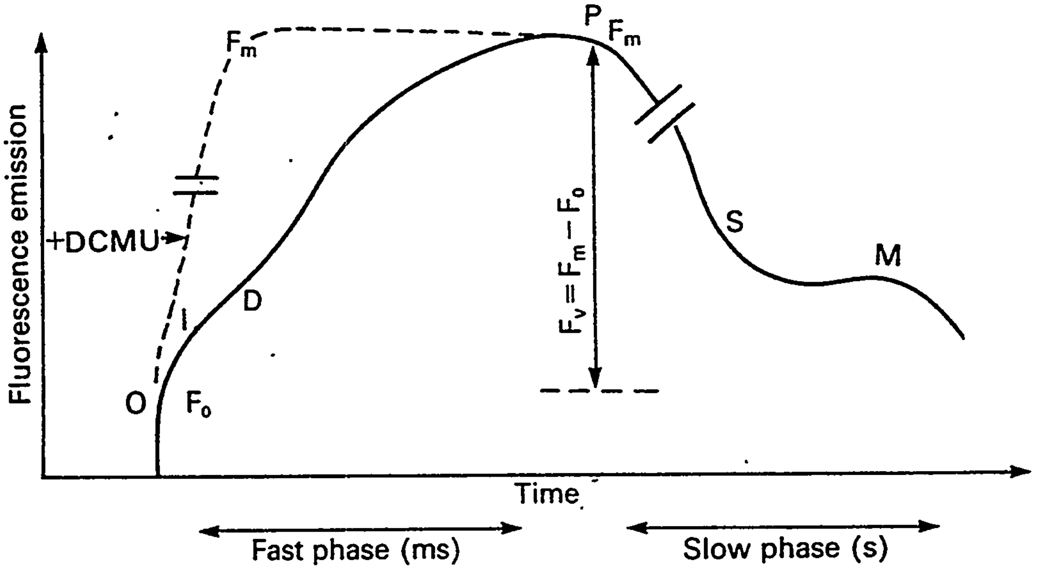
Chl *a* fluorescence

Fast phase chlorophyll *a* fluorescence kinetics were measured using a Hansatech Plant Efficiency Analyzer (PEA) system (Hansatech Instruments Ltd, King's Lynn, Norfolk, England) (Appendix 2) on primary needles from all plants in the study. Measurements were made on intact needles, at 20 weeks plant age, and were performed in the afternoon, after the plant had received plenty of irradiation. Plants were dark adapted for a minimum of 30 minutes in shuttered leaf clips. Measurements were made at ambient temperature (28°C) at PPFD of 1230 μmol/m^2/s. The fluorescence system was of non-modulated type, therefore only fast kinetics were determined: initial fluorescence (F_0), maximal fluorescence (F_m), variable fluorescence or photochemical fluorescence quenching F_v, time to maximal fluorescence (T_m), and area-above-curve between F_0 and F_m. The photochemical efficiency of open reaction centres or photochemical yield of PSII (F_v/F_m) and F_v/F_0 parameters were derived from the measurements. PEA software was assessed for T_m and area-above-curve calculation robustness.

After dark adaption of plant photosynthetic tissue, illumination produces a characteristic chlorophyll *a* fluorescence emission time-course known as the Kautsky curve **(Figure 3)** [Bolhar-Nordenkampf and Oquist 1993, Hall and Rao 1994]. Over a matter of seconds after illumination, photosynthesis reaction centre traps change from open (F_0) to closed (F_P) - observed as minimal to peak fluorescence - through intermediate peaks and troughs, until a terminal state of fluorescence emission is reached..

Fluorescence reaches F_0 in milliseconds, where photochemistry is quenched to a minimum. The value of F_0 is affected by any environmental stress that causes structural alterations at pigment level of PSII, it represents emission by excited Chl *a* molecules of the PSII antennae structure. Q_A is fully oxidised at F_0. (Whole of illuminated leaf clip area needs to be filled in order to compare F_0 values between specimens.) Fluorescence then rises in about three-tenths

Figure 3: Characteristic fluorescence induction kinetics, the Kautsky curve
Source: Hall and Rao 1994



of a second via an intermediate level F_I to a peak level F_P at which photochemistry is no longer quenched. From F_P, fluorescence gradually decays down through lesser peaks and troughs to a terminal level T, taking a few minutes to reach this value. Peak fluorescence F_P equals maximal fluorescence F_m when light is saturating. At F_m, Q_A is fully reduced and thus requires saturating light intensity. (Again, whole of illuminated clip area needs to be filled in order to compare F_m values between specimens.)

The difference between F_0 and F_m is termed the variable fluorescence component F_v and is usually lowered by stresses which cause thylakoid damage. The ratio F_v/F_m has been shown to be proportional to the quantum yield of photochemistry and correlates well with the quantum yield of net photosynthesis of intact foliage. Thus fluorescence can be utilised to compare the photosynthetic capacities of plants grown under different conditions via the efficiency of the open PSII reaction centres for electron transport [Jones 1992]. F_v/F_m is independent of leaf area. T_m is the time at which the maximal fluorescence occurs. Area-above-curve between F_0 and F_m is proportional to the pool size of the electron acceptors Q_A on the reducing side of PSII, and is reduced when electron transfer from reaction centres to the quinone pool is inhibited.

Due to the shape and size of the loblolly pine needles, the entire exposed area of the leaf clip was not able to be filled and thus a non-uniform area was sampled for chlorophyll *a* fluorescence, though attempts were made to use needles of similar thickness to aid comparison of fluorescence measurements amongst plants.

Needle chlorophyll and carotenoids

Pigment was extracted from 100 mg of sectioned tissue of primary needle samples from each plant using DMSO (dimethyl sulfoxide) to solubilize the pigments [Hiscox and Israelstam 1979, Barnes et al 1992, Porra et al 1989], and chlorophyll and carotenoid ratios and concentrations were determined spectrophotometrically (HP 8451A Diode Array UV-Vis Spectrophotometer) (Appendix 2). Optical densities at 648 and 666 nm (Chl *a* and *b*), and 470 nm (carotenoids) were measured for the calculations (calculations detailed in Appendix 3).

Seedling dry matter production

Plants were harvested at 20 weeks and sectioned into above-ground (stems and foliage) and below-ground biomass, and dried in a forced draught oven at 55°C. Roots were observed for possible restrictions in the pot and for presence of mycorrhizae. Only total seedling biomass data are detailed here.

Needle surface areas

Needle surface areas were determined using 5 "diameter" measurements per needle to assess the extent of taper and also by regression analysis of data from the glass bead method [Thompson and Leyton 1971]: glass microbeads (Class V Precision Grade microbeads, Cataphote Inc., Jackson, Mississippi) were used to coat whole needles and sections used for gas exchange measurements with a bead monolayer whose weight was subsequently determined and used to calculate the surface area of the needle (calculations detailed in Appendix 3). (Beads: US sieve size -120+140 or 125-105 μm, single-bead density: 2.42-2.50 g/cm^3 and diameter: 115 μm.)

Statistical methods

The significance of the treatments and the two-way interactions between them were evaluated using Microsoft Excel spreadsheet V6.0, by means of two-factor analysis of variance (ANOVA) and if a treatment effect was observed, a t-test was employed. Separate evaluations were performed for comparisons between ambient and elevated CO_2 treatments at each nitrogen level. Statistical significance α=0.05 in all cases. Treatment differences were evaluated for significance using two-sample t-tests assuming unequal variance and ratios were arcsin transformed prior to evaluation to meet the assumption of normality. [Reference: Practical Statistics for the Physical Sciences. Havlicek and Crain 1988, ACS Professional Reference Book.]

Results

Leaf chloroses became apparent after about 8 weeks of seedling growth and were most likely caused by the onset of a nutrient deficiency. However, on the most part, these visual symptoms had disappeared by 20 weeks, when the seedlings were harvested.

Gas exchange : steady-state leaf photosynthesis

Net, light-saturated photosynthesis means ranged from 3.45 + 0.30 to 7.79 + 0.68 µmol CO_2 m^{-2} s^{-1} (mean + s.e.) (**Figure** 4) (results detailed in Appendix 1). Photosynthesis was reduced with increased growth CO_2 concentration and with increased nitrogen treatment, but enhanced with increased Ca.

For low nitrogen-grown plants, when photosynthesis was measured at ambient CO_2, the CO_2 treatment means for A_{net} were significantly different at P=0.06. When photosynthesis was measured at elevated CO_2, the CO_2 treatment means were significantly different at P=0.04. The average enhancement of photosynthesis of low nitrogen-grown plants by +200 µmol mol^{-1} CO_2 was 1.43, that is, 43% higher at Ca=580 than at Ca=380 µmol mol^{-1} , and photosynthesis was significantly lower in elevated grown plants compared to ambient-grown controls.

For high nitrogen-grown plants, when photosynthesis was measured at ambient CO_2, the CO_2 treatment means for A_{net} were NOT significantly different. Similarly, when photosynthesis

Figure 4a: Needle net photosynthesis among treatments, measured at a common Ca

Measured at Ca=380 umol mol^{-1}.

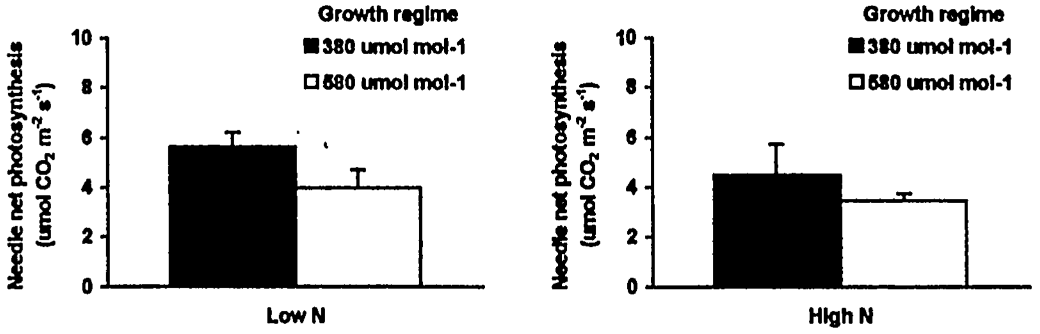
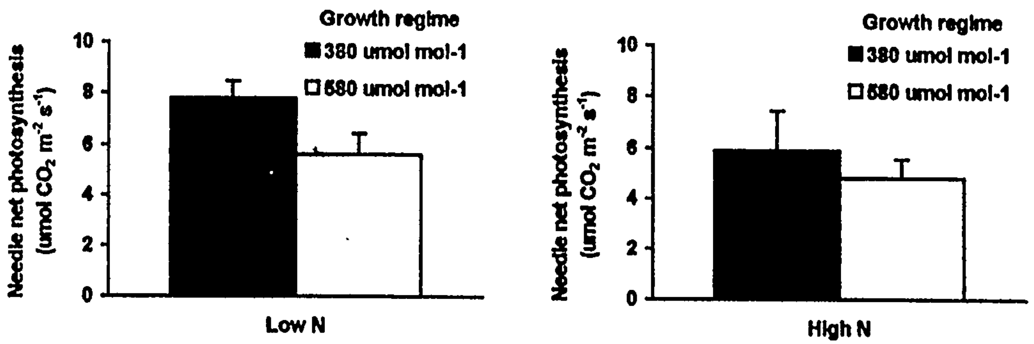


Figure 4b: Needle net photosynthesis among treatments, measured at a common Ca

Measured at Ca=580 umol mol^{-1}.



was measured at elevated CO_2, the CO_2 treatment means for A_{net} were again NOT significantly different. Again net photosynthetic rates were lower in elevated-grown plants compared to ambient-grown controls but not significantly.

For gas exchange at Ca=380 μmol mol^{-1}, there was NO nitrogen effect (nitrogen treatment means were not significantly different). There is a trend toward lower A_{net} in plants grown under high nitrogen, relative decline in photosynthesis (high nitrogen/low nitrogen) was 0.83, but variability between seedlings was also high, hence no statistical difference.

Chl *a* fluorescence

There was a significant CO_2 effect on F_0 (P=0.002), T_m (P=0.031), F_v/F_m (P=0.002) and F_v/F_0 (P=0.001) but no significant nitrogen effects or CO_2-nitrogen interactions. CO_2 was significant on the quinone pool size (area-above-curve) (P=0.015) and there was a slight CO_2-nitrogen interaction (P=0.041). F_v and F_m did not show any significant CO_2, nitrogen or CO_2-nitrogen effects across the treatments. There was a significant CO_2 effect on F_0 at high nitrogen ($P<0.01$) but not at low nitrogen (P=0.05). There was a significant CO_2 effect on T_m at low nitrogen only (P=0.01). CO_2 effect on F_v/F_m was more significant at low nitrogen ($P<0.01$) than at high nitrogen (P=0.05). The area-above-curve parameter only showed a significant CO_2 effect at low nitrogen ($P<0.01$). In summary, there were no significant effects of nitrogen on chlorophyll fluorescence, though F_0, T_m, F_v/F_m, area-above-curve and F_v/F_0 were all significantly different between ambient and elevated CO_2 treatments at low nitrogen only, except for F_0, where CO_2 treatment means were significantly different at high nitrogen only. F_0 was increased in elevated-grown plants, compared to ambient-grown controls, while

T_m, F_v/F_m, area-above-curve and F_v/F_0 were all decreased in elevated-grown plants compared to ambient-grown controls. The data showed high variability in direction and magnitude of change in response to increased CO_2 and to increased nitrogen.

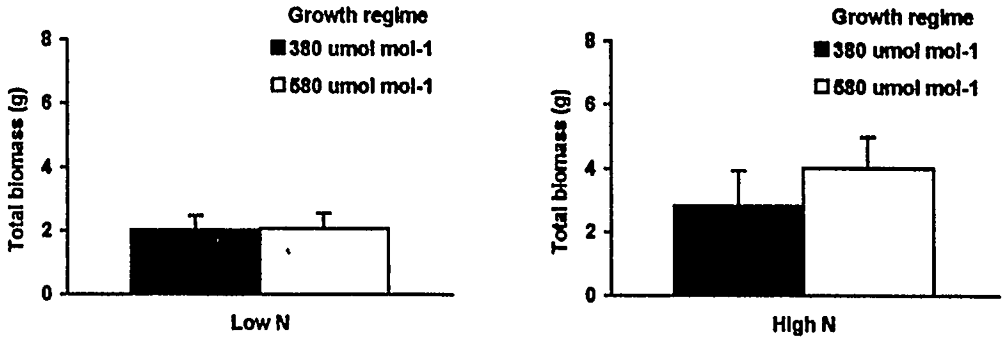
Needle chlorophyll content

Chlorophyll content was observed to decrease with increased CO_2 and with decreased nitrogen, though the treatment means were not significantly different for all chlorophyll parameters. There was a significant nitrogen effect on Chl *b* $(P=0.035)$ and total Chl $(P=0.045)$, though no significant CO_2 effect or CO_2-nitrogen interaction. There was a significant CO_2 effect on Chl *a* $(P=0.034)$ at the low nitrogen treatment only. Chlorophyll content was highest in high nitrogen, ambient-grown plants and lowest in low nitrogen, elevated-grown plants. Both Chl *a* and Chl *b* content increased with increased nitrogen, though only Chl *b* increased significantly. Chl *b*, however, showed negligible response to increased growth CO_2 concentration whilst Chl *a* content decreased, though only significantly in low nitrogen-grown plants.

Seedling dry matter production

There was a significant nitrogen effect on seedling total biomass $(P<0.001)$, a significant effect of CO_2 $(P=0.013)$, and the two-way interaction between nitrogen and CO_2 treatments was also significant $(P=0.024)$ (**Figure 5**). CO_2 was significant at high nitrogen $(P<0.01)$,

Figure 5: Seedling dry matter production



biomass was increased by elevated CO_2, but not at low nitrogen ($P>0.05$). Nitrogen was significant at ambient CO_2 ($P=0.024$), biomass was increased by high nitrogen, and highly significant at elevated CO_2 ($P<0.01$), biomass was nearly doubled in the high nitrogen treatment compared to the low nitrogen treatment.

Discussion

Nutrient addition rate

Constraints exist on the interpretation of results from plant studies where nutrition level is a factor under investigation or control, when a non-constant level of nutrient limitation is maintained [Ingestad and Agren 1992]. When there is a constant nutrient supply rate relative to the rate of uptake by the plant (irrespective of absolute nutrient concentration), growth rate will adjust to the supply rate, with possible differences in growth rates amongst organs observed during the interim period of adjustment. Growth rate and internal nutrient status will stabilise at levels corresponding to optimal, supraoptimal or limiting supply [Ingestad and Lund 1979]. An insufficient nutrient addition rate to maintain a constant limitation (or stress condition), has a feedback of reducing relative growth rate with consequently decreased uptake and depletion rates indicative of a non-true steady state [Ingestad 1982]. In this case, the true growth-limiting factor is an insufficient nutrient addition rate and should be interpreted as, for example, modifying the growth capacity and quantitative nutrient requirements of the plant under question [Ingestad and Agren 1992].

Nutrients were supplied at a constant level of addition throughout the study, regardless of rate of uptake, and thus internal nutrient concentrations may not have been maintained steady (since supply was not necessarily meeting demand) and plant growth rates may consequentially have declined [Linder and Rook 1984]. This needs to be considered as an important factor influencing interpretations from the data. However, maintaining internal nutrient status is not practicable in most cases, and thus, studies involving constant addition rates are common and need to be interpreted in terms of the nonconstant nutrient status of the plant.

Photosynthesis

Leaf photosynthetic capacity is related to leaf nitrogen content primarily because the majority of leaf nitrogen is found in Calvin cycle and thylakoid proteins, and within species there are strong linear relationships between nitrogen and Rubisco, and nitrogen and chlorophyll [Evans 1989]. However, there was no signifcant nitrogen effect observed on photosynthesis in this experiment, perhaps this is a reflection of non-steady-state nutrient levels in the nitrogen treatments.

Comparing net photosynthesis at growth concentrations **(Table 3)**, the relative photosynthetic enhancement (elevated/ambient) was approximately 1.0 at either nitrogen treatment, indicating complete acclimation of photosynthetic rates of elevated-grown plants to the level of ambient-grown plants. This was substantially lower than the average increase in net photosynthesis of 44% (1.44) observed in elevated treatments in a broad review by Gunderson and Wullschleger (1994) of 39 tree species exposed to elevated and ambient CO_2

levels. However, complete acclimation was observed in some studies, for example, *Pinus ponderosa* [Grulke et al 1993] and *Pinus taeda* [Tolley and Strain 1985].

Table 3: Needle photosynthetic rates, measured at (a) growth and (b) ambient Ca. Case study results compared to studies reviewed by Gunderson and Wullschleger (1994)

Species	Growth [CO_2] µmol mol^{-1}	Rel. photosynthetic enhancement (elevated/ambient)	Pot size cm^3	Photosynthesis at amb [CO_2] (elevated/ ambient)	Refs.
(a)					
P taeda	385,585	1.04			Fig 4, App 1
P taeda	350,675	0.94			Tolley ea 1985
P ponderosa	350,700	1.18			Grulke ea 1993
(b)					
P taeda	385,585		2120	0.74	Fig 4, App 1
P nigra	350,800		6000	0.80	Kaushal ea 1989
P ponderosa	350,700		314	0.67	Grulke ea 1993
P radiata	340,640		4000	0.77	Hollinger 1987
P taeda	350,500		3600	0.78	Fetcher ea 1988

Comparing net photosynthesis of plants at a common Ca (ambient) (**Table 3**), however, showed a 26% reduction (0.74) in photosynthesis of elevated-grown plants compared to ambient-grown controls. This compares well with the 24% average downregulation in photosynthetic potential of elevated-grown plants compared to ambient-grown plants (at an ambient Ca) observed in the studies reviewed by Gunderson and Wullschleger (1994). In this review, *Pinus nigra*, *P radiata*, *P taeda* and *P ponderosa* (**Table 3**) all exhibited relative enhancements of photosynthesis (elevated/ambient), at ambient Ca, of between 0.80 and 0.67 over a comparable range of pots sizes and growth CO_2 concentrations to those used in this case study. The reduction in net photosynthesis in elevated grown plants may be a result of facilitated assimilation increasing C/N ratios in the leaf and thus reducing nitrogen per unit leaf surface area, leading to less Rubisco and chlorophyll per unit surface area, with the consequence of reduced surface area-based photosynthetic rates under elevated CO_2. In addition, increases in fine root length, which can be substantially enhanced under elevated CO_2 [Berntson et al 1993], may have become restricted in the pots, potentially leading to downregulation of photosynthesis in the plants with the greatest increases in growth of fine roots. However, evidence to support this hypothesis, from detailed harvest observations, is not available.

Chlorophyll *a* fluorescence

There were no significant effects of nitrogen on chlorophyll fluorescence, though F_0, T_m, F_v/F_m, area-above-curve and F_v/F_0 were all significantly different between ambient and elevated treatments. Thus nitrogen was not observed to affect photosynthetic apparatus at this level.

Needle chlorophyll content

Chlorophyll and accessory pigments are necessary for radiant energy capture in photosynthesis. Chlorophyll is usually considered non-limiting unless the plant is under severe nutrient stress, chlorophyll deficiencies are a common symptom of unbalanced or inadequate supply of nutrients, though drought and temperature extremes may also lead to decline in chlorophyll content [Linder and Rook 1984]. Chlorophyll content and inorganic nitrogen level have been observed to be linearly related in both hardwoods and conifers

[Linder and Rook 1984]. Chlorophyll content has been observed to decrease with decreasing nitrogen content in yellow-poplar trees in treatments with progressively increased CO_2 levels [Norby et al 1992]. Chlorophyll content of loblolly pine seedlings grown for two years at two CO_2 levels and two nitrogen levels (phosphorus was also investigated) was reduced under elevated CO_2 though only significantly in the case of high nitrogen-grown plants [Tissue et al 1993]. In addition, chlorophyll content was found to be significantly higher in high nitrogen-grown plants when compared to low nitrogen plants at each CO_2 treatment. Furthermore, nitrogen content of needles was well correlated with chlorophyll content [Tissue et al 1993]. In contrast, *Pinus radiata* seedlings grown for 8 weeks at two CO_2 treatments [Conroy et al 1986], showed no significant differences in Chl *a*/*b* or surface-area based chlorophyll content between CO_2 treatments, though the extremely low conductances exhibited by these seedlings suggest they may not be comparable.

Total chlorophyll and Chl *b* content were found to be significantly different between nitrogen treatments but no significant CO_2 effects or two-way interactions were found between nitrogen and CO_2. There was a significant decrease in Chl *a* with elevated CO_2, though this was only significant at low nitrogen, and similarly to the two year loblolly pine study, Chl *a* content was higher in high nitrogen plants compared to low nitrogen-grown plants. These results were not all statistically significant but do suggest that potentially greater nitrogen availability may be expressed in the chlorophyll, particularly Chl *b*, content of the plant (or nitrogen stress reduces investment in chlorophyll) and that Chl *a* content may decline with elevated CO_2, perhaps corresponding with changed C/N ratios. In addition, this suggests that the decline in needle chlorophyll content with increased CO_2 is mainly attributable to a reduction in the content of Chl *a*. Moreover, the results observed may reflect the non-steady-state nutrient levels used in the study as well as potential degradation of the chlorophyll extracts with handling and storage [Ingestad 1982, Barnes et al 1992].

Biomass

Nutrient stress did prevent a total plant biomass response to elevated CO_2 **(Figure 5)**. However, biomass was increased by 40% with increased nitrogen at the ambient CO_2 treatment and by 94% at the elevated CO_2 treatment. There was a 42% increase in biomass with elevated CO_2 at high nitrogen. Thus both nitrogen and CO_2 facilitated a very significant biomass response in the loblolly pine seedlings, with a further enhancement when both factors were increased, indicating simultaneous limitations to growth. This suggests that high levels of nitrogen need to be made available to plants in order to enable them to respond fully to elevated CO_2 and that nitrogen stress may be a substantial constraint to any growth enhancement under elevated CO_2 at all.

Taken together, results from this study indicate that the photosynthetic response of *Pinus taeda* seedlings to elevated CO_2 is not significantly correlated to nitrogen treatment, although studies indicate that there is a correlation of leaf photosynthetic capacity with leaf nitrogen content and thus with nitrogen availability [Tissue et al 1993]. Biomass however was substantially enhanced under high nitrogen and at elevated CO_2. Thus nitrogen had the strongest effect on total biomass accumulation but no significant effect on photosynthesis, regardless of CO_2 treatment. The largest (and hence fastest-growing) plants, the high nitrogen, elevated CO_2-grown plants, had the lowest photosynthetic rates by the time they were harvested. The evidence indicates that these plants have downregulated photosynthesis during the course of the experiment, though we would need to check the intermediate harvest data to confirm this. This would confirm the initial hypothesis that plants with the most resources compensate more under elevated CO_2 [Chapin et al 1987]. However, this is most probably due to an initial stimulation of growth early in the experiment enabling the high nitrogen, elevated CO_2 plants to have had a higher initial biomass investment such that they could have downregulated photosynthesis later on in the experiment and still ended up as the largest plants in the experiment. The downregulation of photosynthesis may be the result of a feedback response where enhanced growth and/or carbohydrate accumulations under elevated CO_2 eventually result in dilution of needle nitrogen (and Rubisco) and hence reduced photosynthetic rate at any given Ca. Active gene regulation may also have been causing the apparent downregulation in photosynthesis under elevated CO_2. These results suggest that the potential for the terrestrial biosphere to feedback on the atmospheric CO_2 reservoir will not be fully realised since growth stimulations may occur under elevated CO_2 but will be limited by nutrient constraints and the onset of downregulation in photosynthetic rates in the long-term. It remains to be seen whether mature trees of loblolly pine will respond similarly, and several such experiments are under way in the US (Hendrey et al, Duke FACE experiment; and Teskey 1995 and Dougherty et al 1995, branch bag experiment).

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Overview

The results of the case study indicate that growth stimulations occur under elevated CO_2 and as a result of increased nitrogen. Since nutrient limitations to the growth of forest species are widespread, particularly nitrogen limitation (as discussed in the first chapter), growth stimulations are expected under elevated CO_2 but will not be fully realised due to nutrient constraints on further growth stimulations and the likely onset of downregulation in rates of photosynthesis. However, small stimulations in relative growth in trees at the early stage can lead to large differences in the size of individuals at the end of first year growth, or more years, due to the larger initial investment. Such initial growth stimulations under elevated CO_2 are proposed to differ between species, perhaps according to relative growth rates, successional status or seed size [Oechel and Strain 1985, Bazzaz and Miao 1993] and may consequentially control the structure of forest communities under elevated CO_2.

It is hypothesised that changes in nitrogen availability will occur as a result of climate change, with consequences of altered vegetation distributions and modified growth responses of forest species [Kramer 1981, Eamus and Jarvis 1989]. While there is still debate over the magnitude and mechanism of this possible change, evidence indicates that plant nutrient demand and nutrient-use efficiency will change under elevated CO_2. Hence even if nutrient availability remains similar in the future, nutrients may potentially have a substantial impact on the response of forests to elevated CO_2 [Pastor and Post 1988]. However, internal and external nutrient cycling processes need to be quantified and related to stand carbon and water dynamics [Mooney et al 1991].

Many CO_2-enrichment studies have been conducted on small trees and seedlings [Eamus and Jarvis 1989, Kramer and Sionit 1987]: the relevance of data from seedlings to the response of mature trees or forests is not known. Differences in nutrition and physiology between juvenile and adult plants need to be considered when attempting to extrapolate conclusions from seedling data. In addition, there are field microclimate-induced variations in nutrient availability and greater nutrient mineralization and growth responses have been observed in controlled environments than in the field; controlled environment study inferences may not even be comparable to forest conditions [Musselman and Fox 1991]. Another, much-debated aspect of study design is that of container size and the relevance to growth limitations in terms of sink capacity [McConnaughay et al 1993, Arp 1991]. Small plants take longer to exploit soil fully and thus developmentally younger plants - those grown at ambient CO_2 compared to elevated-grown plants - would not run into potential root sink capacity constraints as early as developmentally maturer plants. Thus many factors may be influencing observations of plant growth responses to elevated CO_2. A required area for future research would therefore entail field studies in natural soils conducted on trees growing in competition and without potential pot restrictions.

Long-term forest dynamics may completely confound the short term physiological responses observed in experiments, thus more long-term investigations are needed to predict potential changes in carbon storage and biosphere-atmosphere feedbacks under increasing CO_2. Hence, the results of long-term experiments on mature trees (for example: Hendrey et al, Duke FACE experiment; and Teskey 1995 and Dougherty et al 1995, branch bag experiment) should provide valuable information on the direction and magnitude of responses expected under elevated CO_2 and should provide a solid database to support and drive climate change policies in the future.

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Appendices

Experimental details from the case study, **Chapter 3**.

Appendix 1: Results

- Gas exchange: steady-state needle photosynthesis
- Chlorophyll *a* fluorescence
- Needle chlorophyll content
- Seedling dry matter production

Appendix 2: Equipment/Methodology

- Conviron E15 plant growth chambers
- Chlorophyll content: HP8451A Diode Array Spectrophotometer
- Chlorophyll *a* fluorescence: Hansatech Plant Efficiency Analyser
- Photosynthesis: CIRAS-1 gas exchange system

Appendix 3: Calculations

- Needle surface area determinations
  - Chord technique
  - Glass bead technique
- Needle chlorophyll content

Appendix 1: Results

Gas exchange : steady-state needle photosynthesis

Photosynthesis units µmol CO_2 m^{-2} s^{-1} (mean + s.e.)
Measurement conditions PFD >= 1400 µmol m^{-2} s^{-1}; T_{air} = 28.3 °C

Treatment	A_{net}	**Ci**
Low nitrogen plants		
Photosynthesis at ambient CO_2:		
(Ca = 380 µmol mol^{-1})		
Ambient-grown	5.61 + 0.60	302
Elevated-grown	3.99 + 0.70	298
Photosynthesis at elevated CO_2:		
(Ca = 580 µmol mol^{-1})		
Ambient-grown	7.79 + 0.68	463
Elevated-grown	5.64 + 0.82	434
High nitrogen plants		
Photosynthesis at ambient CO_2		
(Ca = 380 µmol mol^{-1})		
Ambient-grown	4.49 + 1.25	307
Elevated-grown	3.45 + 0.30	313
Photosynthesis at elevated CO_2:		
(Ca = 580 µmol mol^{-1})		
Ambient-grown	5.89 + 1.55	460
Elevated-grown	4.83 + 0.73	475

Chlorophyll *a* fluorescence

(mean + s.d.)

Treatment	F_0	F_m	F_v	T_m	F_v/F_m	Area-ab -curve	F_v/F_0
Low nitrogen plants							
Ambient-grown	118.2	371.4	253.2	162.4	0.67	4030	2.232
	+ 24.68	+ 80.4	+ 80.1	+ 38.51	+ 0.09	+ 1829	+ 0.84
Elevated-grown	138.2	305.6	167.4	126.2	0.532	2147	1.266
	+ 26.81	+ 68.22	+ 64.32	+ 21.19	+ 0.116	+ 749	+ 0.586
High nitrogen plants							
Ambient-grown	101.6	314.6	213	136.7	0.669	2965	2.158
	+ 30.22	+ 92.30	+ 73.48	+ 19.52	+ 0.09	+1107	+ 0.577
Elevated-grown	139.2	352.1	212.9	131.44	0.586	2792	1.6
	+ 29.48	+ 88.96	+ 84.84	+ 35.29	+ 0.114	+ 1250	+ 0.761

Needle chlorophyll content

Chlorophyll units: ratio of mg Chl/g dry wt. (mean + s.d.)

Treatment	Chl *a*	Chl *b*	Total Chl
Low nitrogen plants			
Ambient-grown	2.329 + 0.554	0.848 + 0.319	3.177 + 0.707
Elevated-grown	1.91 + 0.308	0.811 + 0.341	2.721 + 0.582
High nitrogen plants			
Ambient-grown	2.585 + 0.831	1.112 + 0.27	3.697 + 0.988
Elevated-grown	2.311 + 0.642	1.059 + 0.442	3.37 + 1.011

Seedling dry matter production

Biomass units: g (mean + s.d.)

Treatment	Total biomass
Low nitrogen plants	
Ambient-grown	2.011 + 0.469
Elevated-grown	2.071 + 0.486
High nitrogen plants	
Ambient-grown	2.814 + 1.127
Elevated-grown	4.008 + 0.986

Appendix 2: Equipment/Methodology

Conviron E15 plant growth environment chambers

Two controlled environment chambers were used in the study and a number of variables were controlled: CO_2 concentration was monitored and maintained by means of a PP Systems EGM-1 and CIM-1 apparatus set for 585 μmol mol^{-1} in the elevated chamber with the ambient chamber CO_2 measured to be a nominal 385 μmol mol^{-1}; temperature was cycled between 18 °C and 28 °C; relative humidity set for 60 and 70 % RH; and, light levels were controlled by 1000 W high intensity discharge lamps, three metal halide and three high pressure sodium per chamber incorporating a polycarbonate barrier for optimal transmittance of PAR wavelengths. Chamber dimensions are 0.8 m depth x 2 m length x 1.3 m height and pots were moved between the chambers once a week to reduce chamber variability effects.

Chlorophyll content: HP8451A Diode Array Spectrophotometer

Pigments were extracted from about 100 mg fresh weight of primary needles from each seedling in the study, solubilized in DMSO as sectioned tissue, and then incubated at 55 °C in a drying oven for >12 hours. Extracts were stored at 0-4 °C in the dark until ready to be analysed. Extracts were made up to 10 ml with fresh DMSO and 1 ml of the resultant solution was transferred to a 1 ml microtube. Absorptances were read at 470, 648 and 666 nm using an HP8451A diode array spectrophotometer. Pigment concentrations were calculated according to Barnes et al 1992, Hiscox and Israelstam 1979 and Porra et al 1989.

Chlorophyll *a* fluorescence: Hansatech Plant Efficiency Analyzer (PEA)

Primary needles from the main stem of each seedling in the study were placed in shuttered leaf clips and dark adapted for > 30 minutes before fast fluorescence kinetics were measured using the Hansatech PEA at 1232 μmol/m^2/s and 15 s sample time. The chlorophyll fluorescence system used was of the non-modulated type therefore fast phase fluorescence elucidation only was possible, with calculated parameters: F_0, F_m, F_v, F_v/F_m, T_m, F_v/F_0 and area above curve between F_0 and F_m.

Photosynthesis: PP Systems CIRAS-I gas exchange system

An open, differential-type portable gas exchange system was used, flowing air (reference gas) into a single leaf cuvette and analysing the outlet air (analysis gas) for changes in the quantity of CO_2 and H_2O. Air is dried before passing through the leaf cuvette to counter the effects of water vapour on outlet gas analysis and on calculations of assimilation rate. Curves of assimilation rate against intercellular CO_2 concentration were compiled by manipulating CO_2 concentration (set CO_2) passing through the leaf cuvette and thus over the pine foliage. A Parkinson needle-type leaf cuvette was used on individual fascicles (three secondary needles per fascicle) with adequate water being supplied to the plant throughout measurement duration.

Appendix 3: Calculations

Needle surface area determinations

Chord technique :

Assumes all needle surfaces are photosynthetic, 3 needles form a cylinder.

Surface area = 2 pi x r x l = 2 pi x l x c/2sin60

l = segment length
r = length of (two) longest sides of cross-section, the arc being the remaining side
c = chord of cross-section, measured under scope

Glass bead technique :

Assumes perfect bead packing, uniform bead monolayer, accurate standard curve construction.

Surface area = 3 M / pi x rho_b x r_b

M = mass of bead monolayer
rho_b = single-bead density
r_b = bead radius

Needle chlorophyll content

Chl *a* (μg/ml) = 14.85*A665 - 5.14*A648
Chl *b* (μg/ml) = 25.48*A648 - 7.36*A665
Total Chl (*a* + *b*) = 7.49*A665 +20.34*A648
(A = absorptance @ xyz nm)

Unit conversion:
Chl *a* (mg/g d.w.) = Chl *a* (μg/ml)*ml solvent/g dry mass*mg/1000μg

Chl *a* mol. wt. = 892 g/mol
Chl *b* mol. wt. = 906 g/mol