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EFFECTS OF NITROGEN AVAILABILITY AND ATMOSPHERIC CO$_2$ ENRICHMENT ON GROWTH, WATER USE, AND NUTRITION OF SEEDLINGS OF BOREAL TREES

BY

KEVIN R. BROWN ©

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF FOREST SCIENCE

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled Effects of Nitrogen Availability and Atmospheric CO_2 Enrichment on Growth, Water Use and Nutrition of Boreal Trees, submitted by Kevin R. Brown in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

Seedlings of two boreal tree species were studied. *Populus tremuloides* dominates early successional, relatively fertile sites; *Picea glauca* dominates later successional sites with slower rates of nutrient turnover.

Seedlings were grown for 100 days at ambient (350 ul l^{-1}) or high (750 ul l^{-1}) levels of atmospheric CO_2 and fertilized with high-N (15.5 mM-N), medium-N (1.55 mM-N), or low-N (0.155 mM-N) solutions. High CO_2 increased leaf and total mass of high-N *Picea*, and root mass of low-N *Picea* after 100 days. High CO_2 increased mass, height, and leaf area of *Populus* at 30 days in the high-N regime, at 40 days in the medium-N regime, and at 60 days in the low-N regime; in each treatment, effects did not persist to the following harvest. High CO_2 accelerated the time-dependent decreases in concentrations of N and P (all N treatments) and Ca and Mg (high-N and medium-N treatments) to deficient levels, preventing the continuation of growth enhancement.

In a second experiment, the effects of CO_2 enrichment to 650 ul l^{-1} were examined with plant N concentrations held constant at high or low levels using the relative addition technique of nutrient supply. In the high-N regime, elemental concentrations were higher in *P. tremuloides* than in *P. glauca* and were unaffected by CO_2-enrichment. Relative growth rates (RGR) and net assimilation rates (NAR) of *Picea* (high-N regime) and of *Populus* (high-N, low-N) increased with CO_2-enrichment. The absolute effect of CO_2-enrichment on NAR increased with foliar N content and was greater in *Populus* at a given foliar concentration of N. Nitrogen status had greater effects on root:shoot ratios of *Picea* than of *Populus*; CO_2-enrichment did not affect root:shoot ratios in either species. High CO_2 reduced transpiration by 60 % in *Populus* under both N regimes, but did not affect transpiration by *Picea*. Nitrogen productivity of *Populus* may have increased with CO_2-enrichment.

Thus, methods of nutrient supply may affect plant nutrient status over time and therefore affect plant response to CO_2-enrichment. The effect of CO_2-enrichment increases with nutrient status and is potentially greater in early successional boreal trees, given adequate nutrient supplies.

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CHAPTER 1. INTRODUCTION

Carbon dioxide (CO_2) occurs naturally in the atmosphere where it serves as a resource for plant growth and as a determinant of the earths thermal balance (Clark et al. 1982). Global atmospheric CO_2 levels have increased by 1-1.5 ul l^{-1} $year^{-1}$ since detailed measurements began in the late 1950s (Woodwell 1984; Figure 1). Depending upon where measurements are made, pronounced seasonal amplitude in CO_2 levels reflecting seasonal patterns of terrestrial metabolism may be observed (Woodwell 1984). The present-day concentration of 350 ul l^{-1} is expected to double by late in the 21st century (Clark et al. 1982). Because increased levels of atmospheric CO_2 may affect the earth's climate (Dickinson 1986) and may also directly affect plant growth (Gates et al. 1983), there is much interest in how increasing levels of atmospheric CO_2 may affect terrestrial ecosystems.

The overall objective of this study is to examine the effects of CO_2 enrichment on growth, nutrition, and water use of two species of boreal trees, as modified by the availability of nitrogen (N). The purpose of this introduction is to develop an overview of (1) trends in atmospheric CO_2 levels, (2) the relative importance of different global C sources and sinks in

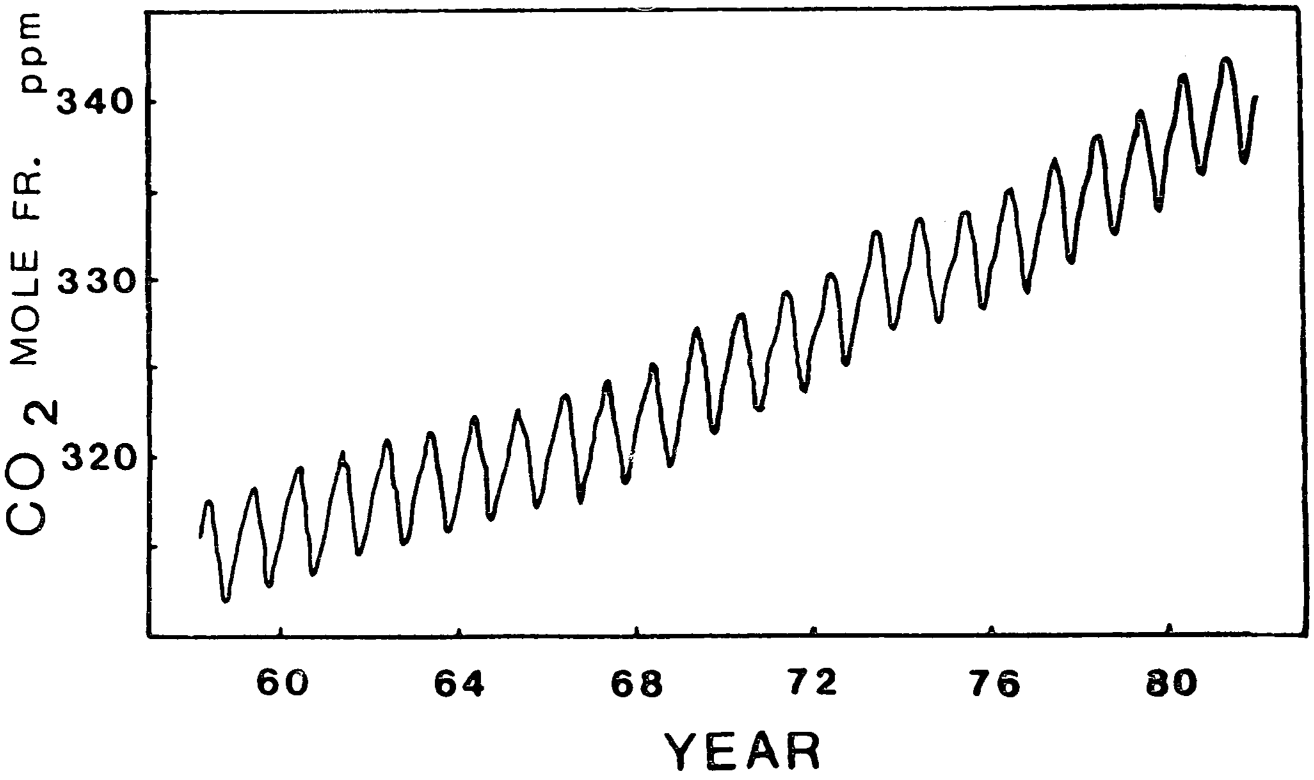

Figure 1. Seasonal and annual trends of atmospheric CO_2 partial pressure, based on direct measurements made at the Mauna Loa Observatory, Hawaii. (Reprinted by permission of Westview Press. From CO_2 and Plants: The Response of Plants to Rising Levles of Atmospheric CO_2, edited by Edgar R. Lemon. Published by Westview Press, Boulder, Colorado, 1983.)

affecting atmospheric CO_2 levels, and (3) possible effects of increasing CO_2 levels on climate. I then focus on the question of how rising CO_2 levels may directly affect vegetation, develop the rationale for my choice of topic, and outline the general operating hypotheses underlying the study.

TRENDS IN ATMOSPHERIC CO_2 LEVELS

Predictions of future atmospheric CO_2 levels can be enhanced by accurate descriptions of past trends and an understanding of what sources and sinks in the global carbon cycle contribute to the consistent, observed increases in CO_2 levels.

Global CO_2 trends prior to the 1950s have been reconstructed using (1) published values of direct measurements of atmospheric CO_2, (2) direct measurements of CO_2 in air bubbles trapped in accumulated ice in Antartica and Greenland, and (3) ratios of $^{13}C/^{12}C$ in individual tree rings. Data generated with these techniques indicate that atmospheric CO_2 levels were approximately 290 ul l^{-1} in 1900 and ramged from 260-290 ul l^{-1} prior to the advent of the Industrial Revolution (ca. 1750) (Callendar 1958; Stuiver et al. 1984; Pearman et al. 1986).

The three techniques used for reconstructions of past atmospheric CO_2 levels have different strengths and weaknesses. Taken together, they provide evidence for exponentially increasing CO_2 levels since 1750. Published measurements of free atmospheric CO_2, which date back to the early 19th century (Fonselius et al. 1956), eliminate the problems of accurate dating of samples. However, direct measurements prior to 1880 were variable, ranging from 250-550 ul l^{-1}. The measurements appear upwardly biased and encompassed a variety of sampling techniques and locations (Fonselius et al. 1956). Thus, many of the early data are of limited use in assessing historical trends (Callendar 1958).

Direct measurments of CO_2 trapped in ice cores and measurements of $^{13}C/^{12}C$ in tree rings have been most useful for estimating atmospheric CO_2 levels prior to and during the early Industrial Revolution. Levels of CO_2 trapped in Antarctic ice cores indicate that average CO_2 levels were 260-280 ul l^{-1} from the mid-17th to the mid-19th century (Neftel et al. 1985; Raynaud and Barnola 1985; Pearman et al. 1986) and were clearly increasing by 1850 (Pearman et al. 1986). Atmospheric CO_2 levels decreased during the 16th, 17th, and 18th centuries before beginning their present trend (Raynaud and Barnola 1985) and have varied more during the past 15000-20000 years than during the past 200 years (Berner et al. 1980; Delmas et al. 1980; Dansgaard 1981). Accuracy in dating ice in the sample cores increases when samples are taken from areas with abundant snowfall and little melting (Raynaud and Barnola 1985).

Estimates based upon $^{13}C/^{12}C$ ratios in individual tree rings, indicate that CO_2 levels in 1900 were approximately 290 ul l^{-1} (Farmer and Baxter 1974) and 260-290 ul l^{-1} in the early 18th century (Stuiver et al. 1984). These values are consistent with those from early direct atmospheric measurements and from ice cores. Carbon in the atmospheric CO_2 pool consists of 98.9% and 1.1% of the stable isotopes ^{12}C and ^{13}C, respectively, with trace amounts (10^{-14}%) of the radioactive isotope ^{14}C (O'Leary 1981). Ribulose biphosphate carboxylase (RUBISCO), the initial enzyme in carbon fixation by C_3 plants, discriminates against ^{13}C (relative to ^{12}C) during carbon fixation (Park and Epstein 1960). Thus, the ratio of $^{13}C/^{12}C$ in organic material, whether live or fossilized (e.g. coal and petroleum) vegetation, is depleted relative to that in the atmosphere (Freyer 1979). If CO_2 released during the past 250 years resulted from fossil fuel combustion and changes in land use, the $^{13}C/^{12}C$ ratio in the atmosphere should decrease over time and should be further depleted in the individual rings of trees (Farmer and Baxter 1974), other factors being equal. This trend has been observed in trees of the Northern Hemisphere, but not in the Southern Hemisphere (Francey 1981).

The $^{13}C/^{12}C$ ratios in tree rings may be affected by environmental conditions which affect photosynthesis (e.g. vapor pressure deficit and availability of light and nutrients), the fractionation varying with the relative importance of stomatal versus nonstomatal limitations (Francey and Farquhar 1982). Climatic variations may therefore affect the fractionation of the two isotopes (Leavitt and Long 1983), although increasing CO_2 levels may not (Farquhar 1980). In addition, $^{13}C/^{12}C$ may increase locally around a sample tree as a result of respiration by other trees, particularly in young, dense stands. Ideally, trees sampled for reconstruction of past CO_2 levels should be in the open or above the rest of the canopy and in areas not subject to stress from low humidity, nutrient supply, or irradiance (Freyer 1979; Francey and Farquhar 1982). Stuiver (1982) suggested normalizing $^{13}C/^{12}C$ to constant tree ring areas to reduce variation which might result from local conditions. This approach reduced variation in $^{13}C/^{12}C$ by approximately 25% (Stuiver et al. 1984).

CONTRIBUTIONS TO INCREASES IN ATMOSPHERIC CO_2 LEVELS

These three lines of evidence demonstrate that global atmospheric CO_2 levels have increased consistently since the mid-19th century, but do not indicate the underlying causes for the CO_2 increase. Such insight requires additional information and understanding of the global carbon cycle.

Major compartments and transfer processes of the global carbon cycle have been identified (e.g. Figure 2), but the magnitudes of carbon fluxes between terrestial biota, the atmosphere, and the oceans are not well known. There is greater certainty regarding CO_2 emissions from cement production and combustion of fossil fuel. The airborne fraction of CO_2 (the amount of CO_2 retained in the atmosphere relative to the total emitted) is ca. 0.50 during the past 100 years (Bolin 1986), indicating that the ocean and possibly the terrestrial biosphere are absorbing a significant fraction of CO_2 emitted from fossil fuels.

Emissions of CO_2 from fossil fuels and cement production have been estimated since 1929 from United Nations fuel production data and extrapolated to earlier dates (Keeling 1973). Such emissions were minimal prior to ca. 1860, but increased exponentially to at least 1980 (Rotty 1981; Marland and Rotty 1984). If these emissions were assumed to be the only source of CO_2 increases since 1860, the pre-Industrial Revolution CO_2 levels would have been approximately 295 ul l^{-1} (based on extrapolations of CO_2 increases measured since the 1950s), levels clearly higher than the 260-280 ul l^{-1} reported from ice cores. This discrepancy indicates the presence of an additional net source of CO_2 during the 19th and 20th centuries, thought

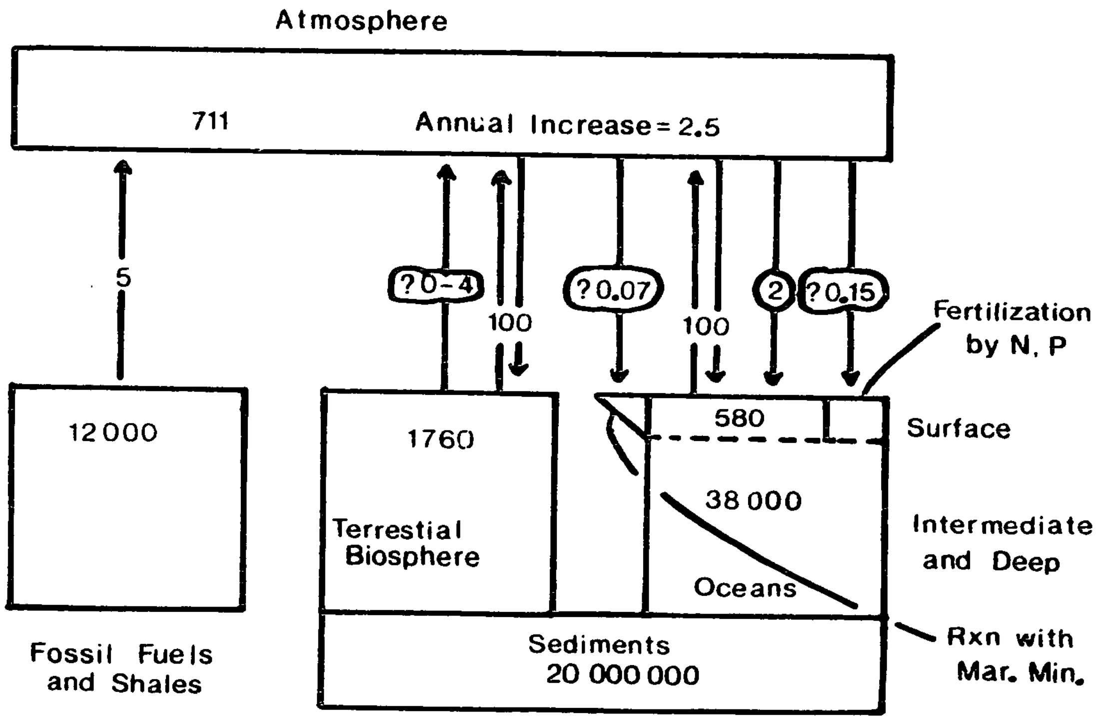

Figure 2. Global carbon reservoirs (in gigatons C) and fluxes between reservoirs (gigatons year^{-1}). Circled fluxes are anthropogenic in origin. Mar. Min.=marine minerals. Redrawn from Liss and Crane (1983).

to be land clearing associated with expansion of agriculture and world population (Siegenthaler and Oeschger 1987).

Emissions of CO_2 from terrestial biota have been estimated using published data on population growth, land alteration rates, and carbon content changes in soil and vegetation (Houghton et al. 1983; Woodwell et al. 1983; Houghton et al. 1987), ratios of $^{13}C/^{12}C$ in tree rings (Freyer 1979), and a combination of carbon cycle models and $^{13}C/^{12}C$ in tree rings (Peng 1985) or ice cores (Siegenthaler and Oeschger 1987). Different estimates for the contribution of biospheric CO_2 to the atmospheric CO_2 pool have been made using these techniques. Woodwell et al. (1983) suggested that land use changes (e.g. deforestation) released more CO_2 than did fossil fuel combustion prior to 1960 and that the biospheric CO_2 emissions increased exponentially at less rapid rates than did emissions from fossil fuel combustion and cement manufacture. These estimates were probably high (Enting and Mansbridge 1987). The differences in estimates of net biospheric CO_2 emissions calculated from the direct measures and those calulated from the combined use of CO_2 concentrations, ^{13}C levels, and carbon cycle models were reduced by assuming less release of soil carbon after deforestation (Houghton et al. 1987).

Estimates of net biospheric CO_2 emissions, based on carbon cycle models and $^{13}C/^{12}C$ in ice cores (Siegenthaler and

Oeschger 1987) or tree rings (Peng 1985), indicate that net biospheric CO_2 emission rates were relatively constant during the 19th and early 20th centuries. Biospheric CO_2 emissions have decreased since 1920. Since 1950, the emissions are less than estimated from present rates of deforestation, indicating increased rates of CO_2 uptake by remaining vegetation.

Relative contributions of CO_2 from land use changes and from cement manufacture and fossil fuel combustion can also be estimated from ratios of $^{14}C/^{12}C$ and $^{13}C/^{12}C$ measured simultaneously in tree rings. In contrast to the stable isotope ^{13}C, which is present in fossilized or live organic matter and depleted in each relative to the atmosphere, ^{14}C is a radioactive isotope (half-life of 5730 years) which is not detectable in fossil fuels. Thus, CO_2 released from fossil fuel combustion (but not from biospheric sources) should decrease the ratio of $^{14}C/^{12}C$ in the atmosphere (Freyer 1979). The CO_2 emissions from fossil fuel combustion and biospheric sources should equally decrease $^{13}C/^{12}C$ in the atmosphere and in tree rings. The difference between CO_2 released from both sources (indicated by $^{13}C/^{12}C$) and that released from fossil fuel combustion (indicated by $^{14}C/^{12}C$) should then be attributable to biospheric CO_2 releases. While attractive in principle, the technique has not been widely used. It is not useful for periods after 1950, when atmospheric ^{14}C levels increased with the advent of nuclear weapons testing (Lorius and Raynaud 1984) and may be less accurate for the preceding 100 years than are extrapolated estimates of fossil fuel consumption. However, ^{14}C has provided a means for tracing carbon distribution in the oceans (e.g. Bolin 1986).

Numerous assumptions implicit in each technique may contribute to different estimates of biospheric CO_2 release. The direct estimates by Woodwell et al. (1983) assumed a percentage reduction in soil carbon content after deforestation (a value later reduced by Houghton et al. 1987); a degree of biomass regrowth after cutting of primary forests; and a correlation between estimated population growth and deforestation rates. Woodwell et al. relied on published rates of deforestation, different estimates of which resulted in different estimates of net biospheric CO_2 release. They assumed that additional carbon storage by vegetation as a result of increasing CO_2 levels did not occur. However, levels of ^{13}C and CO_2 in ice cores combined with carbon cycle models (Siegenthaler and Oeschger 1987) indicate otherwise. More accurate estimates of deforestation and regrowth, based on LANDSAT imagery (e.g. Woodwell 1984), should improve the resolution of these direct estimates of biospheric CO_2 contributions.

Estimates of biospheric CO_2 release, based on a combination of $^{13}C/^{12}C$ in ice cores or tree rings, atmospheric CO_2 levels, and carbon cycle models, assume that samples are accurately dated, that CO_2 uptake by the ocean is accurately known (Woodwell et al. 1983) and that net non-fossil fuel inputs of CO_2 are of biospheric origin and not from oceanic CO_2 inputs (Siegenthaler and Oeschger 1987). Those using $^{13}C/^{12}C$ ratios from tree rings are subject to errors from other factors affecting $^{13}C/^{12}C$ fractionation, as mentioned previously.

Estimates of CO_2 uptake by the oceans are uncertain. Uptake is typically calculated through modelling procedures which attempt to estimate the equilibrium capacity of seawater, the rate at which "excess" CO_2 crosses the air-sea interface into surface layers, and the rate of mixing of surface waters with deeper layers (Liss and Crane 1983). It is particularly difficult to simulate geographical variation in CO_2 transfer between the atmosphere and ocean surface and to model transfer of carbon between surface and deeper layers, such that observed rates of movement of radioisotopes (e.g. ^{14}C) can be described (Liss and Crane 1983). Different modelling approaches calculate different airborne fractions of CO_2 at given rates of CO_2 input (Bolin 1986) and often calculate airborne fractions in excess of those actually observed (Liss and Crane 1983). This suggests that oceanic uptake of CO_2 uptake is underestimated or that other sinks exist (e.g the biosphere) for CO_2 emitted from fossil fuel combustion. However, the discrepancy between model-calculated and observed airborne fractions of CO_2

increases if the biosphere is assumed to be a net source of CO_2 (Enting and Mansbridge 1987). Such uncertainty (Figure 2) regarding the magnitudes (and even direction) of CO_2 exchanges makes projections of future CO_2 levels tenuous.

Despite this uncertainty on a global scale, certain biomes may become important sinks for carbon as atmospheric CO_2 levels increase. These include forests, because of their high proportion of wood, with its high C:N and C:P ratios (Peterson and Melillo 1985). The tundra and boreal forests may become larger sinks than at present if primary productivity increases more than do decomposition rates (e.g. Billings et al. 1982;1983). Assuming a lack of disturbance, which could alter this balance, the role of these biomes as net sources or sinks of carbon may depend on the availability of nutrients to support additional growth and on climatic changes which may be associated with CO_2 increases (Billings 1987; Billings et al. 1982; 1983; Miller et al. 1983).

POTENTIAL CLIMATIC CHANGES ASSOCIATED WITH INCREASING CO_2

Alterations of regional and global climates have been recognized as a potential consequence of rising CO_2 levels since the 19th century (e.g. Aarhenius 1896; Chamberlain 1898). I present here an overview of projected climatic effects associated with a doubling of CO_2 levels, as temperature and precipitation changes may negate or enhance direct effects of CO_2 on vegetation (Gates 1983).

Oxygen and nitrogen, which together comprise 99% of the atmosphere, strongly absorb radiation of wavelengths less than 300nm. In contrast, heteroatomic molecules such as CO_2, H_2O, CH_4, O_3, and chlorofluoromethanes strongly absorb radiation at wavelengths greater than 800 nm, i.e. the infrared spectrum (Fleagle and Businger 1963; Sestak et al. 1971). Thus, the atmosphere is relatively transparent to incoming shortwave solar radiation.

Much of the shortwave radiation reaching the earth's surface is absorbed by the earth, then reradiated as longwave (infrared) radiation. It either escapes to space or is absorbed by the gases listed above and is reradiated, largely back to earth (Figure 3). Other things being equal, an increase in infrared opacity will cause the temperature of the earth's surface and atmosphere to increase until the emission of radiation from the planet again equals the absorbed solar energy (Hanson et al. 1981).

Projections of average global climatic change are complicated by uncertainty surrounding feedback effects on climate associated with a doubling of CO_2 concentration. Increased surface temperatures could increase rates of evaporation and increase the amount of water vapor contained in

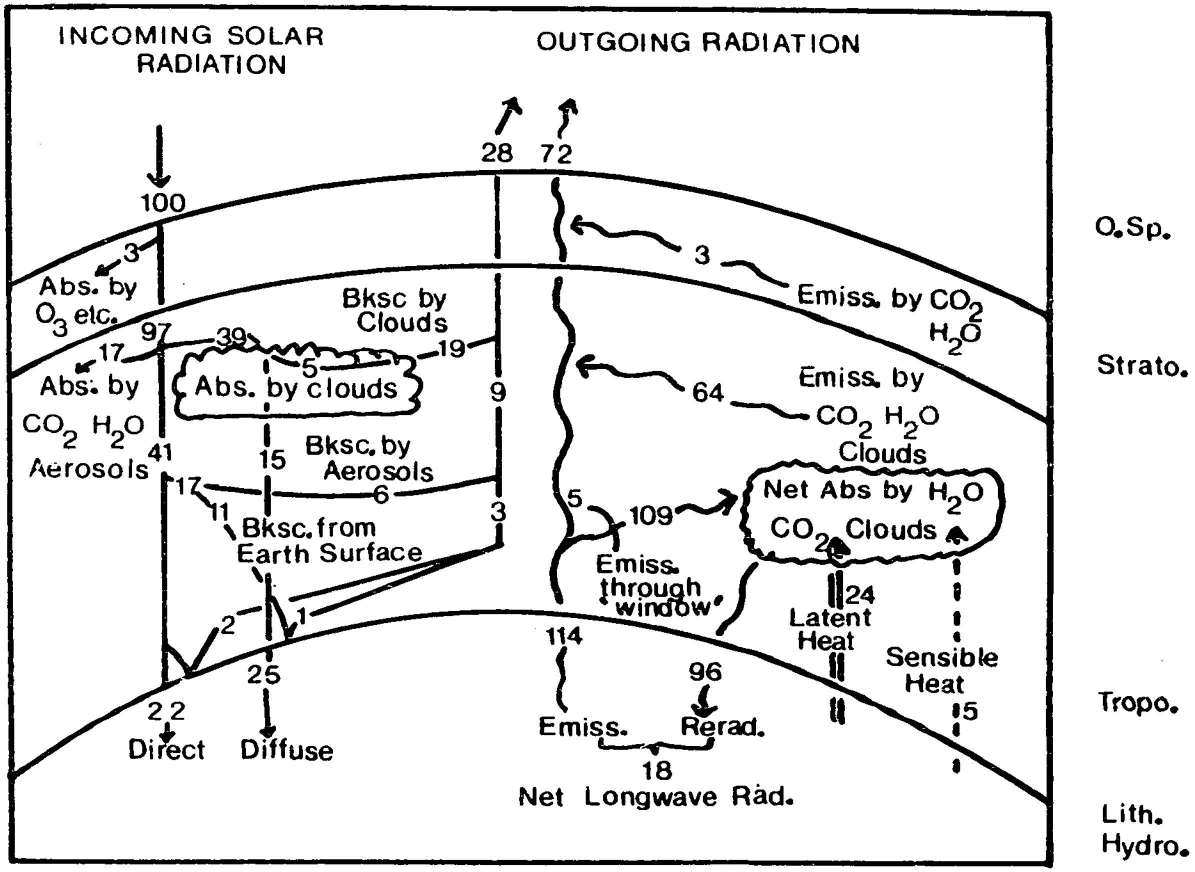

Figure 3. Average radiation and heat balance of the earth-atmosphere system, relative to 100 units of incoming radiation. Abbreviations: Abs.=absorption; Bksc.=backscatter; Emiss.=emission; Rerad.=reradiation; O.Sp.=outer space; Strato.=stratosphere; Tropo.=troposphere; Litho.=lithosphere; Hydro.=hydrosphere. Redrawn from Bach (1984).

radiation emitted from the earth's surface, resulting in additional heating (positive feedback). Alternatively, increases in cloud cover associated with higher concentrations of water vapor might reduce insolation at the earth's surface and increase global albedo (reflectivity), which would act as negative feedback (Fleagle and Businger 1980). Increased temperatures could also reduce the extent of the polar ice packs and reduce global albedo (Dickinson 1986; Dickinson et al. 1987).

The global climate system involves energy transfers between a three-dimensional turbulent and radiatively active atmosphere and spatially heterogeneous land, ocean, and cryosphere surfaces (Dickinson 1986). The most appropriate climatic models for dealing with this complexity are general circulation models (GCMs) (Manabe 1983). To date, however, GCMs have poor resolution for predicting regional climates and are based on simplistic assumptions regarding cloud formation, ice-albedo relations, and oceanic heat transport (Dickinson 1986). Despite such problems, GCMs can reproduce a number of significant parameters of present-day global climate such as seasonal variation in surface temperatures of the northern hemisphere, global average surface temperatures (Manabe and Stouffer 1980), and seasonal variation in sea ice (Barry et al. 1984). They also reasonably simulate past climates, although validation data are often limited (Dickinson 1986).

Two GCMs which simulate climatic change from a doubling of CO_2 and are realistic in their assumptions (Dickinson 1986) are those of Hansen et al.(1984) and of Washington and Meehl (1984). For northern latitudes (e.g. 60 N. latitude), both models projected mean temperature increases of 6-7 C from December-February and 3-4 C from June-August. The greater temperature increases in winter reflect increased summer heat storage and melting of ice in the ocean (Washington and Meehl 1984). Additionally, increases were predicted in absolute humidity, low clouds, and precipitation, with the greatest increase in the winter (Washington and Meehl 1984). Such climatic changes may negate or enhance the direct effects of CO_2 increases on vegetation. This problem will be discussed further in the final chapter.

DIRECT EFFECTS OF CO_2 ENRICHMENT ON VEGETATION

Since CO_2 is the substrate for photosynthetic carbon fixation, its availability will affect plant productivity. Increased productivity has been demonstrated in numerous studies and for numerous species, primarily crops (Kimball 1983). Attempts to project responses of unmanaged vegetation to increased CO_2 levels (c.f. Shugart and Emanuel 1985; Kohlmaier et al. 1987) may, however, be limited by: (1) uncertainty as to how availability of other required resources will modify the effects of CO_2 enrichment and (2) a limited basis for projecting how the magnitude of the response will vary among ecologically associated species, particularly woody species.

The law of the minimum (Blackman 1905) states that organism processes which depend upon many distinct environmental factors are limited by that factor whose value is furthest from process requirements. Therefore, limited supplies of water, irradiance, or mineral nutrients may minimize the enhancing effects of CO_2 enrichment (Kramer 1981), unless there is an increase in the availability or the efficiency of use of those resources (Luxmoore 1981; Norby et al. 1986).

Such effects occur under CO_2 enrichment. Carbon dioxide enrichment may reduce rates of photorespiration, decrease the light compensation point, and thereby compensate for low levels of irradiance (Pearcy and Bjorkman 1983). Conversely, the absolute enhancement of photosynthetic rates by CO_2 enrichment may be greater at high irradiance (Gaastra 1963).

CO_2 enrichment commonly induces partial stomatal closure in plants and rates of transpiration relative to photosynthesis (Morison 1985). It may therefore compensate for decreased concentrations of CO_2 in the leaf (C_i) under moderate moisture stress or might reduce the consumption of water at a given size of plant, thereby delaying the onset of moisture stress.

Productivity per unit N may increase under CO_2 enrichment if less N is allocated into photosynthetic enzymes (e.g. RUBISCO) and more is allocated for biosynthesis (e.g. Wong 1979; Downton et al. 1980; Norby et al. 1986). Efficency of use of N and other mineral nutrients could further increase if the retention time of the element in the plant (Berendse and Aerts 1987) increased with CO_2 enrichment. Availability of N, other mineral nutrients, and water might increase to the plant with enhanced growth of root systems under CO_2 enrichment (Norby et al. 1986). The proportionate enhancement of growth by CO_2 enrichment may be as great at low rates of N supply as at higher rates (Wong 1979), but absolute increases in biomass under CO_2 enrichment should increase with increasing nitrogen supply (Wittwer 1983). Clearly, CO_2 enrichment may stimulate productivity when other resources are readily available, but enrichment may also stimulate productivity when other resources are present in otherwise limiting quantities.

Different plant species occurring in the same ecosystem or biome may have different capacities for responding to CO_2 enrichment. Plants dominating sites of abundant resource availability, such as early successional stages of continuously productive habitats, exhibit characteristics facilitating rapid growth and capture of resources (Table 1)(Grime 1979). Species associated with habitats chronically low in resources tend to have slower maximum growth rates, are less plastic in their

Table 1. Selected physiological and growth characteristics postulated for plant species occurring in resource-abundant and resource-restricted sites (adapted from Grime 1979; Chapin 1980).

	RESOURCE AVAILABILITY	
TRAIT	ABUNDANT	RESTRICTED
Maximum potential:		
Relative growth rate	high	low
Photosynthetic rate	high	low
Nutrient absorption capacity	high	low
Nutrient allocation at high nutrient availability	to growth	to storage
Leaf, root turnover rates	rapid	slow
Nutrient retention	less	more
Effect of nutrient stress:		
Relative growth rate	greater reduction from maximum	less reduction
Photosynthetic rate	greater reduction	less
Root:Shoot ratio	greater increase	less
Nutrient concentration	greater decrease	less

responses to increased resource availability, and have anatomical and / or physiological characteristics which maximize their efficiency of resource use (Table 1). Resource availability may be limited by soil characteristics, climate, or change with succession (Grime 1979).

These generalizations may be useful for assessing the direct effects of increasing CO_2 on productivity of boreal trees. The boreal forest is a large sink for atmospheric CO_2 (Miller et al. 1983). However, productivity in the North American boreal forest is often limited by reduced availability of N and P (e.g. Krause et al. 1978), a consequence of low soil temperatures (Heilman 1966; Van Cleve and Dyrness 1983), poor drainage, podzolization, and/or sandy soils (Bringmark 1980; Larson 1980). Low soil temperatures may result from topography or vegetational changes during succession. Reduced nutrient availability raises the question of whether productivity in the boreal forest should increase with increasing CO_2 levels.

Deciduous hardwoods (Populus tremuloides, Populus balsamifera, and Betula papyrifera) characteristically dominate in early successional stages on sites with warmer, well-drained soils, whereas conifers (Picea glauca, Picea mariana, and Larix laricina) dominate in wetter, colder sites or in later succession on warmer, well-drained sites (Viereck et al. 1983). The stands dominated by deciduous hardwoods have higher rates of production and nutrient turnover (Viereck et al. 1983; Van Cleve et al. 1983). Seedlings of the three deciduous hardwood species common to the forests of interior Alaska (and of the northern boreal forest in general) show greater increases in productivity with increasing supply of P than do the coniferous species (Chapin et al. 1983), a response predicted by Grime's hypotheses.

STUDY HYPOTHESES

For this study, I hypothesized that (1) boreal deciduous hardwood trees, typically dominant in early sucessional, relatively fertile sites, should increase productivity more in response to CO_2 enrichment than the coniferous species, which typically dominate later successional and less fertile sites. I further hypothesized that (2) the enhancement of productivity by CO_2 enrichment would increase more in the deciduous hardwoods as N availability increased.

Nitrogen availability should affect the response of plants to CO_2 enrichment because of its major role in amino and nucleic acids, protein, chlorophyll, and growth regulators (Lavender and Walker 1979). Increased availability of N may directly increase photosynthetic capacity through increased production of chlorophyll and photosynthetic enzymes and may indirectly affect photosynthesis through alteration of the source-sink balance (Moorby and Bestford 1983). Increased availability of N may increase leaf area and mass relative to roots (Moorby and Bestford 1983; Mooney and Chiarello 1984), further increasing whole-plant productivity.

Photosynthetic rates of temperate deciduous hardwoods are generally greater than those of evergreen conifers (Small 1972; Larcher 1980; Chabot and Hicks 1982) and may increase more with N fertilization (Linder and Rook 1984). The lower photosynthetic capacity of the evergreens may result from greater resistance to CO_2 diffusion, lower ratios of internal:external surface area (Gambles and Dengler 1974; Dengler and McKay 1975; Chabot and Hicks 1982), and lower concentrations of chlorophyll and RUBISCO (Chabot and Hicks 1982) at optimum N levels.

In this study, I examined the responses of seedlings of *Populus tremuloides* (Michx.), trembling aspen, and *Picea glauca* (Moench) Voss, white spruce, to CO_2 enrichment and nitrogen supply. The two species are widespread throughout the North American boreal forest (Little 1978). They generally occupy different ecological roles, but commonly cooccur. The two species are dominant in the well-drained mesic uplands of the boreal forest in Alberta and Saskatchewan, with *P. glauca* most prominent in older stands (Rowe 1972). *Populus tremuloides* is seral (Van Cleve and Viereck 1981; Strong and La Roi 1983) with the ability to regenerate rapidly via seed or suckering after fire. *Picea glauca* often invades stands of *P. tremuloides*, becoming dominant after ca. 100 years, at least in interior Alaska and western Canada (Van Cleve and Viereck 1981; Strong and La Roi 1983).

Growth of P. tremuloides is more sensitive to P stress or fertilization than is P. glauca, as indicated previously. It is also more sensitive to reduced irradiance (Kramer and Kozlowski 1979) and grows faster under optimal conditions (Okafo and Hanover 1978; Pollard and Logan 1976). These limited data suggest that the two species may be representative of the species classifications in Table 1.

The following four chapters describe two major experiments addressing the general hypotheses and questions described above. Chapters 2 and 5 deal primarily with growth responses to CO_2 enrichment under different conditions of nitrogen supply, while chapters 3 and 4 address plant nutrition under the particular experimental regimes. In chapter 6, I attempt to synthesize this information and place it in a context relevant to the larger question of how boreal forests may respond to increasing atmospheric CO_2 levels.

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CHAPTER 2. EFFECTS OF CO_2 ENRICHMENT AND CONSTANT SUPPLY OF N ON GROWTH OF *POPULUS TREMULOIDES* AND *PICEA GLAUCA* SEEDLINGS[1/]

INTRODUCTION

The objective of this first experiment was to characterize the growth responses of *P. tremuloides* and *P. glauca* to atmospheric CO_2 enrichment and constant, but varied levels of N supply.

In the preliminary study (Appendix 2), the relative and absolute effects of CO_2-enrichment on total dry mass (TDM) of both *P. tremuloides* and *P. glauca* increased as solution N concentrations increased from 0.155 mM to 15.5 mM. Within an N regime, the relative effects of CO_2-enrichment on dry mass were similar in *P. tremuloides* and *P. glauca*, but the absolute effect of CO_2 increased more with increasing N in *P. tremuloides*. This preliminary data supported the original hypotheses posed in Chapter 1. However, the rapid growth of *P. tremuloides* supplied with 15.5 mM-N (high-N regime) led to wilting and some death prior to the end of the experiment (33 days). Since the effects of CO_2-enrichment may not persist as plants age (Kramer 1981), it was essential to lengthen the study period. In contrast with

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1/ A version of this chapter has been published as Brown, Kevin, and K.O. Higginbotham. 1986. Tree Physiol. 2:223-232.

the preliminary study, the study period was lengthened, only one seed source for each species was used, only N was varied in solutions, and the volume of containers for P. tremuloides was increased to prevent the rapid onset of moisture stress.

METHODS

The study was conducted in two controlled environment rooms with a 22 / 17 C (day/night) temperature regime and an 18 hour thermoperiod and photoperiod. A mixture of sodium and mercury vapor high intensity discharge lamps supplied 400 umol m^{-2} s^{-1} photosynthetic photon flux density (PPFD). Maximum PPFD in the boreal region may exceed 1000 umol m^{-2} s^{-1} during the growing season (Linder and Troeng 1980). However, 400 umol m^{-2} s^{-1} PPFD is slightly less than saturating for photosynthesis of individual leaves of P. tremuloides (Lawrence and Oechel 1983). Air flow velocity across the bench was approximately 0.5 m s^{-1}. Daytime relative humidity was 45 (VPD=1.40 kPa).

Atmospheric CO_2 levels in the two rooms were 350 ul l^{-1} (ambient) or 750 ul l^{-1} (enriched). Carbon dioxide levels were monitored for alternate 5 minute intervals in the two rooms with a Beckman 864 infrared gas analyzer (IRGA). Set-point solenoid controls automatically allowed injection of bottled CO_2 into the high (750 ul l^{-1}) CO_2 room as necessary and also allowed the CO_2 treatments to be alternated between the two rooms.

Populus tremuloides (trembling aspen) seeds were collected from a single tree at the University of Alberta Devonian Botanical Garden near Edmonton; the Picea glauca (white spruce) seeds were collected in bulk near Footner Lake in northern Alberta. Seeds were germinated at 20 C and seedlings were grown in the ambient-CO_2 room until they were large enough to transplant (2.5-3.5 cm long).

Seedlings were transplanted into acid-washed sand with a particle size range of 0.25-0.6 mm. Picea glauca was grown in plastic 160 cm^3 Ray Leach Conetainers (Ray Leach Nursery, Canby, OR.,USA); the P. tremuloides seedlings were grown in 5 cm internal diameter PVC pipe (500 cm^3). The larger containers were required to accomodate the rapid root growth of P. tremuloides as determined from preliminary trials (Appendix 2). On transplanting, plants from each species were randomly assigned to each CO_2 x N treatment combination.

Design of the nutrient solutions allowed manipulation of nitrogen level without altering availability of other elements (Table 2). The high-N solution contained a mass balance of elements suggested as optimal for a variety of species (Ingestad

Table 2. Macronutrient source, concentration, and balance by mass (relative to N) used in the three macronutrient solutions.

Salt	Element	High-N		Medium-N		Low-N	
		Rel. Mass	Conc. (mM)	Rel. Mass	Conc (mM)	Rel. Mass	Conc (mM)
NH_4NO_3	N	100	15.50	10	1.55	1	0.16
KH_2PO_4	P	18	1.26	18	1.26	18	1.26
K_2SO_4	S	13	0.88	13	0.88	13	0.88
KCl,KOH	K	50	2.78	50	2.78	50	2.78
$MgSO_4$	Mg	5	0.43	5	0.43	5	0.43
$CaCl_2$	Ca	5	0.27	5	0.27	5	0.27
	Cl	27	1.70	27	1.70	29	1.80
pH			5.8		5.8		5.7
Conductivity (mS/m)			140		60		45

1979). The medium-N and low-N solutions contained 10% and 1% , respectively, of the nitrogen present in the high-N solution (Table 2). The pH of solutions was first adjusted with KOH after which concentration of K was readjusted with KCl. Thus, the nutrient solutions were virtually identical in all respects except concentrations of N and Cl, and in conductivity. Nutrient solutions were added six times per week (25 ml per Conetainer, 75 ml per PVC pipe container). Containers were flushed with distilled H_2O before every other application of solution to prevent salt buildup.

Groups of seven randomly selected plants were harvested from each treatment at 0, 10, 30, 40, 50, 60, and 100 days after transplanting. Stem length, branch number, needle length, and needle number (P. glauca) and stem length, leaf number, and leaf area (P. tremuloides) were measured on freshly harvested plants. Surface areas of individual needles were calculated using regression equations which related area to needle length (Appendix 3). The data were derived with the glass bead technique (Thompson and Leyton 1971). Whole-plant needle areas were estimated by multiplying needle numbers by mean needle area. At the last harvest, a 10 % sample of needles was used to estimate whole-plant needle area. Projected leaf areas of P. tremuloides were determined with a LiCor 3100 leaf area meter.

Harvested plants were dried for 24 hours at 70 C and dry mass was determined for roots, stems, and leaves. Specific leaf area (SLA), root weight ratio (RWR), and leaf weight ratio (LWR), all indices of dry matter allocation, were calculated for each plant (Appendix 1). Mean relative growth rates (RGR) were calculated for each harvest interval (Appendix 1); RGR, net assimilation rate (NAR), and partitioning coefficients for leaf area and leaf mass (LAP and LWP, respectively) were calculated for the period of exponential growth in each treatment by the functional approach of Potter and Jones (1977) (Appendix 1). The period of exponential growth in each treatment was estimated after fitting linear regressions to ln-transformed total mass and leaf area data from successively increasing numbers of harvests, up to and including the final harvest. Net assimilation rates estimate productivity, while LAP and LWP describe the rate of disposition of dry matter into foliage (Potter and Jones 1977).

The CO_2 treatments were regularly rotated between the two rooms to equally expose plants from the two CO_2 regimes to any other differences in room environments. Treatment effects on ln-transformed primary growth measures and on SLA, RWR, and LWR for a specific harvest were evaluated by a two-way analysis of variance with a randomized design. The two species were analyzed separately. Mean separation was accomplished using Duncan's multiple range test ($P=0.05$) (Steel and Torrie 1980).

RESULTS

Picea glauca

Mineral nitrogen supply significantly affected all the primary growth parameters of *P. glauca* measured in the final three harvests (Table 3). Growth differences between seedlings in the medium-N (1.55 mM) and high-N (15.5 mM) treatments were not detectable until the final harvest; seedlings fertilized with the high-N solution were significantly taller and had greater stem mass, leaf area, and numbers of branches (Table 3).

Carbon dioxide level significantly increased biomass but had no effect on height or branch number of plants measured in the last two harvests. Carbon dioxide enrichment significantly increased root mass of the low-N seedlings at the final harvest (Table 3). The only significant effect of CO_2 enrichment on the seedlings fertilized with the high-N solution was to increase leaf mass; the increased leaf mass was partly a result of decreased specific leaf area (SLA) and, possibly, increased leaf area in the CO_2-enriched atmosphere (Table 3).

The CO_2 x N interaction was significant for leaf and root weight ratios (LWR,RWR) of seedlings at the final harvest. Under CO_2 enrichment, seedlings fertilized with the low-N solution

Table 3. Means of measured and calculated growth parameters of Picea glauca after 30, 40, 50, 60, and 100 days of treatments. Mean values followed by the same letter, within a column and harvest, do not differ significantly at $P \leq 0.05$ ($n=7$). (CO_2-control = 350 ul l^{-1} CO_2; CO_2-enriched = 750 ul l^{-1} CO_2; height=stem height; SLA = specific leaf area; LWR = leaf weight ratio; RWR = root weight ratio).

Treatment (mM-N)	Mass (g)				Height (cm)	Branch no.	LA (cm^2)	SLA ($cm^2 g^{-1}$)	LWR	RWR
	Root	Stem	Leaf	Total						
30 days										
CO_2-control										
15.5	0.005a	0.002bc	0.014c	0.021bc	2.5ab	2.1cd	11c	756ab	0.68c	0.23a
1.55	0.005a	0.001a	0.009b	0.015b	2.3ab	1.1b	7b	812abc	0.59b	0.33b
0.155	0.003a	0.001a	0.004a	0.008a	2.0a	0a	3a	874c	0.45a	0.42c
CO_2-enriched										
15.5	0.011b	0.003d	0.017c	0.031d	3.2c	2.7d	12c	704a	0.56b	0.35b
1.55	0.009b	0.002c	0.015c	0.026cd	2.5ab	1.9c	12c	761b	0.58b	0.35b
0.155	0.008b	0.001ab	0.006b	0.015b	2.3ab	0a	4a	733ab	0.41a	0.50c
40 days										
CO_2-control										
15.5	0.009a	0.003ab	0.030b	0.043bc	3.1b	2.7b	19b	652ab	0.74d	0.20a
1.55	0.011ab	0.003ab	0.027b	0.040abc	3.0ab	2.4b	17b	695ab	0.67c	0.25ab
0.155	0.008a	0.002a	0.009a	0.018a	2.5a	0.4a	6a	676ab	0.48b	0.42c
CO_2-enriched										
15.5	0.014ab	0.004bc	0.033b	0.051bc	3.0ab	4.4b	24b	767b	0.65c	0.26ab
1.55	0.019b	0.005c	0.042b	0.066c	4.0c	3.4b	25b	609a	0.63c	0.29b
0.155	0.011ab	0.002a	0.010a	0.023ab	2.7ab	0.4a	6a	654ab	0.42a	0.49d
50 days										
CO_2-control										
15.5	0.030bc	0.010b	0.101bc	0.140bc	4.7b	7.9c	54cd	542a	0.72c	0.22a
1.5	0.030ab	0.008b	0.075b	0.113b	4.6b	4.3b	40b	558a	0.66c	0.26a
0.155	0.018a	0.002a	0.016a	0.036a	2.5a	0.9a	8a	540a	0.44a	0.50c
CO_2-enriched										
15.5	0.041c	0.010b	0.108c	0.158c	4.7b	8.4c	63d	585a	0.68c	0.26a
1.55	0.042c	0.007b	0.074bc	0.123bc	4.2b	4.4bc	41bc	555a	0.60b	0.34b
0.155	0.020ab	0.003a	0.020a	0.043a	2.9a	1.0a	10a	540a	0.46a	0.48c
60 days										
CO_2-control										
15.5	0.081bc	0.032b	0.219b	0.331b	6.9b	12.3b	117b	551cd	0.68b	0.23a
1.55	0.055bc	0.019b	0.141b	0.215b	5.5b	5.6b	72b	513bc	0.65b	0.26a
0.155	0.021a	0.004a	0.027a	0.052a	3.3a	1.3a	11a	417a	0.52a	0.40b
CO_2-enriched										
15.5	0.118c	0.044b	0.264b	0.426b	6.8b	13.9b	150b	579d	0.65b	0.26a
1.55	0.080c	0.023b	0.200b	0.303b	5.3b	7.7b	85b	431a	0.67b	0.26a
0.155	0.030ab	0.003a	0.035a	0.068a	2.9a	2.1a	16a	480ab	0.50a	0.45b
100 days										
CO_2-control										
15.5	0.352cd	0.123c	0.830b	1.305b	12.4d	28.0c	400c	474d	0.63cd	0.28ab
1.55	0.298c	0.069b	0.595b	0.962b	10.0bc	14.7b	220b	381bc	0.62c	0.31b
0.155	0.059a	0.009a	0.059a	0.127a	4.0a	1.0a	20a	365abc	0.46a	0.47d
CO_2-enriched										
15.5	0.483d	0.149c	1.280c	1.912c	11.5cd	35.1c	506c	399c	0.67d	0.25a
1.55	0.428cd	0.069b	0.656b	1.153b	9.1b	11.9b	196b	312a	0.57b	0.37c
0.155	0.081b	0.011a	0.076a	0.167a	4.2a	2.1a	25a	326ab	0.45a	0.48d

had significantly lower LWR and higher RWR than did those fertilized with the high-N or medium-N solutions (Table 3). At ambient CO_2 levels, RWR did not differ between seedlings of the medium-N and high-N treatments, but CO_2 enrichment significantly increased RWR and decreased LWR of the seedlings grown in the medium-N regime. Thus, CO_2 enrichment significantly increased total dry mass of seedlings grown in the high-N regime; it may also have affected biomass partitioning slightly, with the photosynthate preferentially directed to leaves when N was abundant and to roots when N was moderately deficient.

Populus tremuloides

Nitrogen supply significantly affected growth parameters of *P. tremuloides* at all harvests. Leaf area and root, leaf, and total dry mass of *P. tremuloides* increased with increasing N. Stem mass was greatest in the high-N treatments through 60 days, but seedlings in the high-N and medium-N treatments did not differ at 100 days (Table 4). Stem height did not differ between seedlings in the high-N and medium N treatments after 50 days. Seedlings grown in the high-N regime had well-developed sinker and lateral roots, those grown in the low-N regime had fine, fibrous roots, and those grown in the medium-N regime were intermediate.

Table 4. Means of measured and calculated growth parameters of <u>Populus tremuloides</u> after 30, 40, 50, 60, and 100 days of treatments. Mean values followed by the same letter, within a column and harvest, do not differ significantly at $P \leq 0.05$. (CO_2-control = 350 ul· l^{-1} CO_2; CO_2-enriched = 750 ul l^{-1}) Other abbreviations as in Table 3.

Treatment (mM-N)	Mass (g)				Height (cm)	LA (cm^2)	SLA ($cm^2\,g^{-1}$)	LWR	RWR
	Root	Stem	Leaf	Total					
30 days									
CO_2-control									
15.5	0.073b	0.017c	0.136c	0.226c	4.4b	49cd	336a	0.60bc	0.33a
1.55	0.043b	0.008b	0.056b	0.107b	3.1b	19b	316a	0.51ab	0.42b
0.155	0.003a	0.001a	0.005a	0.009a	0.9a	2a	316a	0.55abc	0.38ab
CO_2-enriched									
15.5	0.207c	0.058d	0.373d	0.638d	9.1c	100d	274a	0.59bc	0.32a
1.55	0.055b	0.009bc	0.057b	0.120bc	4.0b	20bc	341a	0.48a	0.45b
0.155	0.006a	0.001a	0.009a	0.015a	1.4a	4a	303a	0.60c	0.32a
40 days									
CO_2-control									
15.5	0.442bc	0.158d	0.786d	1.386d	16.5c	203c	258b	0.57a	0.31a
1.55	0.134b	0.036b	0.178b	0.349b	8.1b	60b	340d	0.55a	0.36a
0.155	0.009a	0.002a	0.014a	0.025a	1.9a	5a	316cd	0.57a	0.35a
CO_2-enriched									
15.5	0.646c	0.209d	1.139d	1.994d	17.9c	214c	94a	0.57a	0.33a
1.55	0.248b	0.076c	0.340c	0.664c	13.0c	94b	281bc	0.52a	0.37a
0.155	0.022a	0.005a	0.028a	0.055a	2.9a	8a	283bc	0.52a	0.37a
50 days									
CO_2-control									
15.5	1.290d	0.447e	1.585d	3.322d	24.9d	312d	199b	0.48a	0.39b
1.55	0.259c	0.088c	0.388c	0.735c	14.0c	102c	273b	0.54bc	0.34ab
0.155	0.021a	0.005a	0.033a	0.059a	3.1a	10a	313c	0.57c	0.34ab
CO_2-enriched									
15.5	1.345d	0.535e	1.833d	3.713d	27.8d	304d	167a	0.50ab	0.36ab
1.55	0.386c	0.166d	0.586c	1.139c	17.9c	137c	237a	0.54abc	0.32a
0.155	0.048b	0.016b	0.091b	0.155b	5.7b	21b	247b	0.60c	0.29a
60 days									
CO_2-control									
15.5	2.71d	0.95d	2.34cd	6.00d	34.5c	424c	177b	0.37a	0.47b
1.55	1.09c	0.42c	1.20c	2.71c	29.0c	246c	207bc	0.45b	0.40ab
0.155	0.04a	0.01a	0.06a	0.11a	5.4a	20a	345d	0.55d	0.34a
CO_2-enriched									
15.5	2.34d	0.91d	2.65d	5.90d	35.4c	349bc	130a	0.45b	0.40ab
1.55	1.04c	0.45c	1.28cd	2.77c	28.2c	227bc	178b	0.46bc	0.37a
0.155	0.16b	0.04b	0.20b	0.41b	9.5b	45b	227c	0.53cd	0.37a
100 days									
CO_2-control									
15.5	5.72c	2.06b	4.10c	11.88c	46.3b	558c	141b	0.35a	0.49b
1.55	2.35b	1.24b	2.26b	5.84b	45.0b	349b	156b	0.39a	0.39a
0.155	0.26a	0.09a	0.32a	0.67a	12.7a	75a	238d	0.51b	0.36a
CO_2-enriched									
15.5	5.14c	1.94b	4.81c	11.90c	39.9b	518c	107a	0.41a	0.43ab
1.55	2.37b	1.45b	2.02b	5.83b	38.7b	349b	156ab	0.39a	0.39ab
0.155	0.33a	0.11a	0.40a	0.84a	11.9a	72a	190c	0.50b	0.38a

Carbon dioxide enrichment significantly increased biomass, leaf area, and height of P. tremuloides, but the effects were temporary (Table 4). After 30 days, CO_2 enrichment significantly increased biomass and height of seedlings fertilized with high-N solutions, but these effects were not observed in later harvests. The CO_2 x N interaction was significant for stem height at 50 and 60 days.

Significant increases in growth of the low-N treated seedlings, due to CO_2 enrichment, were observed only in those seedlings harvested at 60 days; effects on growth of the medium-N treated seedlings were noted only at the 40 and 50 day harvests (Table 4). Seedlings grown in the high-N regime had high relative growth rates (RGR) during the second harvest interval, followed by a sharp, continuous decrease in RGR to the final harvest (Figure 4). Seedlings grown in the low-N regime showed a less distinct peak in RGR and a more gradual decrease during the latter half of the study. The seedlings grown in the medium-N regime showed no consistent pattern in RGR.

Populus tremuloides showed symptoms of mineral shortage during the later stages of the study. Leaves of seedlings grown in the medium-N and low-N solutions were yellowish-green; new leaves of seedlings grown in the high-N solution had blackened leaf tips and were abnormally curled, possibly a symptom of Ca

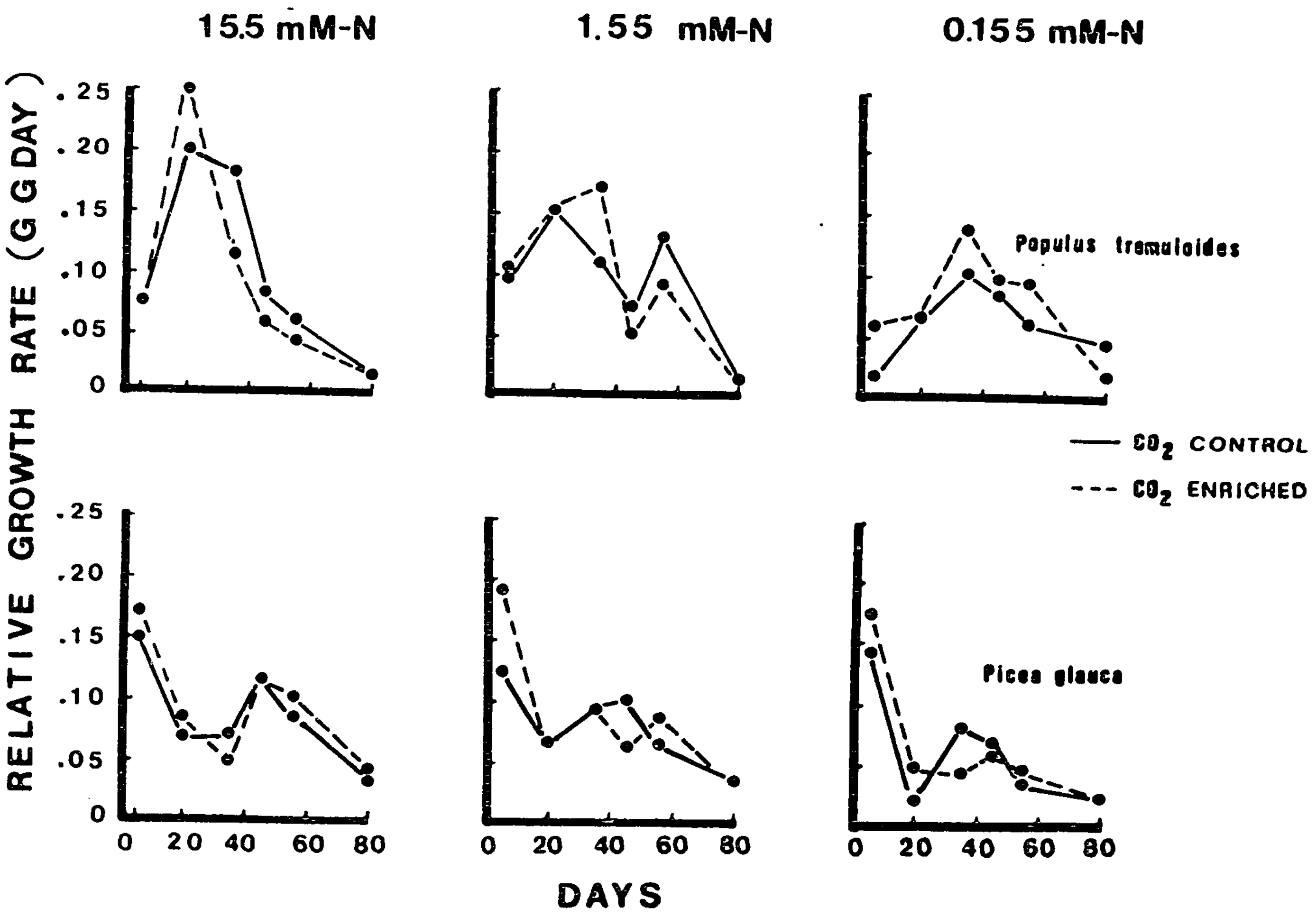

Figure 4. Mean relative growth rates (RGR) by harvest interval for Populus tremuloides and Picea glauca. Means are plotted at the midpoint of each interval between harvests.

deficiency (Erdmann et al. 1979). Leaf senescence began after 30 days. Seedlings in the CO_2-enriched, medium-N regime senesced the most leaf mass and the highest percentage of leaf mass produced (Table 5.)

The functional analysis of seedling growth supported trends inferred from primary growth measures at individual harvests and suggested others. The duration of exponential growth, as indicated by changes in the fit of linear regressions to ln-transformed growth data, was shortest for CO_2-enriched, high-N _P_. _tremuloides_, longer for low-N _P_. _tremuloides_, and longest for _P_. _glauca_, regardless of N treatment (Table 6). Coefficients of determination (r^2) for the regressions exceeded 0.80 in all cases; in 20 of the 36 regressions, r^2 exceeded 0.90. Relative growth rates of CO_2-control P. tremuloides correlated with NAR, but not LAP or LWP, across N regimes. In contrast, the RGR of CO_2-enriched _P_. _tremuloides_ correlated with both NAR and LWP across N treatments (Table 6). Carbon dioxide enrichment increased RGR via increased NAR in all N regimes and decreased LAP and LWP in the low-N regime.

Relative growth rates (RGR) increased less in _P_. _glauca_ than in _P_. _tremuloides_ with CO_2 enrichment and increased N (Table 6). Increased RGR did not correlate with NAR under CO_2 enrichment. Within a N regime, CO_2 enrichment may have increased NAR (Table 6).

Table 5. Senesced leaf mass (g), by harvest interval, of *Populus tremuloides* grown at atmospheric CO_2 levels of 350 or 750 ul l^{-1} (mean ± one 90% C.I.). TLM=final leaf mass.

	HARVEST INTERVAL (Days)				%TLM
TREATMENT	31-40	41-50	51-60	61-100	
350 ul l^{-1}					
15.5 mM-N	0.01(.00)	0.04(.03)	0.04(.05)	0.14(.15)	5
1.55 mM-N	0	0	0.01(.01)	0.04(.04)	2
0.155 mM-N	0	0	0	0.01(.00)	3
750 ul l^{-1}					
15.5 mM-N	0	0.04(.05)	0.04(.08)	0.14(.21)	4
1.55 mM-N	0	0.04(.03)	0.02(.01)	0.55(.38)	21
0.155 mM-N	0	0	0	0	0

	GROWTH PARAMETERS[1/]													
	DAYS	n	W_0	RGR	r^2	A_0	RAGR	r^2	L_0	RLGR	r^2	NAR	LAP	LWP
P. *tremuloides*														
350ul $l^{-1}CO_2$														
15.5mM-N	40	29	.0012	.166	0.96	.289	.157	0.95	.0010	.162	0.96	83.8	187.3	.743
1.55mM-N	40	29	.0015	.125	0.90	.347	.114	0.89	.0011	.118	0.88	67.0	170.0	.614
0.155mM-N	60	43	.0015	.065	0.82	.301	.062	0.81	.0010	.063	0.79	35.5	174.8	.600
750ul l^{-1}														
15.5mM-N	30	21	.0011	.197	0.94	.248	.188	0.97	.0009	.194	0.94	100.0	187.9	.762
1.55mM-N	40	29	.0014	.143	0.96	.331	.132	0.96	.0011	.135	0.95	74.6	177.3	.643
0.155mM-N	60	43	.0015	.086	0.88	.304	.078	0.84	.0009	.083	0.87	53.7	145.6	.536
P. *glauca*														
350ul l^{-1}														
15.5mM-N	90	41	.0035	.063	0.93	1.93	.057	0.92	.0022	.064	0.92	14.9	380.7	.680
1.55mM-N	90	42	.0027	.062	0.91	1.75	.051	0.89	.0015	.063	0.90	15.1	339.7	.616
0.155mM-N	90	42	.0037	.037	0.87	1.76	.026	0.84	.0017	.037	0.86	12.9	198.6	.456
750ul l^{-1}														
15.5mM-N	90	42	.0037	.066	0.90	1.83	.061	0.89	.0020	.068	0.90	15.9	385.5	.628
1.55mM-N	90	42	.0051	.057	0.93	2.62	.047	0.88	.0027	.059	0.92	17.1	276.5	.589
0.155mM-N	90	42	.0046	.038	0.91	2.07	.027	0.86	.0020	.038	0.89	14.3	188.0	.449

1/ W_0 , A_0 , L_0 represent initial total mass (g), leaf area (cm^2), and leaf mass (g), respectively.
RGR, RAGR, RLGR represent relative growth rate (g/g/d), relative leaf area growth rate ($cm^2/cm^2/d$), and relative leaf mass growth rate (g/g/d), respectively.
NAR= net assimilation rate ($mg/dm^2/d$)
LAP=leaf area partitioning coefficient((cm^2-d^{-1})/(g-d^{-1}))
LWP=leaf mass partitioning coefficient(g d^{-1} / g d^{-1})

Table 6. Growth parameters of *Populus tremuloides* and *Picea glauca* during the period of exponential growth associated with each treatment. Relative growth rate (RGR), net assimilation rate (NAR), and the leaf weight and leaf area partitioning coefficients (LWP,LAP) were calculated as described in Appendix 1.

DISCUSSION

As the supply of N increased, P. glauca allocated more dry mass to shoots, a common response in plants. Nitrogen stimulates leaf production (Fagerstrom and Lohm 1977), possibly through the increased production of cytokinins in root tips and their eventual export to the shoot (Marschner 1983). The functional analysis of growth patterns suggested a similar effect in P. tremuloides during the period of exponential growth, but those trends did not persist. Likewise, root:shoot ratios in P. tremuloides were unaffected by supply of P (Chapin et al. 1983).

My observations of differences in root systems of high-N and low-N P. tremuloides were supported by data from a subsequent experiment (Chapter 4), in which the proportion (dry mass) of roots less than 1 mm diameter increased with decreasing N supply. The large lateral roots of P. tremuloides typically give rise to adventitious shoots (suckers) in natural stands (Brinkman and Roe 1975). An abundance of N may preferentially increase the proportion of these roots relative to other parts of the root system.

Although CO_2 level may have interacted with N supply in affecting root:shoot partitioning in P. glauca, the mechanism is unclear. Brouwer (1966) suggested that the ratio of mineral nutrients:carbohydrates in the plant determined whether the root or shoot grew faster, mineral nutrients accumulating in the roots when nutrient availability was limiting. This would lead to proportionally greater root growth. By extension, CO_2 enrichment should increase root growth, relative to shoot growth, if the ratio of minerals:carbohydrates in tissues decreases past a critical level. In general, CO_2 enrichment may preferentially increase the growth of organs nearest the supply of limiting resources (Baker and Enoch 1983).

Carbon dioxide enrichment affected the growth of *P. tremuloides* and *P. glauca* differently. It significantly increased the leaf and total mass of *P. glauca* only in the high-N treatment and increased root mass only in the low-N regime. In contrast, root, stem, and leaf mass increased in *P. tremuloides* in both the high-N and low-N regimes. My observation that NAR increased more with increased N supply in *P. tremuloides* than in *P. glauca* agrees with photosynthetic responses documented for seedlings of other hardwoods and conifers (Linder and Rook 1984).

Why did the effect of CO_2 enrichment not persist? The effect may be most pronounced in young plants and decrease over time (Kramer 1981). Decreased growth rates may result from long-term inhibition of photosynthesis associated with starch buildup in chloroplasts (Cave et al. 1981; DeLucia et al. 1985). Alternatively, higher CO_2 concentrations may have accelerated the development of mineral nutrient deficiencies, as mineral nutrient supply was constant throughout the experiment. Ingestad (1982) demonstrated that nutrient supply had to increase with plant size if a constant internal nutrient status was to be maintained. Carbon dioxide enrichment may have temporarily accelerated biomass accumulation, resulting in decreased tissue concentrations of nutrients and ultimately reduced RGR. This explanation is supported by data in Chapter 3.

The effects of CO_2 enrichment on growth of P. glauca were less than previously reported. Seedlings grown at 600 ul l^{-1} were 250 % taller and 650 % heavier than those grown at 330 ul l^{-1} (Higginbotham 1983). Root weight ratios were similar at 330 and 600 ul l^{-1} and increased at 1000 ul l^{-1} CO_2, consistent with Brouwer's nutrient:carbohydrate hypothesis.

There are few published data of dry mass accumulation and partioning under various CO_2 and nutrient regimes. Dry mass of shoots of cotton increased two-fold with CO_2 enrichment to 650 ul l^{-1}, across a range of N supplies (Wong 1979). Carbon dioxide enrichment to 1500 ul l^{-1} proportionally increased biomass of Noogoora burr (Xanthium occidentale Bertol.) similarly across a range of N supplies (Hocking and Meyer 1985). Allocation was affected by N supply, but not by CO_2 enrichment. Carbon dioxide enrichment to 675 ul l^{-1} increased total dry mass of soybean, sicklepod, and showy crotelaria, the increases being proportionally greater in plants supplied with 1/2-strength

Hoagland's solution than in those supplied with 1/8-strength solution (Patterson and Flint 1982).

Comparisons of the effects of CO_2 enrichment on growth of *Populus tremuloides* seedlings raised under different N regimes are made difficult by the probable changes in nutrient status of plants over time. The temporary increases in mass of CO_2-enriched plants may or may not have persisted had nutrient supply been adjusted to maintain constant internal nutrient status. It would appear, however, that:

(1) Partitioning of biomass was more strongly affected byN supply in *Picea glauca* seedlings than in *Populus tremuloides*, (2) dry mass accumulation by *P. tremuloides* decreased more with decreasing supply of N, and (3) during exponential growth, CO_2-enrichment and increasing N supply enhanced NAR more in *Populus tremuloides* than in *Picea glauca*.

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Chapter 3. EFFECTS OF CO_2 ENRICHMENT ON NUTRIENT CONTENTS OVER TIME IN *POPULUS TREMULOIDES*

INTRODUCTION

Atmospheric CO_2 levels of 750 ul l^{-1} caused large, but temporary increases in biomass of seedlings of *Populus tremuloides*, trembling aspen, when seedlings were subjected to different, but constant rates of nitrogen supply (Chapter 2). At high rates of N supply, CO_2 enrichment more than doubled total dry mass (TDM) and leaf area of seedlings after 30 days of treatments, but the effects were not significant at 40 days. In contrast, CO_2 enrichment quadrupled the dry mass and doubled the leaf area of seedlings subjected to low rates of N supply (1/100 of the high rate) at 60 days, but the effect disappeared by the 100 day harvest. Similar patterns were not observed in seedlings of *Picea glauca*, white spruce. Possibly, CO_2 enrichment stimulated growth rates of *P. tremuloides*, decreased tissue nutrient concentrations, and ultimately accelerated seedlings of each N regime into a state of deficiency for one or more nutrients.

Ingestad (1982) outlined the relations between nutrient supply, nutrient demand associated with plant growth, and plant nutrient status, and demonstrated the high correlation between relative growth rates (RGR) and plant nitrogen status. Exponential rates of seedling growth require exponential increases in nutrient requirements if plant nutrient concentrations are to remain constant. If the daily requirement for nutrients exceeds the daily supply, then concentrations will decrease, as will RGR. Time-dependent changes in plant nutrient status may therefore affect the interpretation of plant growth studies.

The purpose of this study was to evaluate how concentrations of N, P, K, Ca, and Mg in Populus tremuloides varied with CO_2 enrichment, N supply, and time. Plant tissue collected prior to, during, and after the period of high CO_2-enhanced growth, was analyzed. Concentrations of K, Ca, and Mg were determined only on foliar tissue. Nitrogen concentrations in Picea glauca are also presented.

METHODS

The culture conditions and room environments were described in Chapter 2. Atmospheric CO_2 levels in the two rooms were 350 (ambient) or 750 ul l^{-1} (enriched); the concentrations of N in nutrient solutions were 15.5 mM, 1.55 mM, and 0.155 mM in the high-N, medium-N, and low-N treatments. Seedlings were grown in acid-washed sand. Nutrient solutions were added 6 times per week (75 ml for Populus, 25 ml for Picea). Plants were harvested for growth measurements at 10, 30, 40, 50, 60, and 100 days after initiation of the experiment. All harvested plant material was dried at 70 C for a minimum of 24 hours prior to further processing for determination of mineral contents.

Populus seedlings were small (0.11-0.66g mean total dry mass) when growth responses to CO_2 enrichment were observed. A minimum of 0.2 g (dry mass) of tissue was required for mineral analysis. Thus, the individual parts (root, stem, leaf) of each plant in a given treatment x harvest combination were pooled in some cases; in others, all harvested plants in a given treatment x harvest combination were pooled. If possible, each replicate plant within a given treatment and harvest was divided into root, stem, and leaf and the mineral content was determined for each (Table 7).

Plant tissue was digested in a mixture of H_2SO_4 and H_2O_2 (Thomas et al. 1967) heated to 400 C in a block digestor. The digestor was fitted with reflux columns (Panasiuk and Redshaw 1977) to reduce fume emissions during digestion. The digest was previously deemed suitable for determination of N, P, K, Ca, and Mg (Van Lierop 1976). Appropriate blanks and pine needle tissue internal standards (National Bureau of Standards) were included at the rate of two each per 36 sample digest.

Table 7. Partitioning of plant material for mineral analyses, listed in order of increasing plant size. Parentheses enclose the plant parts pooled in the analyses.

PLANT PARTS ANALYZED	NUMBER OF REPLICATES
(Root + Stem + Leaf)	1 (all plants pooled)
(Root + Stem) + Leaf	1 of each
(Root + Stem + Leaf)	5
(Root + Stem) + Leaf	5 of each
Root / Stem / Leaf	5 of each

Total N and P were measured colorimetrically with an autoanalyzer, while cations were measured using atomic absorption (AA) spectrometry. The presence of SO_4 in the digest mixture may interfere with determination of Ca (Lunyon and Heald 1980); thus, Ca standards included H_2SO_4. Lanthium, as La_2O_3, was added to reduce interference. The analytical coefficients of variation for the internal standard were 4.5 % (N); 4.8 % (P); 5.5 % (K); 6.1 % (Ca); and 6.9 % (Mg).

Statistical comparisons were based upon 95% confidence intervals. The CO_2 treatments were regularly rotated between the two rooms to equally expose the plants from the two CO_2 regimes to any other systematic differences in room environments.

RESULTS

Whole-plant and foliar concentrations of N and P in P. tremuloides decreased over time in all treatments (Figure 5, Table 8). Whole-plant N concentrations decreased more slowly in P. glauca (Figure 6). Foliar concentrations of Ca and Mg decreased over time in the 15.5 mM-N and 1.55 mM-N, but not the 0.155 mM-N regime (Table 8). Foliar concentrations of Ca and Mg, but not K, increased as N supply decreased. Within a CO_2 regime, concentrations of P, K, Ca, and Mg and the ratio of Ca:Mg increased relative to N as N supply decreased (Tables 8 and 9).

Figure 5. Whole-plant concentrations of N and P over time in Populus tremuloides (mean ± one 95% C.I.). If no C.I. is shown, n=1; otherwise, n=5.

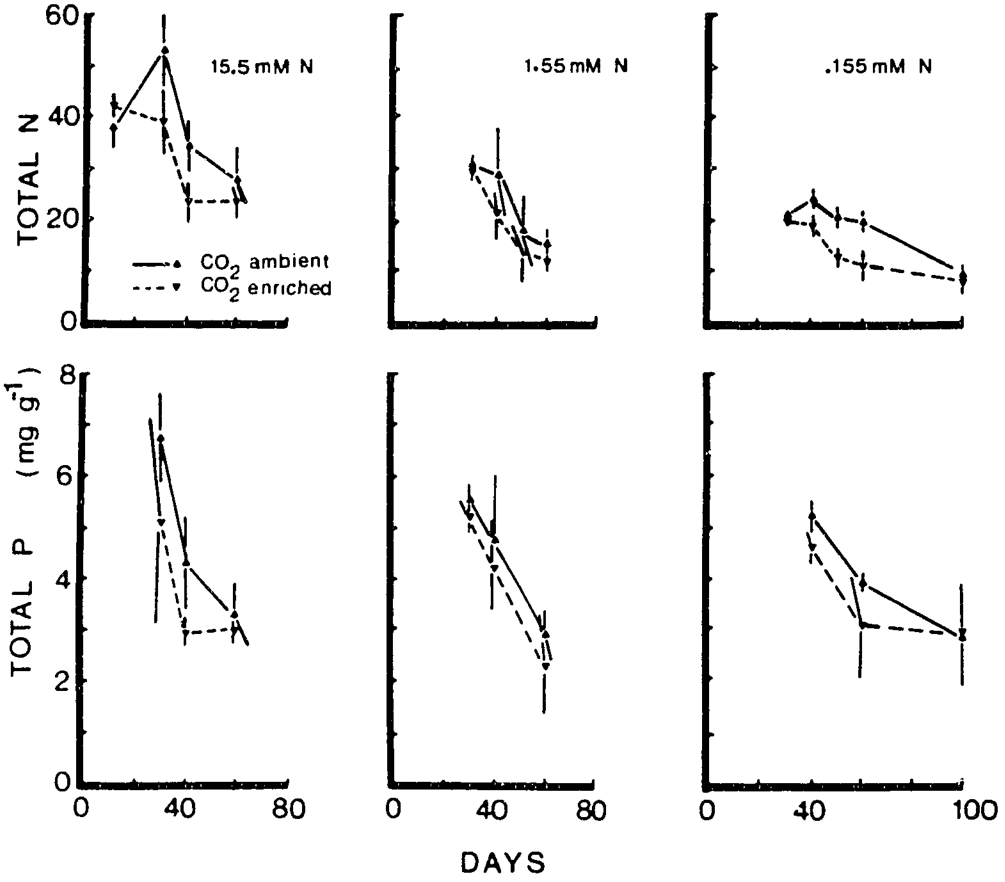

Table 8. Foliar concentrations (mg g^{-1} dry mass) of N, P, K, Ca, and Mg by harvest and relative growth rates (g(g-d)$^{-1}$) during the preceding harvest interval of *Populus tremuloides* fertilized with 15.5 mM-N, 1.55 mM-N, or 0.155 mM-N and grown at atmospheric CO$_2$ levels of 350 or 750 ul l^{-1}. (mean ± one 95 % CI; n=5 for nutrient concentrations; n=14 for relative growth rate). If no CI is shown, n=1.

DAYS	CONCENTRATION (mg g^{-1} dry mass)					RGR (g(g-d)$^{-1}$)
	N	P	K	Ca	Mg	
15.5 mM-N						
350						
30	---	---	---	---	---	0.19(.02)
40	42.0(6.1)	4.7(1.9)	16.6(5.0)	1.8(1.0)	2.4(0.9)	0.19(.03)
60	34.1(5.8)	4.0(1.6)	15.8(3.9)	1.7(0.6)	2.2(0.7)	0.07(.03)
750						
30	45.5(8.9)	5.1(1.4)	16.8(7.3)	2.2(0.8)	2.6(1.2)	0.25(.02)
40	27.4(4.1)	3.0(1.0)	13.2(5.4)	1.0(0.2)	1.4(0.3)	0.11(.03)
60	26.9(6.2)	3.3(0.8)	15.3(4.7)	0.9(0.2)	1.5(0.3)	0.06(.02)
1.55 mM-N						
350						
40	30.2	4.6	23.8	7.7	4.8	0.16(.08)
60	18.9(7.6)	2.8(0.7)	21.1(3.4)	3.5(0.9)	3.0(0.3)	0.13(.03)
750						
40	25.0	4.3(1.2)	19.3(7.3)	5.1(2.7)	3.7(1.7)	0.18(.04)
60	13.5(1.3)	2.2(0.6)	20.4(2.2)	2.1(0.8)	2.2(0.8)	0.10(.02)
0.155 mM-N						
350						
50	22.6	---	18.8	15.9	5.9	0.06(.05)
60	22.7	3.7	22.6	15.1	6.0	0.10(.05)
100	11.4(2.6)	2.2(0.6)	18.8(5.9)	7.6(5.1)	4.0(1.6)	0.04(.01)
750						
50	12.6	4.6	15.4	10.7	4.3	0.13(.07)
60	12.6(2.2)	2.7(0.8)	20.0(5.7)	7.2	4.4(1.6)	0.10(.05)
100	9.5(2.2)	2.2(1.2)	20.4(3.4)	11.9(8.0)	4.9(2.4)	0.02(.01)

Figure 6. Whole-plant concentrations of N over time in *Picea glauca*. Vertical bars represent ± one 95% C.I. about each mean (n=5). If no C.I. is shown, n=1.

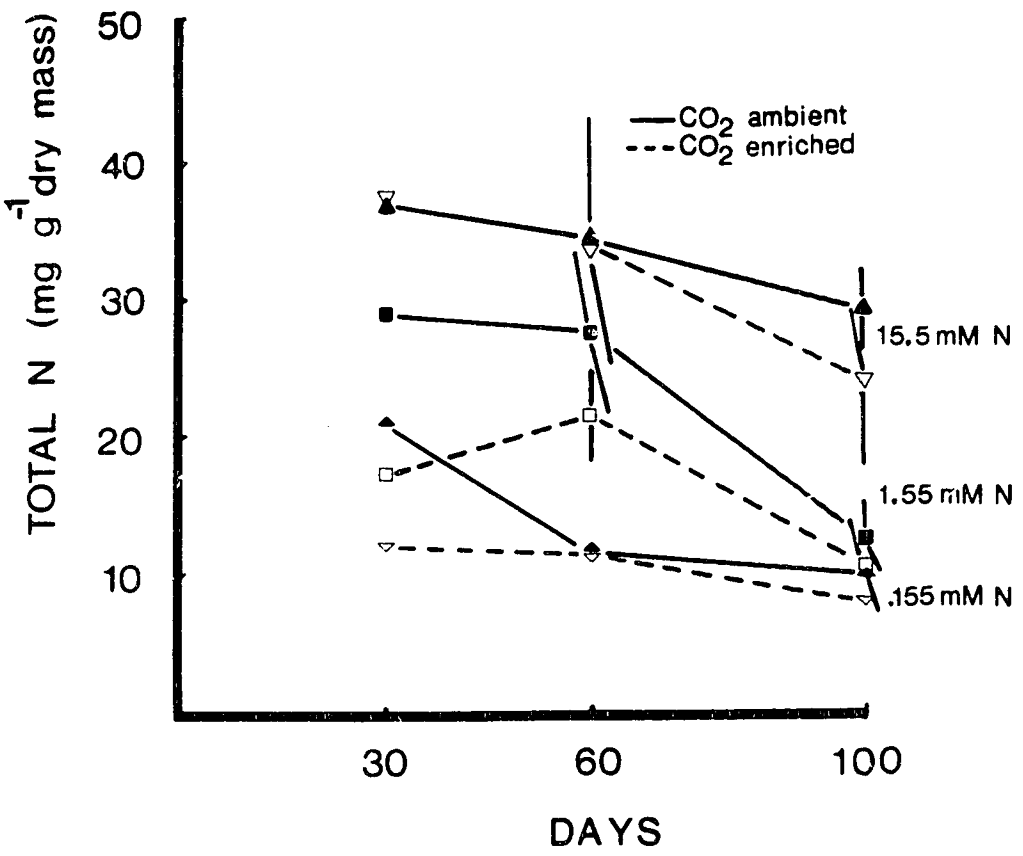

Table 9. Ratios of Ca:Mg in foliage of *Populus tremuloides* as a function of time, CO_2 level, and supply of N. (mean ± one 95 % C.I). If $n \leq 2$, no C.I. was calculated; otherwise, n=5.

TREATMENT	DAYS			
	30	40	60	100
15.5 mM-N				
350 ul l^{-1}	------	0.78(.24)	0.77(.15)	------
750	0.86(.25)	0.73(.28)	0.63(.15)	------
1.55 mM-N				
350	------	1.55	1.15(.27)	------
750	------	1.44	0.99(.24)	------
0.155 mM-N				
350	------	2.68	2.53	1.89(.62)
750	------	2.46	1.62	2.38(.88)

Decreased foliar elemental concentrations accompanied decreases in relative growth rates in both CO_2 regimes. but the decreases occurred sooner in CO_2-enriched seedlings (Table 8). In the 15.5 mM-N regime, CO_2-enrichment increased the mean relative growth rate (RGR) to 30 days and increased biomass at the 30 day harvest. After 30 days, RGR of CO_2-enriched seedlings were lower than those of the controls; the decrease coincided with decreased total N and P and foliar N, P, Ca, and Mg (Table 8). Likewise, CO_2-enriched seedlings in the 0.155 mM-N regime were significantly heavier than controls 60 days into the study, but were not larger at 100 days, the next harvest. The decreased rates of dry matter accumulation by CO_2-enriched plants from 60-100 days accompanied decreased whole-plant and foliar concentrations of N.

DISCUSSION

The data presented here appear to support the original hypothesis. Carbon dioxide enrichment stimulated growth when nutrient supplies were abundant relative to plant requirements, but also accelerated seedlings into a state of nutrient deficiency for one or more elements when plant demands for nutrients exceeded the fixed supply of the system. This interpretation is supported by subsequent work (Chapter 4), which showed that CO_2-enriched _P. tremuloides_ seedlings were larger than controls at high rates of N addition and had consistently higher relative growth rates at restricted rates of N addition, even though the control plants were much larger than in this study. These data do not preclude the possibility that CO_2 enrichment might partially compensate for moderate shortages of N (Wong 1979; Hocking and Meyer 1985) or other elements, while ultimately accelerating the onset of deficiencies.

To definatively ascribe reductions in relative growth rate to induced deficiencies of a specific element is difficult or impossible, because total and / or foliar concentrations of more than one element decreased in any given treatment. Few data relate productivity to elemental concentrations in seedlings or mature individuals of P. tremuloides. In addition, changes in concentrations of one element may affect the critical concentration of others (Bates 1971).

Relative growth rates of P. tremuloides seedlings were 2-4 fold higher(depending on the stage of the experiment) at foliar N concentrations of 34 mg g^{-1} than at foliar N concentrations of 17 mg g^{-1} (Chapter 4). Likewise, RGR of Populus simonii seedlings with foliar N concentrations of 44 mg/g were 3 fold greater than those with concentrations of 21 mg/g N (Jia and Ingestad 1984). Foliar concentrations of P, Ca, and Mg in the slower-growing plants in that study were 2.1, 1.8, and 2.2 mg g^{-1}, respectively. Possibly, all elements except K were at deficient levels during the later harvests in the 15.5 mM-N regime; N and P may have become deficient in the 0.155 mM-N regime. Foliar concentrations of Ca in these seedlings were lower than concentrations reported for natural stands of mature *P. tremuloides* (Coyne and Van Cleve 1977; McColl 1980); this may reflect higher soil solution concentrations of Ca (relative to other elements) than in the nutrient solutions used here.

The low foliar concentrations of Ca observed in response to increasing N and CO_2 enrichment may have resulted from dilution in leaf tissue, decreased uptake, or reduced transport to developing leaves. Preferential uptake of NH_4-N might indirectly reduce the uptake of Ca by decreasing pH in the rhizosphere (Haynes and Goh 1978) and by decreasing the efficiency of the H^+-efflux pump (Marschner 1986). Decreased transpiration rates resulting from CO_2 enrichment (Chapter 3) might also restrict transport of Ca within the plant. Given only these foliar data, it is not possible to determine whether uptake of Ca or transport of Ca within the plant was affected more by N supply and CO_2 enrichment. Elsewhere, CO_2-enriched *Pinus virginiana* and *Quercus alba* accumulated significantly greater quantities of Ca than did controls (Luxmoore et al. 1986; Norby et al. 1986); in both studies, the soils used appeared to be low in N. Allocation of Ca in *Q. alba* was unaffected by CO_2 enrichment (Norby et al. 1986).

The data from this and the associated growth study (Chapter 2) demonstrate the potential problems in interpreting the results of CO_2 enrichment studies when plant nutrient status decreases over time. While these data represent an extreme situation, they do raise the question of whether CO_2 effects reported in similar studies were also confounded with time-dependent changes in plant nutrient status. Carbon dioxide enrichment has been associated with increased concentrations of starch in foliage (Cave et al. 1981; DeLucia et al. 1985), decreased concentrations of mineral nutrients (Porter and Grodzinski 1984), enzyme activity and concentration in leaves (Wong 1979; Porter and Grodzinski 1984), long-term decreases in photosynthetic (DeLucia et al. 1985) and relative growth rates (Tolley and Strain 1984), and increases in ratios of root:shoot biomass (Higginbotham 1983). However, a decrease in nutrient status may induce similar responses (Wong 1979; Ingestad 1982; Linder and Rook 1984; McDonald et al. 1986; Agren and Ingestad 1987). Many studies of the effects of long-term CO_2 enrichment do not indicate whether plant nutrient concentrations changed over time, nor do they define the potential supply of nutrients relative to demand at the time physiological measurements were made. Such information may be essential for interpreting whether physiological responses attributed directly to CO_2 enrichment are, in fact, an unplanned response to decreasing nutrient status.

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Chapter 4. EFFECTS OF CO_2 ENRICHMENT AND PLANT NITROGEN STATUS ON *POPULUS TREMULOIDES* AND *PICEA GLAUCA*. I. MINERAL NUTRITION

INTRODUCTION

Data presented in Chapter 2 demonstrated how the measured responses of plants to CO_2-enrichment may be time-dependent. If the experimental period is short (e.g. 30 days), growth enhancement by CO_2-enrichment of slowly-growing plants (*P. glauca* or low-N *P. tremuloides*) may go undetected. Likewise, if the experimental period is longer (e.g. 100 days), but demands for mineral nutrients exceed supplies, the effects of CO_2-enrichment on rapidly-growing species may also be undetected or underestimated. Detection of effects in such a situation obviously requires frequent harvests. In neither case could the observed effects be extrapolated for field projections. Growing seasons on well-drained boreal sites often exceed 100 days (e.g. National Oceanic and Atmospheric Administration 1980) and naturally occurring plants are not normally subject to drastic decreases in nutrient concentrations (Ingestad 1982).

The purpose of this study was to evaluate the effects of atmospheric CO_2 enrichment on seedlings of _P_. _tremuloides_ and _P_. _glauca_ when plant N concentrations were held constant over time at high or low nitrogen levels. In this chapter, I describe a technique for maintaining steady-state nutrition and examine the effect of CO_2 enrichment and plant N status on long-term accumulation and allocation of N, P, K, Ca, and Mg. Effects on growth and water use are considered in Chapter 5. Whole-plant concentrations of N and P and foliar concentrations of N, P, Ca, and Mg decreased over time and under CO_2 enrichment in experiment 1 (Chapter 3). However, the decreases may have resulted from insufficient supplies of nutrients rather than CO_2-induced reductions in plant nutrient demands. An understanding of whether CO_2 enrichment directly affects the ratios of carbon to N and other mineral elements may be important in projecting long-term trends in productivity, decomposition, and herbivory (Chapter 6).

METHODS

The study was conducted in two controlled environment rooms with a 22/17 C (day/night) temperatures and an 18 hour thermoperiod and photoperiod. A mixture of sodium and mercury vapor high intensity discharge (HID) lamps supplied a photosynthetic photon flux density (PPFD) of 400 umol $m^{-2}s^{-1}$ at canopy level. Air flow velocity across the bench was ca. 0.5 m s^{-1}. Daytime relative humidity was 45 % (VPD=1.40 KPa).

Atmospheric CO_2 levels in the two rooms were 350 ul l^{-1} (ambient) or 650 ul l^{-1} (enriched) and were monitored in each room for alternating 5 minute periods by a Beckman 864 infrared gas analyzer (IRGA). Set-point solenoid controls automatically allowed injection of bottled CO_2 into the high CO_2 (650 ul l^{-1}) room as necessary and also allowed the CO_2 treatments to be alternated between the two rooms.

Populus tremuloides (trembling aspen) and *Picea glauca* (white spruce) seeds were collected from individual trees in the area of Edmonton, Alberta, Canada. Processed seeds were stored at -17 C, then germinated in closed trays in the two rooms. Approximately 200 healthy seedlings of each species were then transferred into trays containing aerated pretreatment solution (equivalent to 1/2-strength start solution; Table 10) and grown until large enough to transplant into containers for initiation of either the "high-N" or "low-N" treatments.

Nutrient Regimes

The relative addition technique (Ingestad 1971; Ingestad and Lund 1986) was used to grow seedlings at high or low nitrogen concentrations maintained constant over time. In the pretreatment and high-N treatment, all elements were supplied in fixed proportions according to plant demand, as determined by pH and conductivity changes in the culture solutions. This technique

Table 10. Macronutrient concentrations and sources in start/conductivity adjustment and pH adjustment solutions. ST/COND= starting and conductivity adjustment solutions

	Concentration (g l^{-1})		
SALT	ST/COND (High-N)	ST/COND (Low-N)	pH ADJUSTMENT (High-N)
NH_3	---	---	19.0
HNO_3	0.8	---	0.8
NH_4NO_3	53.1	---	14.6
KNO_3	18.6	---	3.2
KOH	---	---	8.6
KH_2PO_4	14.3	14.3	14.3
K_2SO_4	11.8	---	11.8
$Ca(NO_3)_2$	7.2	---	7.1
$CaCl_2$	---	4.8	---
$Mg(NO_3)_2$	13.0	---	13.0
$MgSO_4$	---	8.5	---
$MgCl_2$	---	1.6	---
KCl	---	26.0	---

involves growing plants in solutions containing elements in proportions optimal for growth in numerous plant species (Ingestad 1979). When uptake of cations exceeds that of anions, plants maintain charge balance by excreting H^+ (Haynes and Goh 1978). Preferential assimilation of NH_4^+, relative to NO_3^-, will therefore decrease solution pH, if uptake of other cations and anions is balanced. The pH of the solution is then readjusted to the original level with a stock solution containing elements in the proportion assimilated and with a ratio of NH_4-N:NO_3-N equal to or in excess of that assimilated by the plant. Elements other than N may then be replaced by readjusting the solution conductivity to the starting value with a pH-neutral stock solution. The low-N regime, in contrast, consists of adding N at restricted, fixed rates of increase while ensuring that other elements are freely available to the plant. Solutions were not replaced during the experiment. All nutrients were added manually via the stock solutions shown in Table 10. The ratio of NH_4-N:NO_3-N in the pH adjustment solutions was 72:28 and in the start and conductivity adjustment solutons was 38:62.

Seedlings were randomly assigned to different N treatments, weighed fresh, and transplanted into dark plastic pots of 4 L (high-N regime) or 3 L (low-N regime) capacity. Each pot was assigned 4-5 seedlings, with a minimum of 6 pots per treatment. Additional seedlings were harvested for measurement of moisture and nutrient contents. Each seedling was held upright in a closed-cell foam collar inserted into a slot cut into the snap-on plastic lid. This arrangement facilitated the periodic removal of seedlings for fresh mass measurements. Continuous aeration maintained dissolved O_2 levels in excess of that required for maximum growth of P. glauca (Zinkan et al. 1974). This level was assumed to be adequate for growth of P. tremuloides.

Although N addition in the high-N regime began immediately after transplanting, low-N seedlings were first placed into N-free start solutions. Seedlings were grown in the N-free solutions until fresh mass had doubled and tissue N concentrations decreased proportionally. This rapid onset of N stress, followed by initiation of the desired suboptimal rate of N addition, should hasten the onset of steady-state growth and nutrition (Ingestad and Kahr 1985).

The relative N addition rates (RN) used to induce N stress were 0.06 $g\ g^{-1}d^{-1}$ for P. tremuloides and 0.03 $g\ g^{-1}d^{-1}$ for P. glauca. These addition rates were assumed to be ca. 30% of those required to maintain maximum relative growth rates (RGR) of P. tremuloides and P. glauca, based on growth data from Chapter 2. A 1:1 relation between RGR and RN was assumed (Ingestad and Lund 1986). The amount of N to be added on day i for each following 3 day period was calculated as :

$$[1] \qquad \ln N_{i+3} = RN(3) + \ln N_i$$

where N_i and N_{i+3} = the estimated amount of N (mg) in the plant at days i and i + 3, respectively. Whole-plant N concentrations (fresh mass) were assumed to be 0.6 % prior to their placement in the N-free solution. The amount of N to be added per pot was recalculated following each harvest. Nitrogen was supplied from either the high-N pH adjustment or start solutions in order to maintain adequate pH levels; conductivity was adjusted with the N-free start solution.

Carbon dioxide treatments were applied simultaneously to a given N treatment x species combination, but logistics required the different N treatments for the two species to be staggered. Furthermore, the duration of the experimental period, frequency and number of harvests, and number of plants sampled per harvest varied with N treatment and species, to ensure that sufficient plant material for tissue analysis remained at the end of the experiment. Thus, the total number and frequency of harvests was greater, and the duration of the experimental period shorter, for the rapidly growing high-N *P. tremuloides* seedlings than for the slower growing high-N *P. glauca* and low-N *P. tremuloides* and *P. glauca*. A maximum of one seedling per pot was randomly sampled at a given harvest.

Nutrient Analysis

Harvested seedlings were separated into roots, stems, and leaves and dried at 70 C for 24 hours. Immediately prior to the final harvest, samples of foliage used for concuctance measurements (Chapter 4) were extracted in dimethyl sulfoxide (DMSO) and analyzed for total chlorophyll (Hiscox and Israelstam 1978). Dried plant tissue was ground to pass a 20 mesh screen, then digested in a mixture of H_2SO_4 and H_2O_2 heated to 400 C in a block digestor (Van Lierop 1976). Blanks and samples of pine needle standards (National Bureau of Standards) were included at a rate of 2 each per 36 samples. Total N and P were determined colorimetrically with an autoanalyzer and K was measured using atomic absorption (AA) spectrometry.

Although Ca and Mg had previously been measured successfully using this digest and AA analysis (Van Lierop 1976; Chapter 3), the measurements were low and erratic in this study. Therefore, a subset of tissue samples from the same plants was dry-ashed at 500 C for 16 hours in a muffle furnace. Ash contents of samples were determined; samples were digested in 5% HCl, diluted, and analyzed for Mg and Ca using the AA. Coefficients of variation for elemental analysis of the pine needle standard were: 3.4 % (N); 6.0 % (P); 6.9 % (K); 2.4 % (Ca); and 3.7 % (Mg).

Concentrations of N were determined at all harvests. Whole-plant concentrations of Ca and Mg in high-N P. tremuloides were determined at intermediate harvests, but were not measured in the other species and nitrogen treatment combinations. Concentrations of other elements were determined only at the initial and final harvests. Solution concentrations of NH_4-N, NO_3-N, PO_4-P, K, Ca, and Mg were measured at the start and end of the experiment.

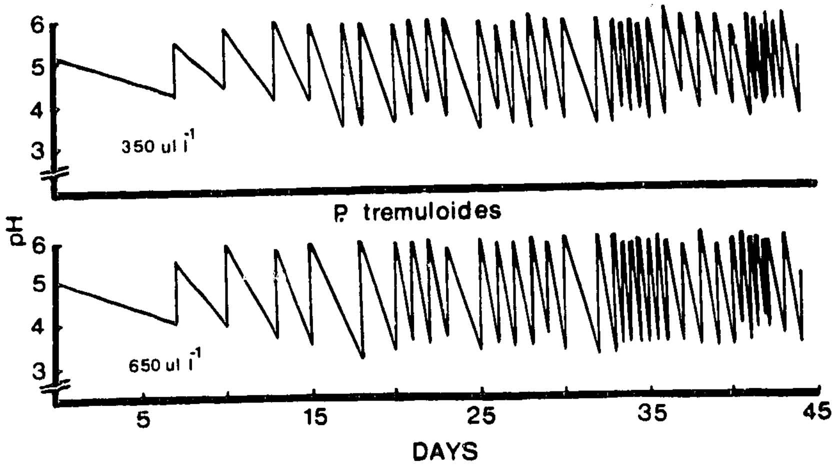
The use of two separate rooms for CO_2 treatments and the staggering of N regimes mean that data analysis using inferential statistics is inappropriate (Hurlbert 1984). I present only means and standard deviations, recognizing the difficulties inherent in inferring treatment effects using that approach.

RESULTS

Solution pH and Nutrient Content

Solution pH was generally maintained from pH 3.5-6.0 for high-N *P. tremuloides* and from pH 3.6-6.0 for high-N *P. glauca* (Figure 7) through periodic harvests and increasing frequency of titrations. Solution pH in the low-N treatments ranged from pH 4.0-6.0. At a given titration, the pH of solutions containing high-N, CO_2-enriched seedlings was lower than that of solutions containing high-N, CO_2-control seedlings. Extrusion of H^+ per dry matter increment may have increased with CO_2 enrichment and appeared greater in *P. tremuloides* than in *P. glauca* (Table 11).

Figure 7. Mean pH of solutions supporting *Populus tremuloides* and *Picea glauca* grown with the high-N regime. Each low point is the mean pH immediately prior to pH adjustment; each high point is the mean pH immediately following pH adjustment (n=7 for *P. glauca*; n=6 for *P. tremuloides* grown at 350 ul l^{-1} CO_2; n=12 for *P. tremuloides* grown at 650 ul l^{-1} CO_2).



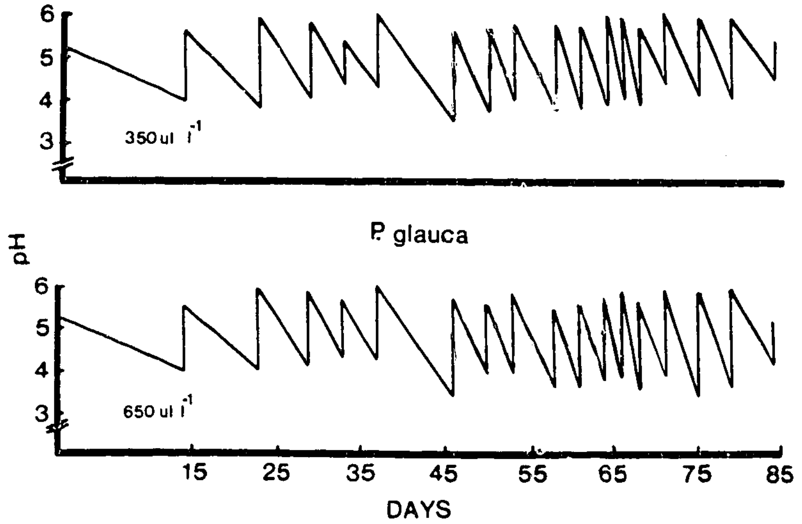


Table 11. Parameters for the linear regressions relating release of H^+ and dry matter increment between adjacent harvests in *Populus tremuloides* and *Picea glauca* grown in the high-N regime. Seedlings were grown at atmospheric CO_2 levels of 350 or 650 ul l^{-1}. Standard errors of the slope are enclosed in parentheses.

	Slope	Y-intercept	r^2	n
P. tremuloides				
350 ul l^{-1}	1.0(0.1)	0.3	0.76	20
650	1.2(0.1)	-0.8	0.84	20
P. glauca				
350	0.3(0.2)	0.8	0.22	18
650	0.5(0.1)	0.2	0.57	21

Elemental concentrations in the high-N culture solutions, measured at the end of the experiment, varied with CO_2 regime and species. Concentrations of all elements except P differed between the start and final solutions (Table 12).

Solutions supporting high-N, CO_2-enriched P. tremuloides had (1) lower concentrations of total N, NH_4-N, and NO_3-N, (2) lower NH_4-N:NO_3-N ratios, and (3) slightly higher conductivities and concentrations of K and Mg than either the start or final solutions supporting CO_2-control P. tremuloides (Table 12). While K, Ca, and Mg accumulated in solutions containing P. glauca, CO_2 enrichment did not affect final concentrations of non-N cations (Table 12). Final solution concentrations of total N, NH_4-N, and NO_3-N decreased with CO_2 enrichment.

Final elemental concentrations of solutions supporting low-N P. tremuloides were either lower than or unchanged from concentrations in start solutions (Table 13). Concentrations of K in solutions supporting low-N P. glauca were lower, but other elements were unchanged compared to the starting solutions. Final concentrations of P and K in solutions supporting P. tremuloides were variable, but lower under CO_2 enrichment (Table 13). Only trace amounts of N were measured in the final solutions. Little adjustment of NH_4-N:NO_3-N was required for maintenance of solution pH.

Table 12. Starting and final concentrations of N, P, K, Ca, and Mg in culture solutions in the high-N regime and at atmospheric CO_2 levels of 350 or 650 ul l^{-1} (mean ± sd).

CONCENTRATION (mg l^{-1})

	START	FINAL			
		P. tremuloides (n=6)		*P. glauca* (n=7)	
		350	650	350	650
NH_4-N	61.8	32.5 (4.3)	22.4 (5.9)	46.5 (5.7)	36.5 (6.6)
NO_3-N	104.7	77.8 (7.3)	63.4(14.6)	108.9(10.2)	89.7 (6.3)
N	166.5	110.3(10.7)	85.8(20.4)	155.4(14.8)	126.2(11.3)
P	24.5	23.9 (1.2)	24.9 (4.0)	25.6 (0.9)	26.2 (0.3)
K	140.4	157.4 (6.0)	175.2(17.8)	165.5 (5.0)	166.8(12.7)
Ca	16.5	18.6 (2.2)	21.6 (3.0)	22.6 (2.0)	21.3 (1.4)
Mg	18.0	22.4 (0.7)	27.4 (3.2)	25.1 (1.0)	23.5 (1.9)
Conductivity (mS m^{-1})	127.5	128.2 (3.2)	146.3(16.6)	128.6 (3.4)	130.3 (2.9)
pH	5.2				
NH_4-N:NO_3-N (added) (N=100)		63(5):37(5)	69(1):31(1)	59(5):41(5)	58(9):42(9)

Table 13. Starting and final concentrations of N, P, K, Ca, and Mg in culture solutions in the low-N regime and at atmospheric CO_2 levels of 350 or 650 ul l^{-1} (mean ± sd).

CONCENTRATION (mg l^{-1})

	START	FINAL			
		P. tremuloides		*P. glauca*	
		350 (n=3)	650 (n=7)	350 (n=7)	650 (n=7)
NH_4-N	0.0	0.2 (0.2)	0.0 (0.0)	0.1 (0.0)	0.0 (0.0)
NO_3-N	0.0	0.4 (0.4)	0.2 (0.2)	0.6 (0.4)	0.4 (0.3)
N	0.0	0.6 (0.6)	0.2 (0.2)	0.6 (0.4)	0.4 (0.3)
P	25.5	26.8 (5.6)	18.4 (4.8)	23.7 (1.3)	23.0 (0.3)
K	166.8	137.2 (23.8)	118.2 (20.8)	142.4 (9.0)	142.7 (2.7)
Ca	16.0	13.4 (2.2)	15.0 (5.0)	14.9 (1.9)	14.5 (0.5)
Mg	16.5	6.2 (1.6)	6.0 (1.8)	15.4 (1.3)	15.2 (0.4)
Conductivity (mS m^{-1})	67.2	51.4 (1.7)	50.7 (0.8)	67.5 (1.8)	67.1 (0.7)
pH	5.0				
NH_4-N:NO_3 (added) (N=100)		42(1):58(1)	40(1);60(1)	38(0):62(0)	38(0):62(0)

Plant Nutrient Contents

Elemental concentrations in plant tissue varied with species and were affected by CO_2 and N regime. Carbon dioxide enrichment decreased concentrations of foliar N in high-N P. tremuloides, but increased concentrations of Ca in roots (Table 14). Carbon dioxide enrichment also decreased foliar concentrations of N and K in low-N P. glauca (Table 15). Concentrations of elements in low-N P. tremuloides and high-N P. glauca, however, were unaffected by CO_2 enrichment. In contrast to those responses, N stress decreased whole plant P without affecting whole plant concentrations of K, Ca, or Mg in P. tremuloides (Table 14). Whole-plant concentrations of P, K, Ca, and Mg in P. glauca all increased with N stress (Table 15). In both species, N contents decreased with N stress. Whole-plant concentrations of N in P. tremuloides and P. glauca did not vary over time (Figure 8), nor did whole-plant concentrations of Ca and Mg in high-N P. tremuloides.

Foliar concentrations of chlorophyll in both species decreased with N status, but less so with CO_2 enrichment (Table 16). The reduction in chlorophyll content with N status, whether expressed on a dry mass or leaf area basis, was greater in P. tremuloides than in P. glauca. Foliar concentrations of chlorophyll decreased proportionately more than did N or Mg under CO_2 enrichment (Tables 14,15,16).

Table 14. Concentration and accumulation of N, P, K, Ca, and Mg in *Populus tremuloides* seedlings maintained at high-N or low-N status and grown at atmospheric CO_2 levels of 350 or 650 ul l^{-1} (mean ± sd). Data are from the final harvest in each treatment.

		CONCENTRATION ($mg\ g^{-1}$)				UPTAKE (mg)	
		ROOT	STEM	LEAF	TOTAL		n
High-N							
350	N	32.5(5.3)	20.9(4.9)	36.9(1.9)	32.4(3.1)	476(189)	5
	P	6.9(1.4)	3.4(3.4)	4.4(1.1)	4.8(1.2)	69 (31)	5
	K	35.2(8.4)	13.8(3.3)	20.0(4.4)	22.3(3.7)	329(176)	5
	Ca	1.2(0.3)	2.5(0.8)	3.8(0.7)	2.5(0.7)	34 (9)	4
	Mg	1.2(0.3)	1.9(0.7)	3.2(0.8)	2.5(0.7)	30 (10)	4
	Ash	80.5(0.3)	39.5(3.7)	56.1(5.0)	58.0(5.1)	---	3
650	N	31.0(5.3)	19.2(3.0)	31.7(3.1)	29.1(2.9)	675(160)	8
	P	7.4(1.6)	3.5(0.7)	4.4(0.8)	5.1(0.8)	115 (35)	8
	K	34.2(7.5)	17.2(5.8)	18.3(3.2)	22.8(4.2)	506(162)	8
	Ca	1.7(0.2)	2.1(0.2)	4.0(0.6)	2.9(0.5)	66 (17)	4
	Mg	1.4(0.2)	2.0(0.3)	2.8(0.3)	2.3(0.3)	52 (16)	4
	Ash	71.0(9.6)	38.7(4.6)	49.4(3.6)	53.3(5.5)	---	3
Low-N							
350	N	13.2(3.7)	9.0(2.3)	18.8(2.9)	14.4(2.5)	120 (26)	6
	P	3.9(0.7)	2.1(0.6)	3.2(0.7)	3.2(0.5)	28 (7)	6
	K	22.3(0.4)	15.0(3.5)	23.0(5.6)	21.0(6.8)	181 (53)	6
	Ca	1.2(0.1)	2.4(0.4)	4.3(0.4)	2.6(0.2)	27 (3)	3
	Mg	1.2(0.2)	1.8(0.2)	3.9(0.4)	2.3(0.2)	24 (4)	3
	Ash	45.0(7.3)	33.0(3.3)	61.7(7.9)	49.3(5.0)	---	3
650	N	15.6(3.8)	12.2(1.4)	18.4(3.2)	15.8(2.4)	86 (48)	6
	P	3.6(0.9)	2.6(0.6)	3.6(0.7)	3.3(0.7)	19 (11)	6
	K	28.8(7.1)	18.8(2.9)	19.4(4.2)	22.6(5.4)	119 (61)	6
	Ca	1.2(0.3)	2.4(0.5)	4.6(1.1)	2.9(0.7)	22 (6)	3
	Mg	1.3(0.2)	2.0(0.4)	3.8(0.5)	2.5(0.3)	20 (7)	3
	Ash	65.3(7.5)	37.3(4.5)	49.3(8.7)	53.3(4.4)	---	3

Table 15. Concentration and accumulation of N, P, K, Ca, and Mg in *Picea glauca* seedlings maintained at high-N or low-N status and grown at atmospheric CO_2 levels of 350 or 650 ul l^{-1} (mean ± sd). Data are from the final harvest in each treatment.

		CONCENTRATION (mg g^{-1})				UPTAKE (mg)	
		ROOT	STEM	LEAF	TOTAL		n
High-N							
350	N	29.0(6.5)	17.1(3.9)	20.0(3.0)	21.1(2.2)	98 (22)	7
	P	4.2(0.7)	2.4(0.3)	2.6(0.4)	2.9(0.3)	13 (5)	7
	K	13.8(3.3)	9.8(1.2)	11.4(1.7)	11.6(1.2)	53 (16)	7
	Ca	1.3(0.2)	1.1(0.2)	1.8(0.4)	1.5(0.3)	7 (1)	3
	Mg	1.6(0.4)	1.1(0.2)	1.2(0.1)	1.3(0.3)	6 (0)	3
	Ash	38.7(3.0)	22.7(3.4)	28.3(1.7)	29.0(2.1)	---	3
650	N	31.4(5.0)	15.7(1.6)	17.9(3.0)	20.3(2.1)	171 (50)	6
	P	5.1(1.0)	2.5(0.4)	2.5(0.3)	3.0(0.4)	26 (10)	6
	K	14.2(2.6)	11.3(1.9)	10.1(2.2)	11.1(1.9)	92 (26)	6
	Ca	1.3(0.2)	1.6(0.8)	1.7(0.7)	1.6(0.6)	15 (6)	3
	Mg	1.5(0.2)	1.6(0.4)	1.3(0.3)	1.4(0.3)	13 (3)	3
	Ash	41.0(5.1)	25.3(4.2)	22.7(2.4)	27.0(2.1)	---	3
Low-N							
350	N	16.2(1.9)	12.4(1.4)	14.2(1.3)	14.7(1.5)	18 (2)	7
	P	5.5(0.8)	2.4(0.3)	2.7(0.4)	3.6(0.5)	4 (1)	7
	K	23.5(3.5)	13.9(1.9)	15.0(2.4)	18.0(2.4)	22 (3)	7
	Ca	1.7(0.2)	1.4(0.0)	2.5(0.1)	2.1(0.2)	3 (0)	2
	Mg	2.9(1.0)	2.0(0.6)	2.3(0.3)	2.5(0.3)	3 (0)	2
	Ash	50.5(8.3)	30.5(4.7)	38.0(3.5)	41.5(5.2)	---	2
650	N	15.2(1.6)	12.0(1.9)	11.3(1.3)	13.2(1.4)	15 (3)	7
	P	5.9(0.7)	2.3(0.4)	2.6(0.7)	3.9(0.6)	4 (1)	7
	K	23.5(4.3)	12.1(2.5)	11.6(2.2)	16.8(2.6)	19 (4)	7
	Ca	1.6(0.0)	1.5(0.0)	2.4(0.2)	2.0(0.1)	2 (1)	2
	Mg	3.5(0.5)	2.1(0.2)	1.9(0.3)	2.6(0.2)	3 (1)	2
	Ash	59.0(8.0)	26.5(4.6)	31.5(4.8)	42.3(2.0)	---	2

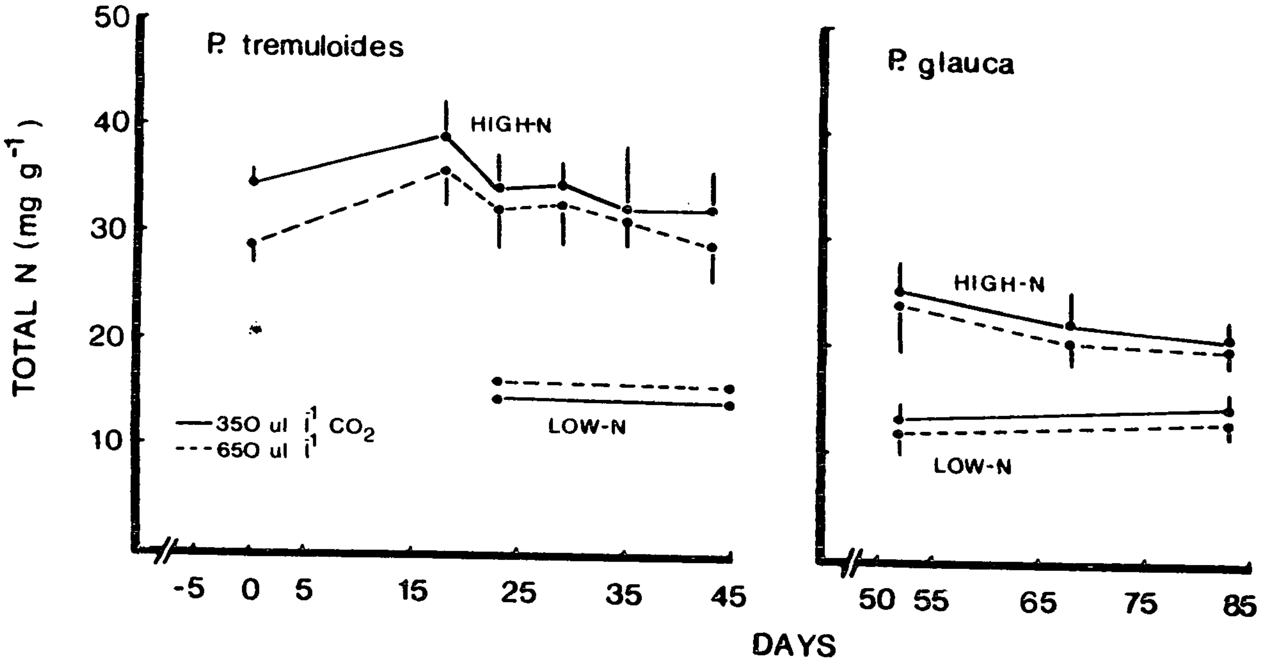


Figure 8. Whole-plant concentration of N over time in *Populus tremuloides* and *Picea glauca* maintained at high-N or low-N status and grown at atmospheric CO_2 levels of 350 or 650 ul l^{-1} (mean ± sd).

Table 16. Foliar chlorophyll concentrations of *Populus tremuloides* and *Picea glauca* seedlings maintained at high-N or low-N status and grown at atmospheric CO_2 levels of 350 or 650 ul l^{-1} (mean ± sd). (fm=fresh mass; dm=dry mass; n=5)

	Chlorophyll concentration		
	mg dm^{-2}	mg g^{-1}(fm)	mg g^{-1}(dm)
P. tremuloides			
High-N			
350	3.6(1.0)	2.2(0.8)	6.1(0.3)
650	2.9(0.6)	1.8(0.2)	4.8(0.8)
Low-N			
350	2.1(0.2)	1.1(0.2)	3.2(0.4)
650	1.8(0.3)	1.0(0.2)	2.5(0.5)
P. glauca			
High-N			
350	0.9(0.3)	0.9(0.3)	3.2(0.8)
650	0.9(0.4)	0.9(0.3)	2.4(0.5)
Low-N			
350	0.6(0.2)	0.5(0.1)	1.8(0.3)
650	0.4(0.1)	0.4(0.1)	1.2(0.2)

Allocation of whole-plant elemental pools varied with element and species and was affected both by CO_2 level and N regime. The fractions of whole-plant Mg and Ca allocated to leaves of P. tremuloides consistently exceeded those of other elements (Table 17). In P. glauca, only Ca was allocated to needles in consistently greater proportions (Table 18). Carbon dioxide enrichment slightly increased allocation of N and Ca to roots in high-N P. tremuloides (Table 17) and increased allocation of N, K, Ca, and Mg to roots of low-N P. glauca (Table 18), but did not affect allocation of elements in either low-N P. tremuloides or high-N P. glauca. The increased allocation of N and Ca to roots of high-N P. tremuloides accompanied slight decreases in allocation to stems and leaves (Table 17); increased allocation of N, K, and Mg to roots of low-N P. glauca was associated with decreased allocation to needles (Table 18).

Nitrogen stress increased allocation of N, Ca, and Mg to roots of CO_2-control, but not CO_2-enriched P. tremuloides (Table 17). In contrast, N stress increased allocation of all elements to roots in P. glauca in both CO_2 regimes. Greater allocation to roots accompanied decreased allocation to leaves in both species (Tables 17,18). Changes in elemental allocation under CO_2 enrichment or N stress were similar to changes in allocation of dry mass, as indicated by the relative lack of change in tissue concentrations of nutrients.

Table 17. Allocation of N, P, K, Ca, and Mg in <u>Populus tremuloides</u> maintained at high-N or low-N status and grown at atmospheric CO_2 levels of 350 or 650 ul l^{-1} (mean ± sd).

		ALLOCATION			
		ROOT	STEM	LEAF	n
High-N					
350	N	0.24(.03)	0.14(.02)	0.62(.02)	5
	P	0.35(.06)	0.15(.02)	0.50(.05)	5
	K	0.38(.05)	0.13(.01)	0.49(.04)	5
	Ca	0.10(.04)	0.18(.04)	0.73(.06)	4
	Mg	0.11(.05)	0.16(.06)	0.72(.09)	4
650	N	0.31(.03)	0.13(.01)	0.57(.05)	8
	P	0.41(.04)	0.14(.02)	0.45(.04)	8
	K	0.42(.04)	0.14(.02)	0.44(.04)	8
	Ca	0.17(.02)	0.14(.02)	0.68(.07)	4
	Mg	0.18(.03)	0.17(.04)	0.64(.09)	4
Low-N					
350	N	0.38(.05)	0.13(.02)	0.49(.05)	6
	P	0.50(.10)	0.14(.03)	0.36(.08)	6
	K	0.44(.09)	0.15(.03)	0.41(.07)	6
	Ca	0.19(.03)	0.18(.04)	0.62(.08)	3
	Mg	0.21(.04)	0.16(.03)	0.64(.08)	3
650	N	0.37(.05)	0.14(.01)	0.49(.04)	6
	P	0.41(.08)	0.14(.03)	0.36(.08)	6
	K	0.48(.10)	0.15(.04)	0.36(.08)	6
	Ca	0.16(.04)	0.15(.02)	0.69(.05)	3
	Mg	0.20(.03)	0.15(.03)	0.65(.08)	3

Table 18. Allocation of N, P, K, Ca, and Mg in *Picea glauca* maintained at high-N or low-N status and grown at atmospheric CO_2 levels of 350 or 650 ul l^{-1} (mean ± sd).

ALLOCATION

		ROOT	STEM	LEAF	n
High-N					
350	N	0.27(.05)	0.16(.03)	0.57(.04)	7
	P	0.30(.07)	0.16(.03)	0.54(.09)	7
	K	0.24(.08)	0.17(.05)	0.59(.12)	7
	Ca	0.17(.02)	0.15(.02)	0.68(.05)	3
	Mg	0.24(.04)	0.18(.04)	0.58(.09)	3
650	N	0.32(.08)	0.13(.01)	0.55(.07)	6
	P	0.35(.11)	0.14(.03)	0.52(.11)	6
	K	0.27(.11)	0.17(.05)	0.56(.12)	6
	Ca	0.19(.09)	0.16(.04)	0.64(.10)	3
	Mg	0.25(.11)	0.20(.04)	0.56(.10)	3
Low-N					
350	N	0.39(.02)	0.09(.01)	0.51(.02)	7
	P	0.53(.04)	0.07(.01)	0.39(.03)	7
	K	0.47(.04)	0.08(.01)	0.45(.04)	7
	Ca	0.28(.02)	0.07(.00)	0.63(.07)	2
	Mg	0.40(.11)	0.08(.03)	0.52(.10)	2
650	N	0.46(.04)	0.11(.05)	0.42(.02)	7
	P	0.60(.06)	0.03(.02)	0.32(.04)	7
	K	0.56(.05)	0.09(.05)	0.35(.03)	7
	Ca	0.34(.00)	0.08(.01)	0.57(.06)	2
	Mg	0.57(.08)	0.09(.02)	0.34(.07)	2

System Nutrient Budgets

Elemental accumulation in culture solutions should accompany decreased tissue concentrations of those elements if the balance of added elements matches that required by the plant. Relative to P (unchanged in solution), the concentration of K in CO_2-enriched, high-N *P. tremuloides* and *P. glauca* may have been slightly lower than in CO_2-control plants (Table 14,15). This would be consistent with its slight accumulation in culture solutions (Table 12,13). However, substantial quantities of added N in the high-N regime, P, K, Ca, and Mg in the low-N regime, and P, K, and Mg in the high-N regime for *P. glauca* were lost from the culture system (Tables 19,20).

Table 19. Nutrient budgets for *Populus tremuloides* (*P. trem.*) and *Picea glauca* grown in the high-N regime and at atmospheric CO_2 levels of 350 or 650 ul l^{-1}. Concentrations of P, K, Ca, and Mg in plant tissue at intermediate harvests were assumed to be the same as in the final harvest (mean ± sd; n=6 for *P. tremuloides*, n=7 for *P. glauca*)

	(mg)				
	N	P	K	Ca	Mg
P. trem.					
350					
START	666	98	562	66	72
ADDED	1516(475)	212(59)	894(251)	140(38)	153(42)
UPTAKE	769(156)	127(28)	593(111)	75(14)	67(17)
FINAL	442 (43)	96 (5)	635 (21)	74 (9)	90 (3)
UNACCT.	971(443)	87(60)	228(235)	58(48)	64(45)
650					
START	666	98	562	66	72
ADDED	1737(195)	232(19)	993(180)	154(10)	167(12)
UPTAKE	1101 (68)	180(36)	825 (70)	107(13)	83(11)
FINAL	412(145)	100(16)	701 (71)	86(12)	110(13)
UNACCT.	890(383)	50(40)	29 (99)	27(18)	46(22)
P. glauca					
350					
START	666	98	562	66	72
ADDED	905(249)	137(36)	553(171)	77(26)	107(34)
UPTAKE	400 (97)	49(11)	195 (68)	26 (5)	22 (3)
FINAL	617 (59)	102 (4)	660 (21)	86 (7)	89 (5)
UNACCT.	554(272)	84(42)	263(178)	28(19)	55(29)
650					
START	666	98	562	66	72
ADDED	1173(423)	182(63)	737(266)	87(24)	95(27)
UPTAKE	481(106)	53(20)	170 (79)	29(13)	25 (7)
FINAL	505 (45)	104 (5)	667 (51)	101 (6)	99 (6)
UNACCT.	853(467)	123(89)	512(408)	35(26)	44(25)

Table 20. Nutrient budgets for *Populus tremuloides* (*P. trem.*) and *Picea glauca* grown in the low-N regime and at atmospheric CO_2 levels of 350 or 650 ul l^{-1}. Concentrations of P, K, Ca, and Mg were assumed to be the same in the final and intermediate harvests (mean ± sd; n=3 (350 ul l^{-1}) or n=6 (650 ul l^{-1}) for *P. tremuloides*; n=7 for *P. glauca*).

			(mg)		
	N	P	K	Ca	Mg
P. trem					
350					
START	0	76	500	48	50
ADDED	409 (44)	195(37)	996(187)	104(20)	126(25)
UPTAKE	384(158)	88(33)	600(269)	69(18)	62(17)
FINAL	0	81(20)	412 (83)	41 (8)	22 (4)
UNACCT.	25 (98)	102(43)	484(245)	42(10)	88(22)
650					
START	0	76	500	48	50
ADDED	349(117)	222(56)	994(306)	118(29)	144(36)
UPTAKE	312(142)	67(32)	446(238)	56(25)	50(22)
FINAL	0	59(17)	380 (74)	50(17)	20 (6)
UNACCT.	37(123)	172(77)	668(400)	60(26)	124(37)
P. glauca					
350					
START	0	76	500	48	50
ADDED	61 (7)	189(48)	970(225)	101(26)	122(31)
UPTAKE	68(12)	18 (4)	86 (21)	10 (1)	12 (2)
FINAL	0	71 (4)	427 (31)	44 (7)	46 (5)
UNACCT.	-7 (7)	176(71)	957(327)	89(29)	114(33)
650					
START	0	76	500	48	50
ADDED	59 (4)	177(42)	918(208)	94(23)	115(27)
UPTAKE	59 (9)	19 (5)	80 (20)	9 (2)	12 (2)
FINAL	0	69 (1)	428 (9)	44 (2)	45 (2)
UNACCT.	0 (3)	165(59)	923(321)	90(24)	107(29)

DISCUSSION

Techniques used to control nutrient availability to plants may alter interpretations of how CO_2 enrichment affects nutrient accumulation and allocation. Typically, nutrients are added at constant rates in CO_2 enrichment studies (e.g. Patterson and Flint 1982; Hocking and Meyer 1985; Chapter 2; Oberbauer et al. 1986). Over time, exponential growth of the young plant may lead to nutrient demands in excess of supply (Linder and Rook 1984) with concommittant decreases in plant nutrient concentration. The decrease may be accelerated by, and wrongly interpreted as, a direct consequence of CO_2 enrichment (Chapter 3). Such difficulties in interpretation of short-term experimental results may render them unsuitable for projecting longer-term effects, particularly on long-lived woody plants. The problem was avoided in this study by adding nutrients at fixed relative rates of increase or commensurate with plant demands via pH and conductivity titrations. Either maintaining an exponentially increasing rate of nutrient addition or providing adequate solution volume to allow continued exponential growth of the root system and prevent nutrient depletion should reduce the potential for time-dependent decreases in plant nutrient concentrations.

When nutrients were freely available, _P. tremuloides_ had higher elemental concentrations of all elements than did _P._

glauca, within a CO_2 regime. Elemental concentrations are often higher in foliage of deciduous tree species than in foliage of associated evergreen species (Ovington 1956; Small 1972; Chabot and Hicks 1982). The lower concentrations in foliage of evergreens may reflect a greater amount of structural material and, under optimum and steady-state nutrition and / or may accumulate only to levels commensurate with lower photosynthetic capacities. The lower photosynthetic capacities could, in turn, be related to greater diffusive resistances associated with coniferous leaf structure. In that case, higher concentrations of N (luxury consumption) would not increase photosynthetic capacity and could increase respiration rates (Bloom et al. 1985). Luxury consumption of N and other elements may, however, be useful to plants growing where nutrient availability is generally low yet unpredictable. Use of only two N regimes in this study precluded any observation of luxury consumption.

Additional N supply regimes would clarify whether plant N status differentially affects elemental accumulation in P. tremuloides and P. glauca, as well as confirming the concentrations associated with luxury consumption. Different patterns of mineral accumulation might be expected in these species, however. When nutrients were freely available, uptake rates of NH_4 and PO_4, but not K, were greater in P. tremuloides than in P. mariana, a species associated with infertile sites (Chapin et al. 1986). Absorption capacity for immobile ions (e.g. NH_4 and PO_4) may not be enhanced in species growing in sites where soil processes strongly limit ion availability. Under such conditions, an increased capacity for absorption would not increase ion uptake and would entail additional energetic and N expenditures associated with maintenance of high absorption capacity (Chapin 1980; Chapin et al. 1986; Clarkson and Hanson 1980).

In this study, CO_2 enrichment had relatively little effect on whole-plant elemental concentrations in either species or in either nitrogen regime. When nutrients were freely available, CO_2 enrichment increased whole-plant uptake such that constant and adequate concentrations were maintained. Uptake rates could increase if elemental concentrations in roots decreased (Glass 1983) with increased translocation to shoots or with increased root carbohydrate concentrations (Marschner 1986).

Other studies have reported varied effects of CO_2 enrichment on nutrient accumulation, including decreased concentrations of whole-plant N (Hocking and Meyer 1985; Norby et al. 1986; Wong 1979), Ca (Oberbauer et al. 1986), K, and P (Luxmoore et al. 1986) and Mg (Porter and Grodzinski 1984). Decreased concentrations of one element may be accompanied by similar decreases (Porter and Grodzinski 1984), a lack of change (Luxmoore et al. 1986; Chapter 3), or increases in concentrations of other elements (Oberbauer et al. 1986). Patterns of nutrient uptake are difficult to establish from those studies. The variation may be species-specific or reflect differences in relative abundance of nutrients available to the plants at the time of harvest in each study.

The data from this study were insufficient to determine whether CO_2 enrichment increased whole-plant nutrient uptake more through increased root growth or via increased absorption rate. In soil, ion supply rate may be the major limitation to nutrient uptake, rather than absorption capacity (Robinson 1986). Carbon dioxide enrichment increased nutrient uptake by *Pinus virginiana*, grown in soil, by increasing root weight duration not absorption rate (Luxmoore et al. 1986).

Carbon dioxide enrichment may increase the uptake ratio of cations:anions, increasing the release of H^+ from the roots and thereby lowering rhizosphere pH (Luxmoore et al. 1986). In this study, the mean pH of solutions supporting high-N *P. tremuloides*, prior to pH adjustment, was always lower under CO_2 enrichment than in the control atmosphere. This could be partly explained by the larger size of the CO_2-enriched plants. It might also result from increases in uptake of NH_4^+ and other cations relative to NO_3^- and other anions. That question cannot be answered because (1) S uptake was not measured and (2) significant quantities of N were unaccounted for in the high-N treatment, preventing the calculation of NH_4 and NO_3 uptake.

Nutrient losses in this study may have resulted from several factors peculiar to the handling techniques and culture system. For example, Pasteur pipettes were used to introduce air into the culture solutions. Changes in line air pressure or damage to pipette tips could result in more vigorous bubbling and splashing of culture solutions out of the containers. Nutrients may have been lost when plants were weighed fresh or were handled during titrations. Losses could become significant over time with exponential increases in root area, if solution losses were related to root area. The additional handling involved in repeated measures of fresh mass might also damage root systems and inhibit root growth (Ingestad and Lund 1986).

Under CO_2 enrichment, whole-plant nutrient use efficiency did not increase, since whole-plant concentrations of nutrients were unchanged. However, N productivity (a measure of relative growth rate per unit whole-plant N) may have increased slightly with CO_2 enrichment (Chapter 5). The increased N productivity largely resulted from greater increases in net assimilation rates per unit foliar N under CO_2 enrichment. Reduced investment of N into chlorophyll and possibly into enzymes of the Calvin cycle (Wong 1979; Pearcy and Bjorkman 1983; Porter and Grodzinski 1984) per unit leaf mass could allow greater allocation of N for biosynthesis of roots and leaves. Carbon dioxide enrichment increased the proportion of the whole-plant N pool allocated to fine roots and leaves (less to stems) in N-stressed _Quercus_ seedlings (Norby et al. 1986) and increased the R:S ratio of total N in Xanthium occidentale (Hocking and Meyer 1985).

An increase in N productivity would not necessarily translate into increased nitrogen use efficiency over a longer time scale. The efficiency of use of N depends upon rates of production per unit N during active growth (N productivity) as well as the retention time of a given molecule in the plant (Berendse and Aerts 1987). Most nutrient losses from plants occur via senescence of leaves and possibly roots. Increased retranslocation would reduce N losses via senescence and would make N use more efficient. Further studies in this area are essential for projecting long-term effects of CO_2 enrichment on vegetation growing in infertile sites.

The lack of change in whole-plant nutrient use efficiency with CO_2 enrichment reiterates the need for supply of N (and other nutrients) to increase if CO_2 enrichment is to enhance productivity in the long term (Norby et al. 1986). Nutrient supply in unmanaged soils might increase if CO_2 enrichment increases mycorrhizal development (O'Neill et al. 1987; Norby et al. 1987), increases rates of mineralization of P (Norby et al. 1986), or acidifies the rhizosphere and increases rates of weathering (Luxmoore et al. 1986).

The nutrient addition technique has potential for investigating how plant nutrient status modifies uptake and utilization of carbon in CO_2-enriched plants, how CO_2 enrichment affects assimilation and allocation of mineral nutrients, and how these effects differ in associated species. Control of nutrient losses and solution pH should allow insight into whether CO_2 enrichment alters the relative preference for NH_4-N versus NO_3-N. Imposition of the CO_2 treatments after the lag phase had ended might better clarify the growth and nutrition responses of low-N *P. tremuloides* to CO_2 enrichment.

Attention should be given to the modifying role of rooting zone temperature and the effect of mycorrhiza so these data are more useful for extrapolation to field situations. Mycorrhizae may not only increase the availability of some elements, but may also alter the relative uptake of NH_4-N and NO_3-N as well as fluxes of other ions (Bledsoe and Rygewicz 1986). Root zone temperatures may differentially affect ion uptake in different boreal tree species (Chapin et al. 1986) and may also affect the responses of plants to CO_2 enrichment (Yelle et al. 1987). Since northern latitudes may warm substantially with increasing global CO_2 levels (Washington and Meehl 1984), an understanding of how changing soil temperatures affect nutrient availability, nutrient uptake, and productivity, in conjunction with CO_2 enrichment, should be important in projecting future responses of boreal trees to increasing CO_2 levels.

In conclusion, differing plant concentrations of nitrogen were induced and held constant over time by using the relative addition technique of nutrient supply. When all nutrients were freely available, *Populus tremuloides* accumulated higher concentrations of N, P, K, Ca, Mg and chlorophyll than did *Picea glauca*. Extrusion of H^+ into the culture solutions per unit dry matter gained, was greater in *P. tremuloides* than in *P. glauca* and may have increased with CO_2 enrichment, an effect attributed to the larger size of CO_2-enriched plants and possibly to an increase in the uptake of cations (including NH_4^+) relative to anions (including NO_3^-). Growth in CO_2-enriched atmospheres did not affect whole-plant elemental concentrations, but did affect their allocation among roots, stems, and leaves. Although nitrogen use efficiency may increase with CO_2 enrichment, increased nitrogen availability will be necessary for long-term enhancement of growth as atmospheric CO_2 levels increase.

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CHAPTER 5. EFFECTS OF CO_2-ENRICHMENT AND PLANT NITROGEN STATUS ON *POPULUS TREMULOIDES* AND *PICEA GLAUCA*. II. GROWTH AND WATER USE.

INTRODUCTION

The objective of this study was to evaluate the effects of CO_2-enrichment and N status on growth and water use when plant nitrogen concentrations were held constant over time, as described in Chapter 4.

METHODS

The plants were grown in controlled environments as described in Chapter 4. Atmospheric CO_2 levels in the two rooms were 350 ul l^{-1} (ambient) or 650 ul l^{-1} (enriched).

Following germination and pretreatment, seedlings were transplanted to dark plastic pots (4-5 plants per pot) containing 4 L (high-N) or 3 L (low-N) of aerated nutrient solution. Within each CO_2 regime, seedlings were assigned to either a high-N regime (all elements freely available) or a low-N regime (all elements except N freely available).

The treatments were designed to maintain steady-state nutrition (Ingestad and Lund 1986) for each element during the experimental period and were described in detail in chapter 4. In the high-N regime, nutrient additions to replace elements assimilated by the plants were based on pH and conductivity changes in the solutions and were accomplished via manual titrations. Nitrogen addition rates in the low-N regime were 0.06 $g\ g^{-1}d^{-1}$ for P. *tremuloides* and 0.03 $g\ g^{-1}d^{-1}$ for *P. glauca*. These addition rates were assumed to be ca. 30 % of those required for maximum growth, based on measurements from Chapter 2

Growth Measurements

Fresh mass of individual seedlings was measured at 5-10 day intervals. Periodically, seedlings were randomly sampled (maximum of one per pot) from each treatment and harvested for measurement of specific growth characteristics and moisture and N contents. The total number of seedlings sampled per harvest and the frequency of harvests were based upon the amount of biomass required for mineral analysis at the end of the experiment.

Stem length, branch and leaf number, and leaf area (*P. tremuloides*) and stem length, branch and needle number, and needle lengths (*P. glauca*) were measured on plants prior to oven drying. Leaf areas of *P. tremuloides* (one-sided) were measured with a Li-Cor 3100 leaf area meter. Whole-plant needle areas of

P. glauca seedlings were estimated after measuring the lengths of at least 10% of the needles on each seedling. Total surface areas of individual needles were calculated with linear regressions which related needle length and surface area (Appendix 3), developed using the glass bead technique (Thompson and Leyton 1971). Total needle area per seedling was then estimated based on the proportion of needles measured.

Harvested seedlings were oven dried (24 hours at 70C). Dry masses of roots, stems, and leaves and whole-plant moisture contents were determined. Oven-dried roots of *P. tremuloides*, collected at the final harvest, were divided into coarse (> 1mm diameter) and fine root (≤ 1mm diameter) fractions and weighed separately. Specific leaf area (SLA), leaf area ratio (LAR), and root, stem, and leaf weight (=mass) ratios (RWR,SWR,LWR) were calculated from primary growth data. Relative growth rate (RGR) was calculated for plants in each replicate pot over the period between weighings as per Evans (1972). Net assimilation rate (NAR) and the leaf area partitioning coefficient (LAP) were calculated as described in Chapter 2. Leaf areas used in the calculations of NAR and LAP were either measured directly (on harvested plants) or indirectly. The latter estimates were derived from regressions of leaf area on total dry mass of harvested plants within a given treatment. Nitrogen productivity, a measure of dry matter accumulation per unit N and per unit time

(Ingestad and Lund 1986) was calculated from growth and whole-plant N concentrations.

Transpiration and Photosynthesis

Transpiration (E) and stomatal conductance (g_s) were measured with a LI-1600 steady-state diffusion porometer on individual leaves of *P. tremuloides* and on the upper 3 cm of terminal shoots of *P. glauca*. Net photosynthetic rates of high-N *P. tremuloides* were determined with an LCA-2 portable IRGA (ADC). Photosynthesis and transpiration were measured on the same leaves and at the atmospheric CO_2 levels maintained during growth. The leaves measured in the high-N *P. tremuloides* were of leaf plastochron index (LPI) 9±2 and 14±2 and in low-N seedlings were of LPI 4 and 11, where LPI represents a description of leaf ontogeny (Larson and Isebrands 1971). Each set of measurements was made in midmorning in the controlled environment rooms and was repeated at least three times over a 3-4 day period.

Water use efficiency (WUE) and internal CO_2 concentrations (C_i) were calculated for individual leaves; conductance of H_2O was assumed to be 1.6X that of CO_2 (Von Cammerer and Farquhar 1981). All gas exchange data were pooled by plant.

The logistics of the experiment prevented simultaneous application of all treatments. Furthermore, the two CO_2 treatments were applied simultaneously to a given species and N regime, but were applied in two different rooms and therefore not truly replicated. Thus, I present only means and standard deviations for measured and calculated values (Hurlbert 1984), recognizing the inherent difficulties in inferring treatment effects using that approach.

RESULTS

Biomass Partitioning and Accumulation

Nitrogen stress increased the root weight ratio (RWR) and decreased the leaf weight ratio (LWR) of both species; in addition, the stem weight ratio (SWR) of *P. glauca* decreased (Tables 21 and 22). Thus, root:shoot ratios (R:S) increased in both species with decreasing N status, but the change in R:S per unit change in whole-plant concentration of N was greater in *P. glauca* (Figure 9). Nitrogen stress increased the proportion of root dry mass as fine ($\leq$ 1 mm diameter) roots (Table 23).

Within a nitrogen regime, CO_2 enrichment had little consistent effect on dry mass partitioning between root, stem,

Table 21. Dry matter partitioning and final total dry mass of high-N and low-N *Populus tremuloides* grown at atmospheric CO$_2$ levels of 350 or 650 ul l^{-1} (mean ± sd). Abbreviations: RWR=root weight ratio; SWR=stem weight ratio; LWR=leaf weight ratio; SLA=specific leaf area (cm^{2}g^{-1} leaf); LAR=leaf area ratio (cm^{2}g^{-1} plant); TDM=total dry mass (g).

	DAYS	n	RWR	SWR	LWR	SLA	LAR
High -N							
CO$_2$							
350	18	4	0.21(.01)	0.16(.01)	0.64(.02)	339(48)	216(37)
650	18	11	0.20(.03)	0.15(.02)	0.66(.03)	241(30)	158(19)
350	23	3	0.19(.02)	0.16(.05)	0.66(.07)	261(17)	172(26)
650	23	9	0.22(.05)	0.14(.03)	0.63(.04)	204(23)	128(13)
350	30	3	0.23(.05)	0.18(.03)	0.59(.08)	228(26)	136(33)
650	30	10	0.25(.04)	0.16(.03)	0.59(.05)	195(24)	115(15)
350	36	8	0.25(.02)	0.19(.04)	0.56(.04)	234(29)	131(19)
650	36	16	0.26(.04)	0.17(.03)	0.56(.05)	184(29)	104(19)
350	44	5	0.24(.03)	0.21(.01)	0.54(.03)	201(28)	109(17)
650	44	8	0.30(.02)	0.19(.02)	0.51(.03)	158(17)	81(12)

Total dry mass (g) after 44 days
350 ul l^{-1} 14.9 (6.8) 650 ul l^{-1} 23.3 (3.6)

	DAYS	n	RWR	SWR	LWR	SLA	LAR
Low-N							
350	23	6	0.38(.03)	0.20(.01)	0.41(.03)	183(11)	76 (7)
650	23	12	0.39(.05)	0.18(.02)	0.43(.05)	163(30)	70(14)
350	40	8	0.42(.07)	0.20(.01)	0.39(.07)	187(22)	73(19)
650	40	23	0.42(.03)	0.19(.02)	0.40(.04)	160(20)	64(10)

Total dry mass (g) after 40 days
350 ul l^{-1} 8.7 (2.6) 650 ul l^{-1} 6.5 (1.7)

Table 22. Dry matter partitioning and final total dry mass of high-N and low-N *Picea glauca* seedlings grown at atmospheric CO_2 levels of 350 or 650 ul l^{-1} (mean ± sd). Abbreviations as in Table 21.

High-N

CO_2	DAYS	n	RWR	SWR	LWR	SLA	LAR
350	52	7	0.18(.04)	0.15(.02)	0.66(.05)	439(85)	294(73)
650	52	7	0.18(.02)	0.13(.00)	0.69(.04)	382(85)	258(48)
350	68	7	0.20(.04)	0.16(.03)	0.64(.06)	373(51)	238(39)
650	68	9	0.18(.02)	0.14(.01)	0.68(.02)	315(27)	216(21)
350	83	13	0.21(.03)	0.19(.03)	0.61(.04)	321(57)	194(36)
650	83	11	0.21(.04)	0.16(.01)	0.63(.04)	268(81)	168(53)

Total dry mass (g) after 83 days
350 ul l^{-1} 4.4 (1.1) 650 ul l^{-1} 7.0 (0.9)

Low-N

350	52	7	0.42(.03)	0.10(.01)	0.47(.03)	292(81)	138(38)
650	52	7	0.36(.04)	0.11(.01)	0.53(.04)	306(122)	160(58)
350	83	14	0.36(.04)	0.11(.02)	0.53(.04)	339(99)	180(59)
650	83	14	0.40(.05)	0.11(.02)	0.48(.06)	272(80)	132(46)

Total dry mass (g) after 83 days
350 ul l^{-1} 1.1 (0.1) 650 ul l^{-1} 1.1 (0.1)

Figure 9. Root:shoot (R:S) ratios of *Populus tremuloides* and *Picea glauca* in relation to whole-plant concentration of N.

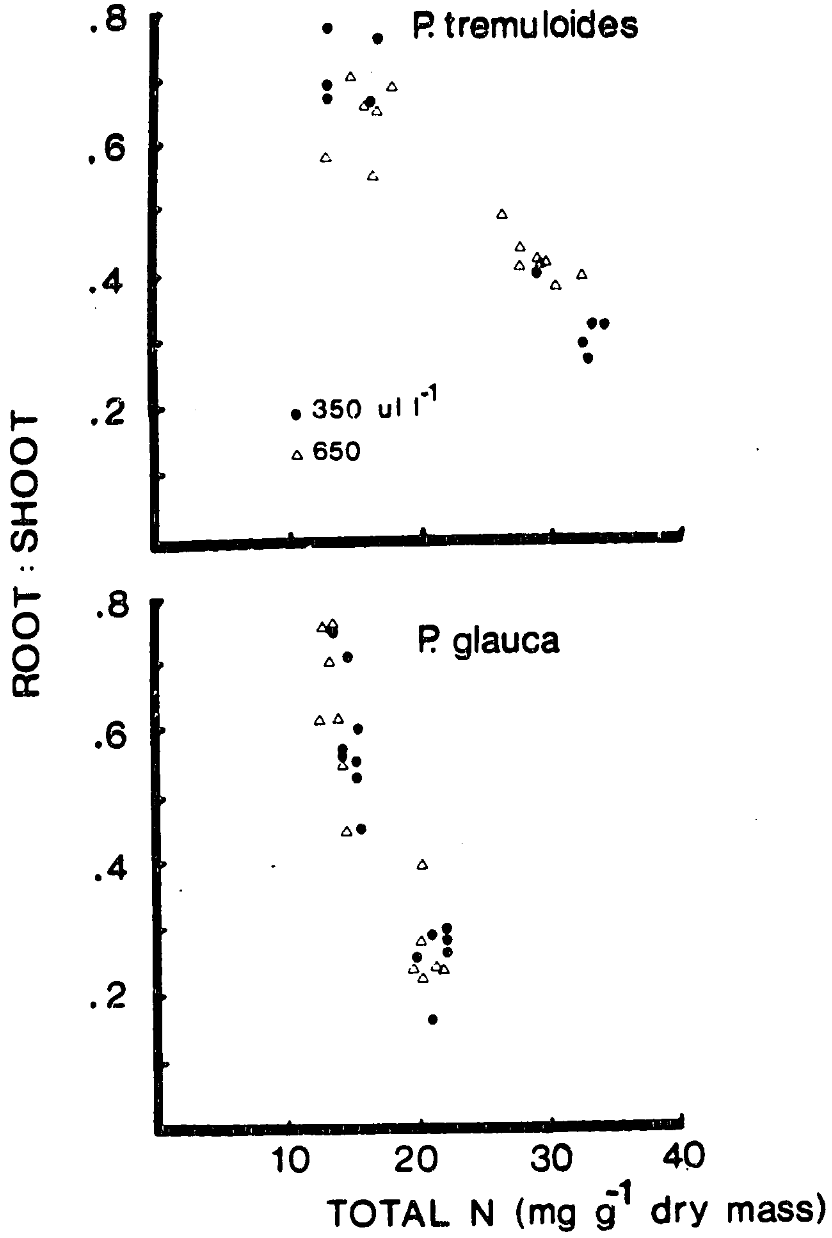


Table 23. The proportion of *Populus tremuloides* dry root mass as fine roots ($\leq$ 1 mm diameter) (mean ± sd).

TREATMENT	n	FINE ROOT FRACTION
High-N		
350 ul l^{-1}	6	0.48 (0.05)
650 ul l^{-1}	5	0.53 (0.03)
Low-N		
350 ul l^{-1}	5	0.69 (0.05)
650 ul l^{-1}	6	0.74 (0.05)

and leaf in either species (Table 21,22). Carbon dioxide enrichment may have increased the proportion of dry root mass as fine roots and decreased specific leaf area (SLA) and leaf area ratio (LAR) of high-N P. *tremuloides* (Table 21,23). Both SLA and LAR decreased over time (Table 21,23). Leaf area of P. *tremuloides* was linearly related to total dry mass (TDM); ln-transformed leaf areas of P. *glauca* were linearly related to ln-transformed TDM (Table 24).

Growth Dynamics

Nitrogen stress depressed relative growth rate (RGR) in both species, once steady-state nutrition was achieved. In high-N P. *tremuloides*, RGR peaked between 17 and 22 days and decreased thereafter; RGR of low-N P. *tremuloides* decreased during the lag phase of the study to a constant rate during the experimental period (Figure 10). Nitrogen-induced increases in growth rates resulted from increases in net assimilation rate (NAR) and from increased allocation of biomass into leaf area expansion (Table 25). Carbon dioxide enrichment slightly increased RGR of high-N and low-N P. *tremuloides* and high-N P. *glauca*, but enhanced growth rates in the latter did not persist. The effect of CO_2 enrichment on RGR appeared most pronounced in larger plants (Figures 11,12). The increased growth rates resulted from increased net assimilation rates (NAR). Net assimilation rates of high-N P. *tremuloides* and P. *glauca* and of low-N P. *tremuloides*

	REGRESSION	n	r^2	RANGE,TDM	S.E.E.	95% CI SLOPE	95% CI INT.
P. tremuloides							
High-N							
350ul l^{-1}	LA=106.23(TDM)+ 97.78	23	0.95	0.35-25.46	150.42	±10.75	±93.77
650ul l^{-1}	LA= 80.66(TDM)+106.15	54	0.96	0.32-26.51	138.44	± 4.82	±51.14
Low-N							
350ul l^{-1}	LA= 50.55(TDM)+ 99.10	14	0.88	0.49-18.25	93.02	±11.98	±92.42
650ul l^{-1}	LA= 52.30(TDM)+ 44.44	35	0.97	0.57=15.91	33.25	± 3.15	±19.89
P. glauca							
High-N							
350ul l^{-1}	Ln(LA)=0.705(LnTDM)-0.749	26	0.70	1.20-6.37	0.221	± 0.20	± 2.03
650ul l^{-1}	Ln(LA)=0.679(LnTDM)-0.535	19	0.89	1.08-10.21	0.183	± 0.12	± 1.30
Low-N							
350ul l^{-1}	Ln(LA)=1.278(LnTDM)-6.640	21	0.93	0.27-1.72	0.195	± 0.16	± 1.48
650ul l^{-1}	Ln(LA)=0.938(LnTDM)-3.722	21	0.88	0.30-1.61	0.209	± 0.17	± 1.50

Table 24. Relationships of leaf area and total dry mass in *Populus tremuloides* and *Picea glauca*. Abbreviations: LA=leaf area ((cm^2), one-sided for *P. tremuloides* and total for *P. glauca*); TDM=total dry mass (g); S.E.E.=standard error of the estimate for the linear regression; INT.=y-intercept of the regression. The leaf areas of P. glauca were multiplied by 10^5 prior to ln-transformation.

Figure 10. Relative growth rates (RGR) of *Populus tremuloides* and *Picea glauca* over time. Day 0 represents the start of the experimental (post-lag) period. Each vertical bar represents one standard deviation about the mean (n=7 for *P. glauca*; n=6 for *P. tremuloides* grown at 350 ul l^{-1} and n=12 for *P. tremuloides* grown at 650 ul l^{-1}.)

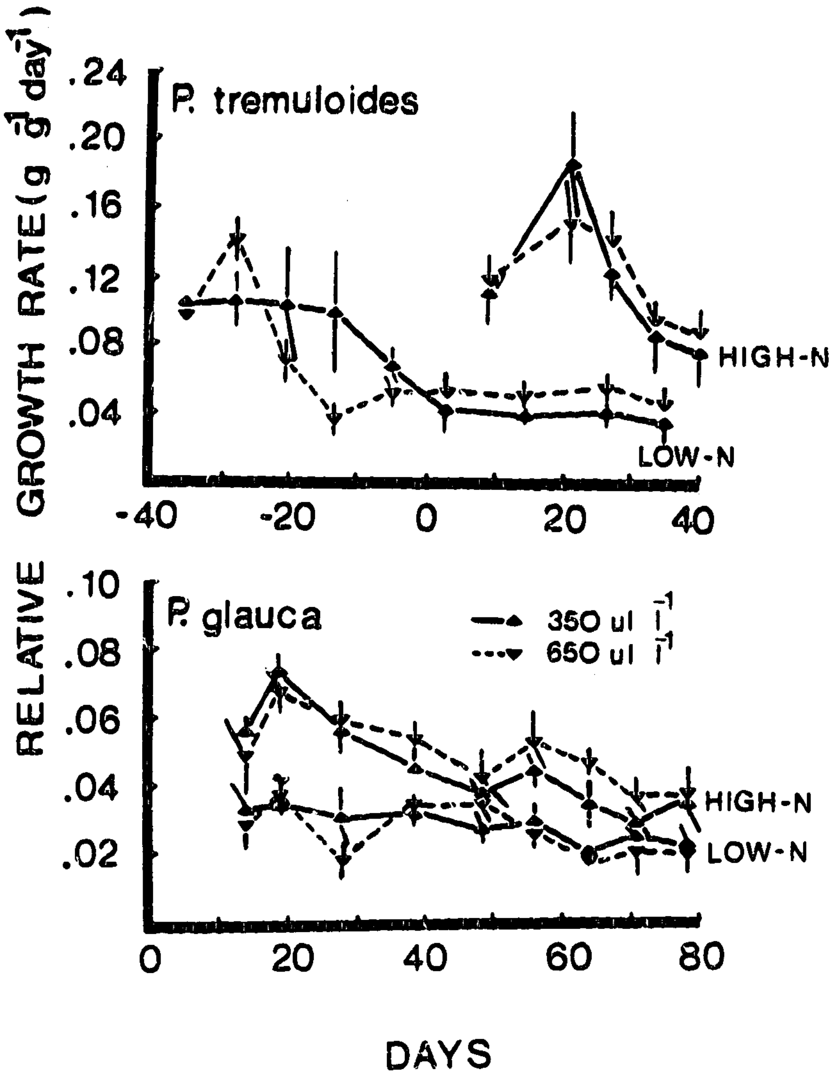


Table 25. Mean relative growth rate ($g\ g^{-1}d^{-1}$), net assimilation rate ($g\ dm^{-2}d^{-1}$), and leaf area partitioning coefficient (cm^2d^{-1} / $g\ d^{-1}$) of *Populus tremuloides* and *Picea glauca* maintained at high-N or low-N status and grown at atmospheric CO_2 levels of 350 or 650 ul l^{-1}. Abbreviations: RGR=relative growth rate; NAR=net assimilation rate; LAP=leaf area partitioning coefficient.

	INTERVAL (Days)	RGR	NAR	LAP
P. tremuloides				
High-N				
350	18-23	0.187	0.095	107.2
650	18-23	0.148	0.088	73.6
350	23-30	0.121	0.091	100.2
650	23-30	0.143	0.131	74.2
350	30-44	0.087	0.074	99.4
650	30-44	0.092	0.104	77.2
Low-N				
350	0-40	0.037	0.050	56.1
650	0-40	0.051	0.069	55.9
P. glauca				
High-N				
350	52-83	0.036	0.016	157.9
650	52-83	0.042	0.022	134.9
Low-N				
350	52-83	0.023	0.014	203.9
650	52-83	0.020	0.013	119.0

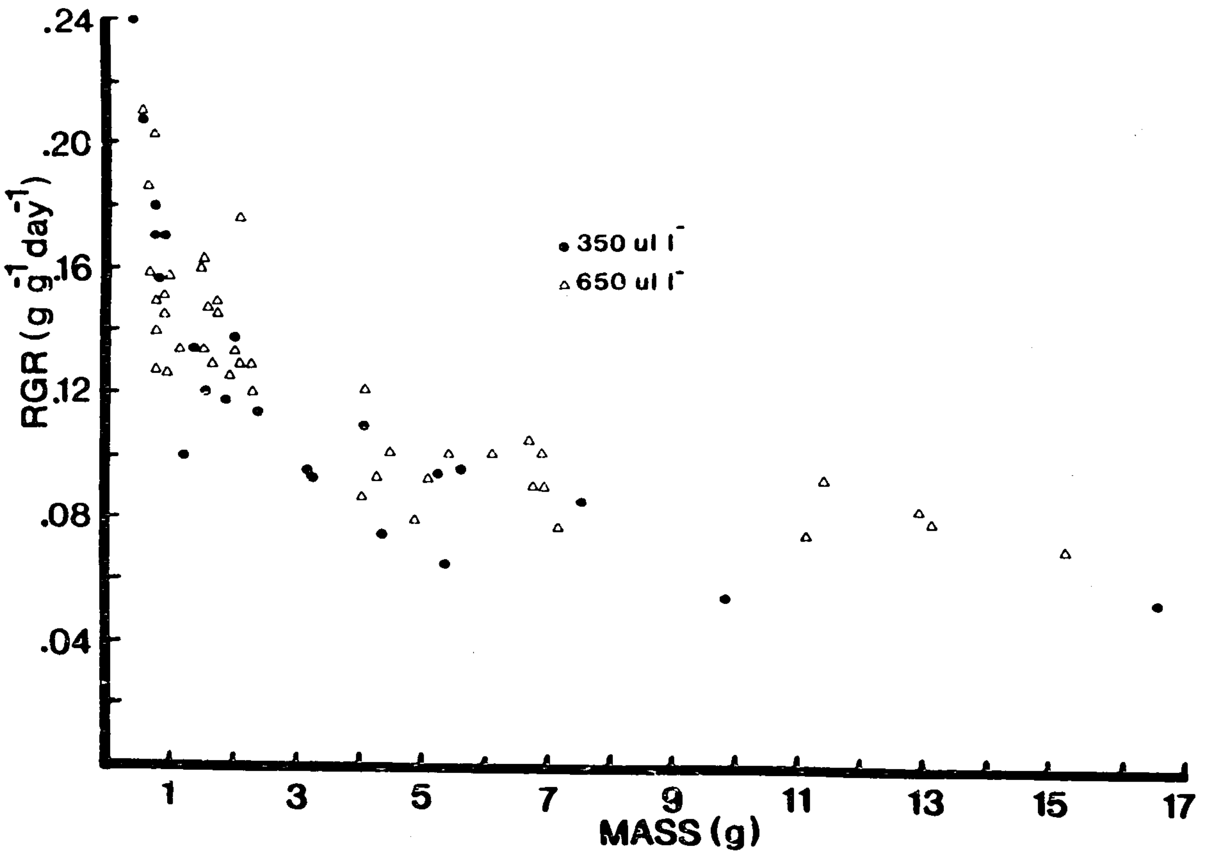


Figure 11. Relative growth rates (RGR) of high-N *Populus tremuloides* as a function of plant mass. The relative growth rate for plants in a given pot over a given measurement interval is plotted against the mean estimated dry mass at the start of the interval.

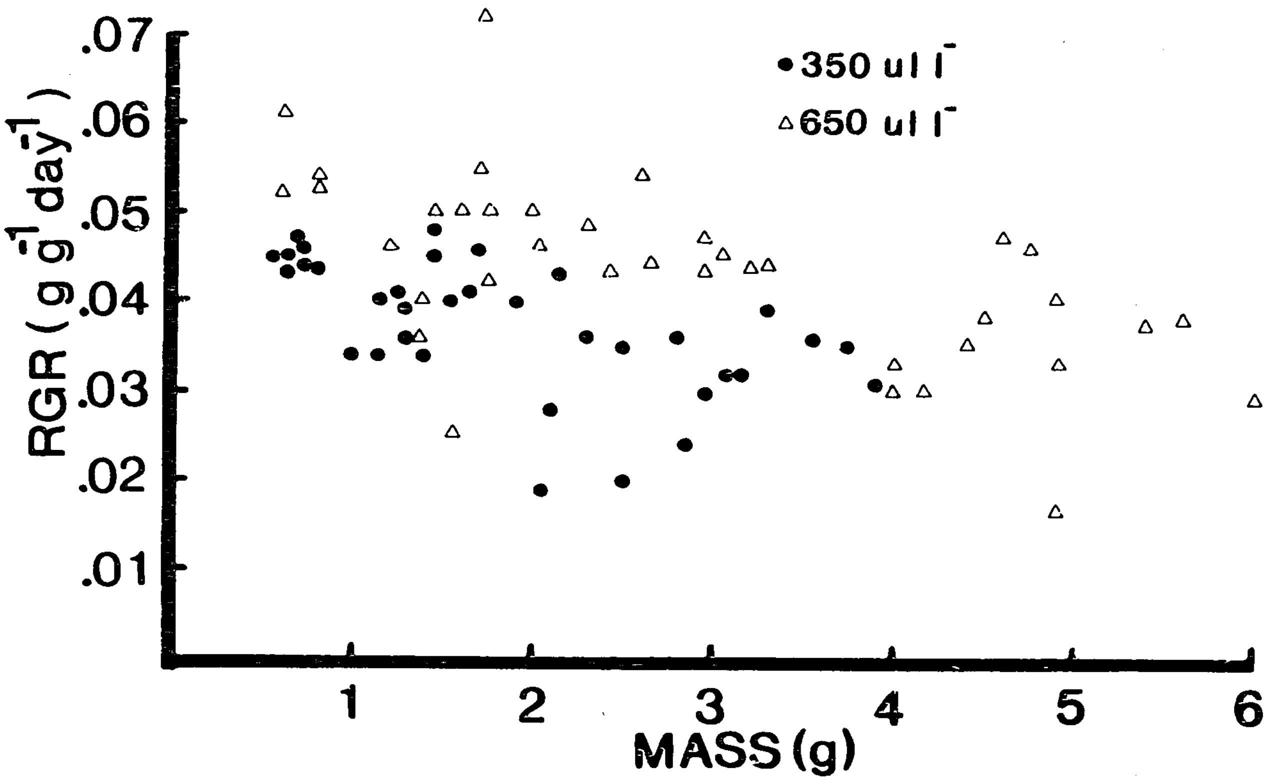


Figure 12. Relative growth rates (RGR) of high-N *Picea glauca* as a function of plant mass. The relative growth rate for plants in a given pot over a given measurement interval is plotted aginst the mean estimated dry mass at the start of the interval.

increased 40 % with CO_2 enrichment; absolute increases were greater in P. tremuloides (Table 25).

The final total dry mass of high-N P. tremuloides and P. glauca increased by nearly 60% with CO_2 enrichment (Tables 21,22). The absolute increase in dry mass, due to CO_2 enrichment, was 8 g over a 44 day period in P. tremuloides and less than 3 g in P. glauca over 83 days (Tables 21,22). Elevated CO_2 did not increase the final dry mass of low-N P. tremuloides and P. glauca. In P. tremuloides, this resulted from different growth rates during the lag phase of the experiment; in contrast, the relative growth rates of low-N P. glauca were unaffected by CO_2 enrichment during the experimental period.

Growth Dynamics and N Contents

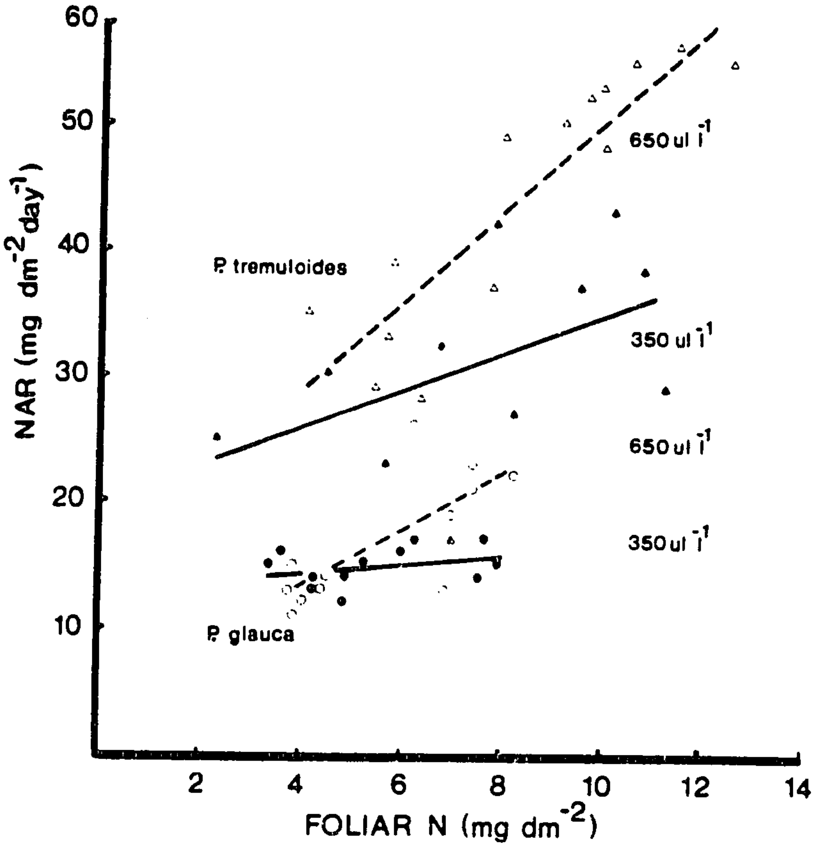
Carbon dioxide enrichment may have increased the N productivity of P. tremuloides (Table 26); if so, the increase resulted from increases in relative growth rate. Nitrogen productivity of high-N plants decreased over time, such that N productivities of high-N and low-N plants were similar late in the experiment (Table 26).

Within a CO_2 regime, P. tremuloides had greater net assimilation rates (NAR) than did P. glauca at a given concentration of foliar N (Figure 13). Within each species, CO_2

Table 26. Nitrogen productivity (g dm g N^{-1} d^{-1}) of *Populus tremuloides* and *Picea glauca* seedlings maintained at high-N or low-N status and grown at atmospheric CO$_2$ levels of 350 or 650 ul l^{-1} (mean ± sd).

	P. tremuloides			*P. glauca*	
High-N					
	Days	N prod.		Days	N prod.
350	0-18	3.0 (0.8)	350	0-52	2.2 (0.3)
(n=6)	18-22	5.0 (1.2)	(n=7)	52-67	1.6 (0.3)
	22-29	3.5 (0.7)		67-82	1.5 (0.3)
	29-43	2.4 (0.5)			
650	0-18	3.5 (0.7)	650	0-52	2.3 (0.4)
(n=10)	18-22	4.6 (1.2)	(n=7)	52-67	2.2 (0.4)
	22-29	4.3 (1.0)		67-82	1.6 (0.4)
	29-43	2.9 (0.3)			
Low-N					
	Days	N prod.		Days	N prod
350	0-23	2.8 (0.6)	350	52-82	1.7 (0.4)
(n=6)	23-42	2.6 (0.6)	(n=7)		
650	0-23	3.1 (0.8)	650	52-82	1.6 (0.4)
(n=9)	23-42	3.2 (1.0)	(n=7)		

Figure 13. Net assimilation rates (NAR) as a function of foliar N concentrations in *Populus tremuloides* and *Picea glauca*. Both NAR and foliar N are expressed on the basis of total leaf surface area.



enrichment may have increased the slopes of the NAR vs. foliar N regression lines by a similar absolute amount; however, the variability about each line was high (Figure 13; Table 27). This suggests that the absolute effect of CO_2 enrichment on NAR at a given level of foliar N was greater for P. tremuloides than for P. glauca.

Transpiration and Photosynthesis

Transpiration rates (E) and stomatal conductance (g_s) of expanded P. tremuloides leaves were strongly reduced by both N stress and CO_2 enrichment (Table 28). Absolute reductions associated with CO_2 enrichment were greater in high-N seedlings, but percentage reductions were greater in low-N seedlings. Transpiration rates and conductance of P. glauca shoots were low in all treatments (Table 28).

Photosynthetic rates (A) of high-N P. tremuloides may have increased under CO_2 enrichment, at least when expressed on a leaf area or unit chlorophyll basis (Table 8). Water use efficiency (WUE) increased 2.8-fold. Carbon dioxide enrichment may have decreased the ratio of internal:ambient CO_2 (C_i/C_a) (Table 28).

Table 27. Parameters of the linear regressions relating net assimilation rate (NAR) and foliar N concentrations of *Populus tremuloides* and *Picea glauca* grown at atmospheric CO_2 levels of 350 or 650 ul l^{-1}. The standard errors of the regression slopes are enclosed in parentheses. Data points are shown in Figure 12.

	SLOPE	Y-INT.	R^2	n
P. tremuloides				
350	1.52(0.90)	19.33	0.27	10
650	3.57(0.75)	14.88	0.79	14
P. glauca				
350	0.33(0.32)	12.97	0.12	13
650	2.23(1.08)	4.26	0.57	12

Table 28. Effects of CO_2 enrichment and N supply on transpiration (E) and stomatal conductance (g_s) of *Populus tremuloides* and *Picea glauca* seedlings and on net photosynthesis (A), C_i/C_a, and water use efficiency (WUE) of high-N *P. tremuloides* (mean ± sd). Seedlings were maintained at high-N or low-N status and grown at atmospheric CO_2 levels of 350 or 650 ul l^{-1}.

TREATMENT	n	E (ug cm^{-2} s^{-1})	g_s (cm s^{-1})
P. tremuloides			
High-N			
350	5	7.2(2.2)	0.58(.16)
650	8	2.9(0.7)	0.24(.03)
Low-N			
350	5	3.2(1.3)	0.23(.12)
650	5	0.9 (.4)	0.07(.03)
P. glauca			
High-N			
350	5	0.6 (.2)	0.04(.01)
650	5	0.6 (.2)	0.04(.02)
Low-N			
350	5	0.8 (.2)	0.05(.02)
650	5	0.7 (.4)	0.05(.03)

	High-N *Populus tremuloides*	
	350 ul l^{-1} n=5	650 ul l^{-1} n=8
A		
(mg dm^{-2} hr^{-1})	13.6(3.1)	15.6(2.9)
(mg g^{-1} hr^{-1})	22.6(7.0)	25.3(8.4)
(mg mg chl^{-1} hr^{-1})	3.7(1.1)	5.4(1.7)
C_i/C_a	0.78(.12)	0.64(.15)
WUE (mg CO_2 g H_2O^{-1})	5.3(1.1)	14.8(3.9)

DISCUSSION

The nutrient addition technique maintained constant whole-plant N concentrations during the experimental period. Thus, the direct effects of CO_2 enrichment on plant growth and transpiration could be separated from indirect effects associated with time-dependent decreases in plant nutrient status. At constant rates of nutrient supply, CO_2 enrichment may temporarily stimulate plant growth, but also accelerate time-dependent decreases in plant nutrient status (Chapters 2,3). Extended exposure to high CO_2 may induce responses similar to those induced by N stress. For example, CO_2 enrichment decreased specific leaf area and leaf area ratios (Patterson and Flint 1982), increased root:shoot ratios (Higginbotham 1983), increased foliar starch contents (DeLucia et al. 1985), and decreased levels of chlorophyll and RuBP carboxylase (Wong 1979). Similar responses may result from a decrease in plant N status (e.g. Wong 1979; McDonald et al. 1986; Agren and Ingestad 1987). Therefore, nutrient supply rates, growth rates, and plant nutrient levels over time should be described in CO_2-enrichment studies to allow better interpretation of CO_2 effects on plant growth. Projections of long-term responses of vegetation to increasing levels of CO_2 depend largely upon data from short-term studies in which factors other than CO_2 level are presumably defined and controlled. Better generalizations of species responses may result.

Even though whole-plant N concentrations remained constant during the experimental period, relative growth rates (RGR) of high-N *P. tremuloides* and *P. glauca* declined over time. The effect was most pronounced in *P. tremuloides* and may have resulted from decreased rates of whole-plant photosynthesis (per unit plant mass) relative to rates of respiration. Low pH in those solutions supporting CO_2-enriched *P. tremuloides* may have reduced root growth. However, *Betula pendula* seedlings had higher RGR when pH fluctuated from 3.4-6.2 (similar to this study) or was maintained at pH 4.5 or 5.0, than at a constant pH of 3.5 (Ingestad 1979).

Relative growth rates of *P. tremuloides* exceeded those of *P. glauca* when N was freely available, but decreased more when N supply rates were reduced to 30 % of optimal. Similar patterns were observed in *Betula pubescens* and *Pinus sylvestris* (Karlsson and Nordell 1987) and agree with the postulated effects of increased nutrient availability on plants associated with habitats of differing fertility (Grime 1979; Chapin 1980). High CO_2 levels had greater effects on growth rates of *P. tremuloides* than of *P. glauca* both when N was freely available and when the supply of N was restricted.

The higher growth rates observed under CO_2 enrichment resulted from increased net assimilation rates. Net assimilation rate, a measure of the net accumulation rate of carbon and other elements per unit leaf area, could increase via increased rates of net photosynthesis and mineral uptake or decreased rates of respiration. Whole-plant respiration rates were not measured, but whole-plant mineral concentrations were unchanged by CO_2 enrichment (Chapter 5). Presumably, CO_2 enrichment increased photosynthetic rates by increasing the partial pressure of CO_2 at the site of carboxylation (C_i). Whole-plant photosynthetic rates of high-N P. tremuloides may have also been increased by CO_2-induced decreases in leaf area ratio, which could have increased the irradiance incident on individual leaves.

Within a nitrogen regime and at given foliar concentration of N, CO_2 enrichment had greater absolute effects on net assimilation rates of P. tremuloides than of P. glauca. Populus tremuloides may allocate a greater fraction of its foliar N to chlorophyll (Chapter 5) and other nitrogenous components of photosynthesis than does P. glauca. The lower stomatal conductances of P. glauca may also restrict net assimilation rates over part or all of the range of foliar N levels induced in this study. Additional N regimes would clearly indicate the levels of foliar N above which NAR would not increase and below which CO_2 enrichment would have no effect.

In contrast to the effects of CO_2 enrichment, N status strongly affected partitioning of dry matter among roots, stems, and leaves. Such changes in allocation are a response for maintaining a certain balance of internal constituents, given varied availabilities of above and below-ground resources; thus R:S often decreases with increased availability of N (Hunt and Nicholls 1986).

It is unclear why R:S changed more per unit change in whole-plant N concentration in P. glauca than in P. tremuloides. The differences between P. glauca and P. tremuloides are like those between the coniferous Picea abies (northern provenance) and the broadleaved Populus simonii and Betula verrucosa (Agren and Ingestad 1987), but they contradict the responses postulated for species of fertile and infertile sites as nutrient availability increases (Grime 1979; Chapin 1980). Use of nonmycorrhizal plants in the controlled nutrition studies may partly account for the discrepancy. Additionally, the proportion of tissue as meristems may differ less between such species when seedlings, than when more mature. Total root biomass may be an inappropriate measure when evaluating R:S plasticity in P. tremuloides, if: (1) the large lateral roots, which give rise to adventitious shoots, function more as storage organs for carbohydrates (Schier and Zasada 1973) and less to absorb minerals and (2) the ratio of coarse (>1mm diameter):fine root mass increases with plant N status. Measurements of fine root production in response to temporally and spatially varied availability of nutrients, more similar to the variation found in nature (Crick and Grime 1987), might be a more sensitive measure of root growth plasticity.

Carbon dioxide enrichment may not affect (Patterson and Flint 1982; Hocking and Meyer 1985) or may strongly affect (Higginbotham 1983; Norby et al. 1986) partitioning of dry mass among roots, stems, and leaves. The effect of CO_2 enrichment may depend upon the relative abundance of mineral nutrients and CO_2. For example, CO_2 enrichment (to 700 ul l^{-1}) seemed to decrease RWR of *Populus euramerica* cuttings with abundant N supply and increase RWR of cuttings with restricted N supplies (Goudriaan and deRuiter 1983).

Carbon dioxide enrichment and N stress decreased transpiration and conductance of *P. tremuloides*, but had no effect on *P. glauca*. Elsewhere, foliar N levels were negatively correlated with conductance (g) in *Pinus radiata* (Sheriff et al. 1986), but were positively correlated with transpiration rates of *Picea abies* (Keller 1972). A positive correlation between g and foliar N might be expected if g is controlled by C_i, the partial pressure of CO_2 in the leaf, via photosynthetic rate (A) (e.g. Wong et al. 1979).

Carbon dioxide enrichment often reduces conductance and transpiration rate (Morison 1985) and may also reduce stomatal density (Woodward 1987). Conductances of both high-N and low-N *P.*

tremuloides in this study were reduced more by CO_2 enrichment than in other species of *Populus* (Regehr et al. 1975; Jurik et al. 1984). If the measured transpiration rates in this study accurately reflected whole-plant transpiration rates, the reductions would more than compensate for the increased leaf area of CO_2-enriched high-N *P. tremuloides*, at least through 44 days of CO_2 enrichment. The insensitivity of stomata to CO_2 enrichment, noted in *P. glauca*, was also noted in *Picea sitchensis* (Beadle et al. 1979), *Pinus contorta* (Higginbotham et al. 1985), *Pinus radiata* (Conroy et al. 1988),and *Pinus taeda* (Tolley and Strain 1985). Stomatal insensitivity to CO_2 level and N status may indicate that stomatal opening in conifers is not tightly coupled to rates of carbon fixation (Jarvis 1980). Conroy et al. (1988) pointed out that whole-plant efficiency of water use by *P. radiata* still increased with CO_2 enrichment; the effect resulted via increased accumulation fo dry matter, not via decreased rates of transpiration. *Picea glauca* may have responded similarly, since transpiration rates were unchanged and net assimilation rates increased under CO_2 enrichment.

Morison (1985) showed that species with greater conductance at ambient CO_2 levels also had greater reductions in conductance at CO_2 levels twice ambient, a pattern consistent with the responses of *P. tremuloides* and *P. glauca* in this study. The ecological implications are unclear, particularly since water stress was not imposed in the study. An increase in WUE might be especially beneficial for establishment of *P. tremuloides* seedlings, which are especially sensitive to moisture deficits (McDonough 1978). Establishment of *P. glauca* seedlings on drier sites might not be enhanced, in contrast, unless root development was preferentially enhanced under higher atmospheric CO_2 levels.

In conclusion, growth rates were greater in *P. tremuloides* than in *P. glauca* when mineral nutrients were freely available, but were reduced more in *P. tremuloides* by a reduction in nitrogen supply and plant nitrogen status. Within a nitrogen regime, absolute increases in growth and net assimilation rates, under CO_2 enrichment, were greater in *P. tremuloides* than in *P. glauca*. This greater response was associated with higher foliar concentrations of chlorophyll and nitrogen, as well as greater stomatal conductance to diffusion of water and CO_2.

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Chapter 6. SYNTHESIS AND FUTURE RESEARCH NEEDS

COMPARISONS OF EXPERIMENTS 1 AND 2

Similar general results were evident from experiments 1 and 2, ignoring the post-exponential growth period of experiment 1:

(1) The absolute effect of CO_2 enrichment on accumulation of total dry mass (TDM) by *Populus tremuloides* and *Picea glauca* increased with increased availability of N.

(2) Within a nitrogen regime, the proportional increase in relative growth rate (RGR), due to CO_2 enrichment, was as great or greater in *P. tremuloides* than in *P. glauca* (Table 29). The absolute effect was consistently greater in *P. tremuloides*.

(3) Root:shoot ratios were unaffected by CO_2 enrichment in either species. Nitrogen availability had greater effects on R:S of *P. glauca* than of *P. tremuloides*.

The results listed in points 1 and 2 support the general hypotheses posed in Chapter 1, whereas point 3 contrasts with postulated responses by the plant groups assumed to be represented by *P. tremuloides* and *P. glauca*. These trends were

Table 29. Proportional effects of nitrogen supply and CO_2 enrichment on relative growth rate (RGR), and net assimilation rate (NAR) of <u>Populus</u> <u>tremuloides</u> and <u>Picea</u> <u>glauca</u> in experiments 1 and 2. Results from the medium-N treatment (experiment 1) are not shown. Other values are not shown if direct comparison was not possible. Values shown for experiment 1 are based on the exponential growth period for a given treatment as discussed in Chapter 2.

	Experiment 1		Experiment 2	
	Populus	Picea	Populus	Picea
Effect of N				
$350 \text{ ul } l^{-1} CO_2$				
RGR	2.6X	1.7X	3.0X	1.6X
NAR	2.4X	1.2X	1.5X	1.1X
750/650				
RGR	2.3X	1.7X	2.5X	2.2X
NAR	1.9X	1.2X	1.5X	1.7X
Effect of CO_2				
High N				
RGR	1.2X	1.0X	1.3X	1.2X
NAR	1.2X	1.1X	1.4X	1.4X
Low N				
RGR	1.3X	1.0X	1.4X	0.9X
NAR	1.5X	1.1X	1.4X	0.9X

observed, despite the following dissimilarities between the two experiments:

(1) Differing atmospheric CO_2 levels in the CO_2-enriched regimes of experiment 1 (750 ul l^{-1}) and experiment 2 (350 ul l^{-1}).

(2) Differing N regimes. In experiment 1, N was added at constant rates of supply, ultimately resulting in uncontrolled changes in plant nutrient status and disappearence of the enhancing effect of CO_2-enrichment on growth of P. tremuloides. In experiment 2, plant N status was held constant at differing levels for the duration of the study.

(3) Differing pretreatment period and initial plant sizes. The initial total dry mass (TDM) of P. tremuloides was 0.002 g and of P. glauca was 0.001 g in experiment 1. In experiment 2, the initial TDMs ranged from 0.05-0.12 g and varied with species and treatment (Table 30). Thus, 25-90 % of the study period of experiment 1 passed before the seedlings were as large as their counterparts in experiment 2. The effect of CO_2 enrichment on growth may decrease as plants age (Kramer 1981; Tolley and Strain 1984).

(4) Differing seed sources.

Table 30. Initial total dry mass (g) of seedlings at the beginning of experiments 1 and 2. The approximate number of days required for seedlings in experiment 1 to grow to the initial total dry mass of seedlings in experiment 2 is enclosed in parentheses.

	TOTAL DRY MASS (g)	
EXPERIMENT 1		
	Populus	Picea
15.5 mM-N		
350 ul l^{-1} CO_2	0.002 (26)	0.001 (47)
750	0.002 (23)	0.001 (46)
0.155 mM-N		
350	0.002 (48)	0.001 (89)
750	0.002 (40)	0.001 (77)
EXPERIMENT 2		
High-N		
350	0.110	0.096
650	0.110	0.101
Low-N		
350	0.047	0.107
650	0.049	0.102

The results of the experiments may therefore not be directly comparable, but the general conclusions are strengthened by the differences in the experiments. The two experiments are complementary in demonstrating how plant nutrient status may alter the effects of CO_2 enrichment.

My data indicate that increased N availability increased the relative growth rates (RGR) of P. tremuloides both through increased net assimilation rate (NAR) and through increased partitioning into leaf area (LAP, LAR), although the N effect on NAR was more pronounced in experiment 1 and in the 650 ul l^{-1} CO_2 treatment of experiment 2. Net assimilation rate of P. glauca was less affected by N supply than was either LAP or LAR. In fact, NAR was less in high-N than in medium-N seedlings (Chapter 2), possibly indicating shading limitations induced by increased leaf area. Likewise, NAR of Betula pubescens seedlings was more responsive to nutrient availability than was NAR of Pinus sylvestris (Karlsson and Nordell 1987).

The greater absolute response of NAR in P. tremuloides (compared to P. glauca) to increased availability of N was accompanied by (1) less resistance to diffusion, (2) higher concentrations of chlorophyll when N was freely available, (3) a greater increase in chlorophyll concentration as N availability increased, and (4) slightly greater changes in allocation of the whole-plant N pool to leaves (but not biomass) at 350 ul l^{-1}

CO_2. Greater diffusive resistance and a leaf arrangement which may result in considerable self-shading could constrain P. glauca to lower maximum photosynthetic rates than possible in P. tremuloides and therefore result in less capacity to utilize higher levels of atmospheric CO_2. A smaller increase in foliar N and chlorophyll levels with increasing N supply could limit the photosynthetic capacity of P. glauca and might reflect an adjustment to constraints imposed by high diffusive resistances (Chapin et al. 1987).

These hypotheses could be tested by analyzing the effects of N status and atmospheric CO_2 levels on allocation of nitrogen and on stomatal and nonstomatal limitations to photosynthesis (e.g. Farquhar and Sharkey 1982) in both species. Growing plants of constant, controlled nutrient status using the relative addition technique of nutrient supply seems a logical start for evaluating the fundamental relations between N status and photosynthesis in various CO_2 regimes and for relating photosynthetic and whole-plant growth responses. Generation of data usable in developing models of whole-plant responses to CO_2-enrichment may be possible. My approach could be improved in future studies if (1) the culture system was modified to allow the study of smaller plants, thereby allowing an assessment of the impact of self-shading and (2) more N addition rates were included in order to better define N concentrations associated with luxury consumption and below which CO_2 enrichment would not affect growth. Application of this approach to other boreal tree species would confirm whether the responses observed in *Populus tremuloides* and *Picea glauca* are typical of other deciduous and evergreen tree species of the boreal forest.

Growth and nutrient relations derived from experiments maintaining steady-state conditions should be extrapolated to field conditions with care, however. For example, responses to localized nutrient pulses may provide a more sensitive measure of R:S plasticity than do responses under steady-state nutrition (Chapter 5). Variable nutrient regimes may better represent conditions to which a plant, growing in soil, is typically exposed (Crick and Grime 1987). Likewise, plants associated with infertile sites may absorb a large percentage of their annual nutrient uptake during seasonal nutrient flushes, rather than under steady-state conditions (Chapin 1980). However, an understanding of simple systems provides a foundation for understanding more complicated ones. In that sense, studies of steady-state nutrition and growth relations are useful.

My data suggest that CO_2-enriched plants will deplete a given supply of nutrients more rapidly than would their counterparts grown at ambient CO_2 levels, increases in nutrient use efficiency notwithstanding. Under ambient CO_2 conditions, rapidly growing species characteristic of more fertile sites (e.g. *P. tremuloides*) exhaust a given supply of mineral nutrients more rapidly than do slower growing species typical of less fertile sites (e.g. P. glauca) (Grime 1979; Chapin 1980). Carbon dioxide enrichment might further accentuate the inherent differences between these two species in consuming a given supply of nutrients. Possibly, CO_2 enrichment could increase the below-ground competitive ability of rapidly growing species relative to slower-growing, more stress-tolerant species. Conversely, the more rapid exploitation of soil nutrients might hasten the demise of the rapidly growing species during succession.

Such speculation assumes that CO_2-enrichment studies of seedlings can be extrapolated to long-term field conditions and that nutrient availability will not change under CO_2 enrichment. Both assumptions are tenuous, since neither can be supported or refuted by existing data. Little is known about the direct effects of CO_2 enrichment on:

(1) different stages of the tree life cycle, including the speed of maturation and lifespan.

(2) competitive relations between associated species

(3) nutrient availability and rates of carbon and mineral nutrient turnover in boreal ecosystems.

(4) allocation of carbon to differing functional classes within the plant and its effect on herbivores and pathogens which may alter the carbon balance of the tree.

(5) The above processes as impacted by possible CO_2-induced climatic changes in the boreal forest.

FUTURE RESEARCH NEEDS

Different Stages of Plant Life Cycles

CO_2 enrichment may accelerate maturation and senescence of plants, at least annuals (St. Omer and Horvath 1983). In addition, several studies, primarily of herbaceous plants, indicate that the greatest effects of CO_2 enrichment on growth occur at the seedling stage (Kramer 1981). Notwithstanding the previously-discussed problem of time-dependent changes in plant nutrient status, changes in growth response to CO_2 enrichment may be associated with decreased leaf area ratio and specific leaf area (Hurd 1968; Neales and Nicholls 1978), decreased net assimilation rate (Mauney et al. 1978; Tolley and Strain 1984), and photosynthetic rate (Aoki and Yabuki 1977; Kreidemann and Wong 1984; DeLucia et al. 1985). Decreased photosynthetic rates over time have been associated with starch buildup (Wulff and Strain 1982; DeLucia et al. 1985), reduced activity and / or concentration of RUBISCO (Wong 1979; Porter and Grodzinski 1983), and reduced concentration of chlorophyll (DeLucia et al 1985). It is not clear whether the reported effects are developmentally related, given the variety of plants and plant ages used, their degree of maturation, and the lack of nutritional information accompanying many of the studies.

Several problems arise in applying such information to trees growing in nature. Tree seedlings may be exposed to higher levels of CO_2 near the ground surface than in the bulk atmosphere (e.g. Williams and Bazzaz 1986), a consequence of soil respiration. Thus, tree seedlings may be exposed to higher concentrations of CO_2 than are older individuals. If CO_2 levels near the ground surface increase proportionally with bulk atmospheric CO_2 levels, seedlings may be exposed to much higher levels than currently assumed when bulk atmospheric CO_2 levels are 650 ul l^{-1}. It may be more appropriate to consider the effects of CO_2 levels greater than 650 ul l^{-1} when projecting the impacts of CO_2 doubling on forests.

Secondly, there are no published studies which have examined the effects of CO_2 enrichment on growth over a significant portion of the tree's life cycle. Age to maturation might be shortened, since conditions favoring rapid juvenile growth also may promote the onset of cone production and flowering in trees (Robinson and Wareing 1969). Such promotion of reproductive maturity might, in turn, decrease the availability of photosynthates and mineral nutrients for vegetative growth (Rook 1985). Relative growth rates of trees decline with age, a result of increased proportions of respiring but non-photosynthesizing tissues (Rook 1985), increased leaf overlap and decreased photosynthetic efficiency (Waring and Schlesinger 1985), and in some cases, more restricted shoot elongation patterns than in juvenile individuals (Rook 1985). In evergreen conifers, the photosynthetic capacity of needles may decrease with age (Linder and Troeng 1980; Hom and Oechel 1983), a consequence of wax buildup in the stomatal antechamber (Jeffree et al. 1971). Juvenile growth rates may (Chapin et al. 1983) or may not (Jones and Schier 1984) be correlated with growth rates of more mature individuals and with stand productivity. Early successional species such as *P. tremuloides* (individual ramets) typically have shorter lifespans than do later successional species such as *P. glauca* (Heinselman 1981). Carbon dioxide enrichment may accelerate a state of canopy closure; it could further accelerate senescence and rate of succession.

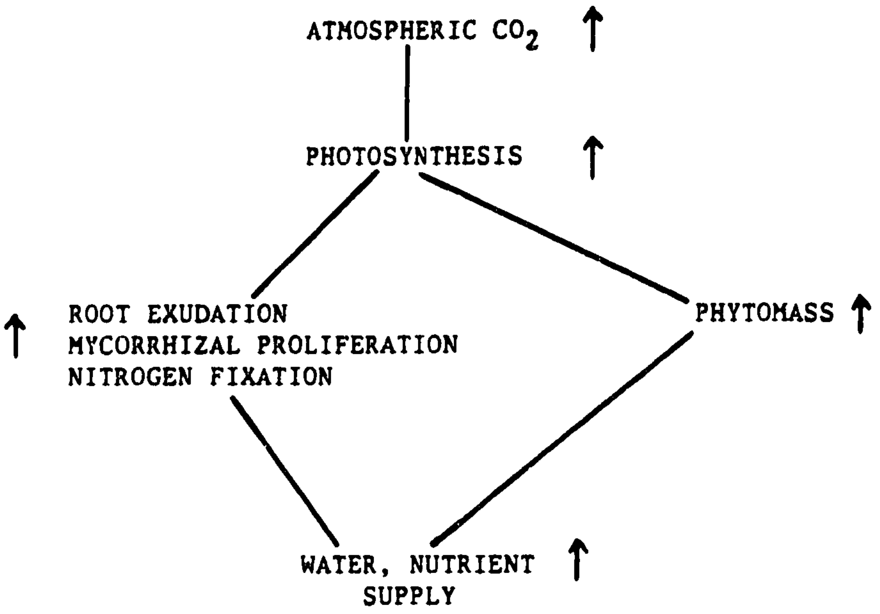
Competitive Relations Among Plants

The effects of long-term CO_2 enrichment on competitive relations between trees or between trees and other vegetation are virtually unstudied, although species responses might be altered from conditions in which plants are grown separately. Comparative data, such as in this study, suggest that growth of the inherently faster-growing of two species, could be enhanced more by CO_2 enrichment, at high or low nutrient availability. The data do not indicate whether or by how much the growth of the slower-growing species would be reduced under competition and CO_2 enrichment. Such information might be important in reforestation planning, given that Picea glauca seedlings are often suppressed by Populus tremuloides or by various deciduous shrubs on well-drained mixed-wood sites (Jarvis et al. 1966) or on cutovers by grasses such as Calamagrostis canadensis. An important consideration dictating competitive outcomes under CO_2 enrichment may be the relative ages and sizes of the competing species (Wray and Strain 1987).

Cycling of Mineral Nutrients and Carbon

Luxmoore (1981) presented a conceptual model outlining various ways in which CO_2 enrichment might alter availability and cycling of nutrients, such that productivity might be enhanced under nutrient-poor conditions. The model (Figure 11) presumes that CO_2 enrichment should increase (temporarily, at least) whole-plant photosynthetic rates, photosynthate production, and allocation of photosynthates to root systems. This, in turn, could stimulate development of mycorrhizal associations (e.g. O'Neill et al. 1987), fine root production (Norby et al. 1986), and exudation of organic acids and other

Figure 14. A possible mechanism by which CO_2 enrichment might increase production of phytomass in nutrient-limited situations. Redrawn from Luxmoore (1981).



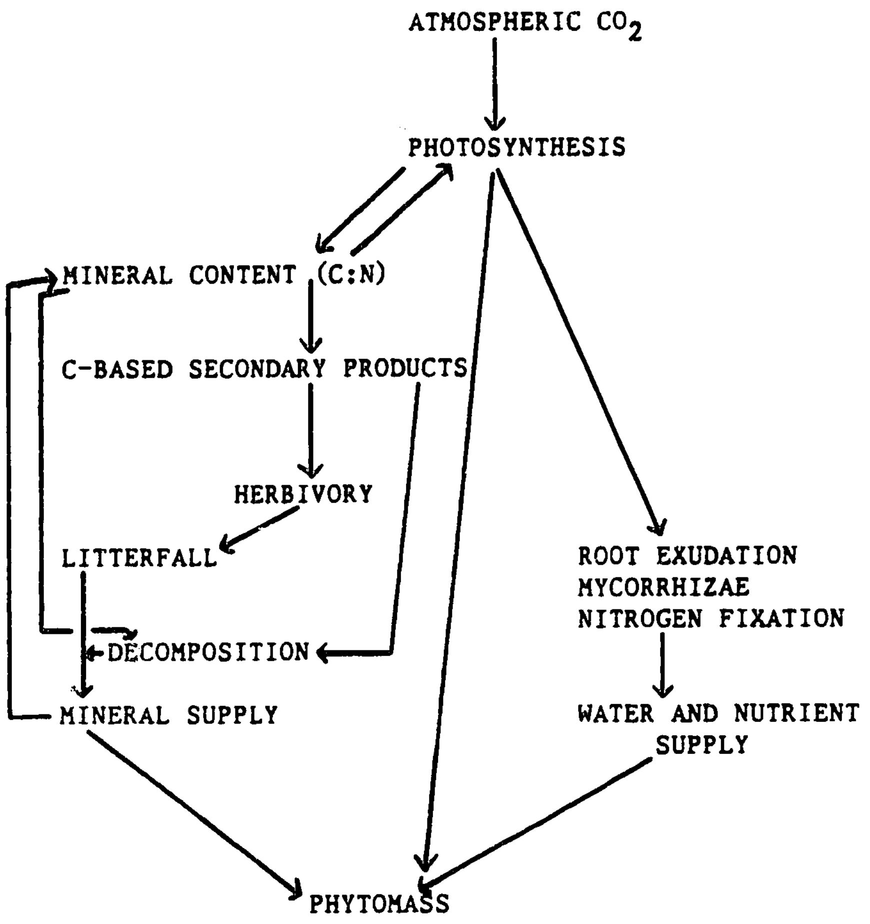
substances from root systems (Norby et al. 1987), all of which could increase nutrient availability to the plant. Increased nutrient availability could then support continued growth enhancement under CO_2 enrichment.

This approach is important in that (1) responses of individual plants are evaluated in the context of the ecosystem and (2) it provides a conceptual framework whose components can be tested experimentally. It is conceivable that CO_2 enrichment may alter various ecosystem properties through its effects on component vegetation; the changed ecosystem characteristics may, in turn, feedback and alter the response of the vegetation to increased CO_2.

The application of such a model to the boreal forest should consider several processes integral to shorter and longer-term aspects of the C and N cycles (Figure 15). Of particular importance should be the effect of CO_2 enrichment on C and N allocation within the plant and its subsequent effects on herbivory and decomposition.

Insect herbivores may be important in regulating primary production in forest ecosystems (Schowalter et al.1986). Consumption of available net primary production may approach 15% (Hodkinson and Hughes 1982). Insect herbivores may increase rates of nutrient cycling by digesting tissue and producing frass,

Figure 15. Possible interactions among productivity, carbon cycling, and mineral cycling in the boreal forest as affected by CO_2 enrichment.



processing green tissue without consumption, and may cause increases in intensity and volume of canopy leaching; they may also induce changes in leaf chemistry which ultimately inhibit decomposition (Choudbury 1988). The impact of vertebrate herbivores is generally much less than that of insects (Waring and Schlesinger 1985), but browsing mammals such as snowshoe hares (Lepus americana) may be an important selective pressure on boreal trees (e.g. Bryant and Kuropat 1980; Bryant et al. 1983) and may cause substantial problems in reforestation efforts (Jarvis et al 1966).

The impacts of herbivores could either increase or decrease with CO_2 enrichment if plant chemistry or "quality" is altered. Rates of herbivory by defoliating insects are often inversely correlated with tissue N concentrations, particulary of soluble N (Mattson 1980; Hodkinson and Hughes 1982). If CO_2 enrichment decreases foliar N concentrations, consumption rates might increase, as demonstrated for soybeans and soybean loopers (Lincoln et al. 1984; 1986) and peppermint and the noctuid moth, Spodoptera eridania (Lincoln 1986). If the plant has C-based defenses (e.g. phenolics, tannins, and their derivatives), a situation typical of boreal trees (Bryant et al. 1983), feeding rates of herbivores may not be related to plant N concentration, but instead may be inversely correlated with the concentrations of those C-based defensive compounds (Bryant and Kuropat 1980). The levels of those compounds may increase as the relative availability of N and C (manipulated via shading) to the plant is modified (Waring et al. 1985; Bryant et al. 1983), which in turn decrease palatability and feeding rates (Bryant et al. 1983; Larsson et al. 1986). Carbon dioxide enrichment may have a similar effect, although the magnitude might vary with tissue N status.

Decomposition rates are a major impediment to nutrient cycling in the boreal forest (Van Cleve and Dyrness 1983) and may be affected by substrate chemistry (Flanagan and Van Cleve 1983) as well as substrate temperature and moisture (Berg et al. 1975; Fogel and Cromack 1977; Fox and Van Cleve 1983). Turnover rates of forest floor material in stands of Picea glauca, P. mariana, Populus tremuloides, and Betula papyrifera were positively correlated to forest floor N and inversely correlated with lignin content (Flanagan and Van Cleve 1983). Decomposition rates of Pinus sylvestris needles were correlated with concentrations of N, P, K, and S to the point at which readily metabolized carbon compounds (soluble sugars, cellulose, and hemicellulose) were degraded; subsequent decomposition rates were not correlated with tissue nutrient concentration, but were instead inversely related to lignin content (Berg and Staaf 1980).

Thus, CO_2 enrichment could slow decomposition rates if nutrient concentrations of litter decreased and / or concentrations of recalcitrant C-based compounds (e.g. tannins, lignin) increased. The one study to date showed that senesced leaves of CO_2-enriched Quercus alba, grown in N- and P-deficient soil, had lower concentrations of N and lignin, but higher concentrations of soluble sugars and tannins (Norby et al 1986). In the absence of actual decomposition studies, such results indicate that short-term and long-term decomposition rates could either increase or decrease.

Clearly, a major problem in predicting effects of CO_2 enrichment on herbivory and decomposition lies in assumptions regarding N contents in affected tissues and the role N level may have in affecting levels of C-based compounds such as phenolics, tannins, and lignins. As previously discussed, N levels may be inadvertently reduced by insufficient supplies during the experimental period. Experiments examining the effect of CO_2 enrichment on tissue chemistry, herbivory and decomposition rates should take that problem into account.

CO_2-Induced Climatic Change

Since productivity, rates of nutrient cycling, and nutrient uptake rates in boreal trees and ecosystems may be strongly affected by soil temperature and moisture, a discussion of the effects of increased levels of CO_2 is incomplete unless the potential impacts of CO_2-induced climatic changes can be addressed as well.

As mentioned in Chapter 1, general circulation models (GCMs) of global climate generally predict increased annual temperature and precipitation at northern latitudes (e.g. 60 N. lat.) with a doubling of atmospheric CO_2 levels. The predicted increases vary seasonally, with the greatest increases predicted for winter months and the smallest increases, in summer. Recent applications of two GCMs to the boreal region of Alberta, Saskatchewan, Manitoba, and the Northwest Territories confirmed these general trends (Wheaton et al. 1988), but there was considerable uncertainty as to the direction and magnitude of precipitation changes. Temperature and precipitation changes could either negate or enhance the direct effects of CO_2 enrichment on boreal trees and ecosystems, as illustrated in the following scenarios.

Increased air temperatures should increase soil temperatures, other factors being equal. Increased soil temperatures may enhance rates of decomposition and nutrient mineralization in boreal soils (Fox and Van Cleve 1983), and increase rates of mineral uptake (Chapin et al. 1986), transpiration, and photosynthesis (Lawrence and Oechel 1983; DeLucia 1986), at least within the range of temperatures observed in the boreal forest. Increased nutrient availability, uptake, and photosynthetic rates should enhance the response to increased CO_2 levels by trees currently occupying cooler, wetter sites. Alternatively, increased air temperatures could accelerate the development of growing season moisture deficits, which might negate direct effects of CO_2 enrichment.

The seasonal changes in air temperatures, predicted by GCMs, may differentially affect evergreen and deciduous tree species, depending upon how much the length of the growing season and / or annual photosynthetic period is altered. Evergreen conifers typically have lower photosynthetic capacities than deciduous hardwoods or conifers (Larcher 1980). Annual production may, however, be as great or greater than in associated deciduous species of similar age; this may result from the greater longevity of leaves and accumulation of total leaf area in evergreens (Matyssek 1986) and / or from a longer photosynthetic period, particularly in areas with mild winters (e.g. Waring and Franklin 1979). Temperature increases during late winter and early spring could accelerate shoot elongation and expansion of new leaves in both deciduous and evergreen species (Kramer and Kozlowski 1979; Lavender 1981). If photoperiod is important in initiating leaf abscission in deciduous trees (Addicot 1982), increased air temperatures during the fall months could increase the annual photosynthetic period of evergreens relative to that of deciduous species. The scenario may be further complicated if warmer winter air temperatures increase winter desiccation of conifer needles.

Conversely, vegetation development, accelerated by higher CO_2 levels and air temperatures, might feed back so as to adversely affect site charateristics and productivity. For example, increased production of litter in sites underlain by permafrost might override the effects of increased soil surface temperatures on the thickness of the active layer (Miller et al. 1983).

The responses of vegetation to CO_2 enrichment, both at an ecosystem and at a landscape level, may be further modified by changes in precipitation and the occurrence of catastrophic disturbances such as fire. Viereck and Van Cleve (1984) suggested that increased temperatures, if not accompanied by increased summer precipitation, could increase the frequency of fire in interior Alaska, an effect also suggested by Wheaton et al. (1988) for the boreal forest of western Canada. A drier forest floor could result in more intense burning of organic material, could increase losses of some nutrients (e.g. N, S), and increase the availability of others to support plant growth. Increased fire frequency might increase the area of warmer, well-drained sites occupied by early successional deciduous species such as *Populus tremuloides*, *P. balsamifera*, and *Betula papyrifera* and result in expansion of closed *Picea glauca* and *P. mariana* forests into open wetland forests.

If summer precipitation increased, fire frequency might decrease, as would the effect of fire on nutrient cycling rates. Productivity might increase in forest types occupying the coldest and wettest or warmest and driest sites (Viereck and Van Cleve 1984); with more favorable temperature and moisture regimes, those sites might experience greater increases in productivity in response to CO_2 enrichment.

Modelling Approaches

The complex interactions and feedbacks alluded to in this discussion should clearly demonstrate the need for a modelling approach to predicting possible long-term effects of CO_2 enrichment and associated climatic change on boreal ecosystems.In fact, several attempts have been made to model the direct effects of increasing CO_2 levels and / or predicted climatic effects associated with increasing CO_2 on various aspects of boreal trees and ecosystems.

Perhaps the simplest approach has been to predict changes in either ecosystem boundaries or biomass in response to climatic changes modelled by GCMs. The predicted climatic measures used typically include a measure of length and intensity of the growing season (e.g. growing degree days) and a measure of precipitation change, Emanuel et al. (1985) used this approach to predict changes in distribution of Holdridge life zones. Their analysis, which excluded the possibility of changes in precipitation or soils, indicated that the boreal forest could virtually disappear with climate change associated with a doubling of CO_2 levels. It would be replaced by cool temperate steppe in drier regions and by cool temperate forest in moister regions. Wheaton et al.(1988) attempted to predict changes in zonation and productivity of the boreal forest in Alberta, Saskatchewan, Manitoba, and the Northwest Territories using bioclimatic indices (growing-degree days, precipitation effectiveness index, evapotranspiration, and soil moisture deficit) calculated from output of two GCMs. They indicated that aspen parkland and boreal temperate forest might replace subarctic or boreal forest in drier and moister regions, respectively. A scenario of climatic warming with increased precipitation increased potential biomass in all but the southernmost site (approximately 50 N. lat.) by 19-118 %. The results were not, however, validated against actual productivity data in the study area. Solomon et al. (1984) used a similar approach, but deemed it unsuitable for projecting changes in forest zones chages in eastern North America (including the boreal and subarctic forests of eastern and central Canada). Subsequent predictions of forest responses to CO_2-induced climate changes using the FORET forest succession simulation model indicated that closed spruce forest would encroach upon open parkland near Arctic treeline, increased biomass and abundance of <u>Picea glauca</u> and <u>Betula papyrifera</u> in the northern boreal forest, and a 30-50 % increase in total stand biomass of the boreal forest.

Modelling the combined effects of CO_2 enrichment and CO_2-induced climate change on the boreal ecosystem is more difficult than simulating the effects of climate change alone. Models appropriate for predicting the effects of climatic changes often can not simulate the direct effects of CO_2 enrichment on plant processes or traits such as photosynthesis, stomatal conductance, photosynthate allocation, litterfall, crown architecture, or foliar C:N ratios (Dahlman 1985; Reynolds and Acock 1985), all of which may affect long-term responses of the boreal forest to CO_2 increases. Models which do simulate these direct effects may have to rely on simpler assumptions than desired, given the paucity of appropriate experimental data. For example, Shugart and Emanuel (1985) represented the effect of CO_2 enrichment on forest succession via fixed proportional increases of the intrinsic growth rates of the different tree species and did not account for possible effects of nutrient limitations. Such assumptions may not be appropriate and could result in misleading predictions. However, simulation models represent the only means for dealing with the large number of possible combinations of direct CO_2 effects and effects of climate change. Models may illustrate areas requiring experimental data which are crucial to the validity of the predictions (Dahlman 1985).

The most comprehensive physiologically based model developed for northern ecosystems is the NECS (Northern ecosystems carbon simulator) model of Miller et al. (1983). The model calculates the storage of carbon in different terrestial pools of northern ecosystems and the fluxes between these pools and the atmosphere. The calculations are based on assumptions concerning the direct effects of CO_2 level and temperature on photosynthesis, respiration, and growth, and their indirect effects resulting from changes in soil temperature and moisture, the relative amounts of frozen versus unfrozen soil organic matter, and nutrient availability. Photosynthesis is assumed to increase linearly with atmospheric CO_2 level above ambient levels, unless total nonstructural carbohydrate (TNC) levels increase past a certain level. Possible differences in species responses seem to be ignored. The prediction that productivity of mid-boreal forests exceeds that of southern boreal forests under ambient conditions is contrary to existing data (Wheaton et al. 1988). Nonetheless, the model provides a useful framework for elucidating experimental needs and for making general predictions of changes in carbon storage in the boreal forest.

In attempting to project long-term effects of increasing atmospheric CO_2 levels (and associated climatic change), two major and general problems exist. One is a lack of appropriate models (Dahlman 1985), i.e. those encompassing processes important to functioning of boreal forest ecosystems, built at levels of hierarchy appropriate for answering specific questions (Reynolds and Acock 1985), and which are capable of extrapolative predictions (Solomon et al. 1984; Reynolds and Acock 1985).

The second general problem concerns the adequacy of data available for validating such models (Solomon et al. 1984; Dahlman 1985). As previously discussed, studies of woody plant responses to CO_2 enrichment have exclusively focused on seedlings or saplings, have generally not involved comparative studies of associated species, and with few exceptions (e.g. Luxmoore et al. 1986; Surano et al. 1986; Prudhomme and Oechel 1984), have not been conducted under natural conditions. Studies of seedling responses are appropriate, in and of themselves, for several reasons: mortality rates are highest during that stage (Solbrig 1980); events during that stage may ultimately influence stand structure (Harper 1977); and slight enhancement of carbon capture by plants relative to resource requirements may reduce mortality under marginal conditions. Seedlings are the most appropriate stage to study when attempting to identify mechanisms underlying responses to CO_2 and nutrients. Furthermore, studies of seedling responses are directly applicable in such areas as nursery production of stock for reforestation. An understanding of seedling responses to CO_2 is not sufficient , however, for predicting the responses of mature trees (Solomon et al. 1984).

A second problem involves scaling of responses measured at the leaf level (e.g. transpiration and photosynthesis) to a whole-tree, stand or ecosystem level. CO_2-induced reductions in transpiration, measured at the leaf, may be lessened at the whole-plant level, and even reversed at a community level (Jarvis and McNaughton 1985; Morison and Gifford 1984; Morison 1985). Similarly, plant nutrient relations may change at different scales, as previously discussed. These problems deserve attention in future studies. From a modeller's perspective, interactions between CO_2 and other environmental factors must be modelled at the levels of physiological processes, but predictions must be made at the ecosystem level. This may create problems with respect to choice of time steps, increasing number of assumptions (and error) and additional difficulties in debugging the models (Reynolds and Acock 1985.

In summary, three general approaches should be pursued in assessing the potential impacts of increasing atmospheric CO_2 levels on the boreal forest. First, field fumigation studies should be instituted to evaluate the responses of representative tree species under natural light, precipitation, and in native soil. Ideally, such studies should extend over a longer period than have previous studies in glasshouses, perhaps to the onset of reproductive maturity. Models of boreal forest responses, based on physiological responses of trees to CO_2, temperature, nutrient availability, and moisture, should be developed. These models could generate the variety of scenarios possible under CO_2 enrichment and could dictate specifically what data is required for the model to produce realistic results and thereby guide experiments in controlled environments. Finally, a better understanding of CO_2 effects on tree growth in response to levels of irradiance, moisture, and nutrients, and to soil temperature is needed to clarify physiological mechanisms of the CO_2 effect. Such studies, perhaps involving several factors, would complement field and modelling studies.

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Appendix 1. Symbols and formulae used for calculations of growth, physiological, and treatment parameters. The number in parentheses indicates in which chapter the symbol or formula is located.

SYMBOLS

A_0	(2)	Initial leaf area (cm^2)
C_a	(5)	Concentration (ul/l) of CO_2 in bulk atmosphere
F	(5)	Volumetric flow rate (cm^3/s)
L_0	(2)	Initial leaf mass (g)
LA	(2,5)	Leaf area (cm^2)
LM	(2,5)	Leaf mass (g)
[N]	(4)	Concentration of nitrogen (mg/g)
RM	(2,5)	Root mass (g)
SM	(2,5)	Stem mass (g)
T	(5)	Temperature (C or K)
t	(2,5)	Time (days)
TDM	(3,5)	Total dry mass (g)
p_a	(5)	vapour pressure in airstream (g/cm^3)
p_c	(5)	vapour pressure in cuvette (g/cm^3)
p_l	(5)	vapour pressure at mesophyll surface (assumed to be at saturation) (g/cm^3)

FORMULAE

A	(5)	Net photosynthetic rate ($mg/dm^2/hr$) $A=C_d(44/22414)(273/T)(600)(F/LA)(P/1013)$ where T=temperature (K) and P=barometric pressure (mb)
C_i	(5)	Internal CO_2 concentration $C_i=C_a-1.6(A/g_s)$
E	(5)	Transpiration rate ($ug/cm^2/sec$) $E=(p_c-p_a)(F/LA)$
g_s	(5)	Stomatal conductance (cm/s) $g_s=1/R_s$, where $R_s=(LA/F)((p_1-p_c)/(p_c-p_a))-0.015$
LAP	(2,5)	Leaf area partitioning coefficient ($cm^2/d/g/d$) $LAP=(RAGR)A_0e^{(RAGR)t}/(RGR)W_0e^{(RGR)t}$
LAR	(5)	Leaf area ratio (cm^2/g) LAR=LA/TDM
LWP	(2,5)	Leaf mass partitioning coefficient (g LM/d/g/d) $LWP=(RLGR)L_0e^{(RLGR)t}/(RGR)W_0e^{(RGR)t}$
LWR	(2,5)	Leaf weight ratio (g/g) LWR=LM/TDM
NAR	(2,5)	Net assimilation rate ($mg/dm^2/d$) $NAR=(RGR)W_0e^{(RGR)t}/A_0e^{(RAGR)t}$
Pn	(5)	Nitrogen productivity (g TDM/g N/day) Pn=RGR/[N]
RAGR	(2,5)	Relative leaf area growth rate ($cm^2/cm^2/day$) $RAGR=(\ln LA_2-\ln LA_1)/(t_2-t_1)$
RGR	(2,5)	Relative growth rate (g/g/day) $RGR=(\ln TDM_2-\ln TDM_1)/(t_2-t_1)$
RLGR	(2,5)	Relative leaf growth rate (g/g/day) $RLGR=(\ln LM_2-\ln LM_1)/(t_2-t_1)$
RN	(4,5)	Relative nitrogen addition rate (g/g/day) $RN=(\ln N_2-\ln N_1)/(t_2-t_1)$
R:S	(5)	Root:shoot ratio (g/g) RM/(SM+LM)
RWR	(2,5)	Root weight ratio (g/g) RWR=RM/TDM
SLA	(2,5)	Specific leaf area (cm^2/g) SLA=LA/LM
SWR	(5)	Stem weight ratio (g/g) SWR=SM/TDM
WUE	(5)	Water use efficiency ($mgCO_2/gH_2O$) WUE=(E/A)(2.78)

APPENDIX 2. Effects of CO_2 enrichment and nutrient supply on growth of *Populus tremuloides* and *Picea glauca* seedlings in sand culture: a preliminary study

INTRODUCTION

Prior to conducting the first experiment (chapter 2), a preliminary study was conducted. The objectives of this trial were to:

(1) Establish a range of N supply rates for use in subsequent experiments
(2) Compare growth responses to solutions deficient in N only with solutions in which all mineral nutrients were reduced by a similar proportion.
(3) Compare growth responses of *P. tremuloides* seedlings from several half-sib sources of seed from the Edmonton area.
(4) Perfect culture techniques for seedlings and estimate time requirements for the first experiment.

METHODS

The study was conducted in two controlled environment rooms with a 22 / 17 C (day/night) temperature regime, 18 hour photoperiod and thermoperiod, canopy level irradiance of approximately 400 umoles/m^2/sec supplied by sodium and mercury HID lamps, and a daytime relative humidity setting of 50%. Atmospheric CO_2 levels were maintained in the two rooms at 350 ul/l (ambient) and 750 ul/l with a Beckman 864 infrared gas analyzer (IRGA) and set point control system which automatically injected bottled CO_2 into each rooms airstream as required.

Sand with a particle size of 0.25-0.6 mm (Grade Sil-4, Sil Silica, Edmonton, Alta.) was used as the rooting medium in this study; the pots were "supercell conetainers" (Ray Leach Conetainer Nursery, Canby, OR.) with a volume of 160 cm^3 and surface area:volume ratio of approximately 0.08 cm^2/cm^3. Prior to seeding, the sand was acid-washed with 3.5% HCl, leached with distilled H_2O, then flushed with dilute nutrient solution (solution #4, see below) to reduce conductivity and raise the pH of the sand. The purpose of the acid wash was to remove any macro- or micronutrient impurities and clay particles which might affect the availability of added nutrients to the plants (Hewitt 1966).

Sand was chosen as a rooting medium because it is chemically inert, because it might physically abrade elongating roots in a fashion similar to that of soil (Asher and Edwards 1983), and because of its apparent ease of maintenance. The disadvantages in using sand include a lack of precise control of the rhizosphere environment (Asher and Edwards 1983) and the lack of water (=nutrient solution) retention after irrigation relative to substrates containing organic matter and / or clay (Rawlins 1979).

Five nutrient solutions were developed (Table 1) for the study. Solution #2 was thought to have both an optimal mass balance of elements for plant growth (Table 2) and an appropriate concentration of elements, based on other sand culture studies (Wild et al. 1974; Chapin et al. 1983). Solutions #1 and #5 had the same mass balance of elements as did #2, but were respectively 10X and 1/10X as concentrated. Solutions #3 and #4 had 1/10 and 1/100 as much N, respectively, as did #2, but had equivalent concentrations of other elements. This was accomplished by supplying N entirely as NH_4NO_3 and by using chloride salts of Ca and K to increase the availability of those cations. Potassium hydroxide (KOH) was added to each solution as necessary to adjust the pH to an appropriate level; KCl was then added to readjust the K levels in each solution.

Table 1. Macronutrient source and concentration(mM) of elements in nutrient solutions 1-5. Balance of each element by mass (relative to N) is indicated by number enclosed in parentheses. Elem.=element; Conc.=concentration (mM)

		SOLUTION				
Salt	Elem.	#1 Conc.	#2 Conc. (mM)	#3 Conc.	#4 Conc.	#5 Conc.
NH_4NO_3	N	155(100)	15.5(100)	1.55(10)	0.155(1)	1.55(100)
KH_2PO_4	P	12.6(18)	1.26(18)	1.26(18)	1.26(18)	0.126(18)
K_2SO_4	S	8.8(13)	0.88(13)	0.88(13)	0.88(13)	0.088(13)
KCl, KOH	K	27.8(50)	2.78(50)	2.78(50)	2.78(50)	0.278(50)
$MgSO_4$	Mg	4.3 (5)	0.43 (5)	0.43 (5)	0.43 (5)	0.043 (5)
$CaCl_2$	Ca	2.7 (5)	0.27 (5)	0.27 (5)	0.27 (5)	0.027 (5)
pH		5.9	5.8	5.8	5.7	6.1
Conductivity ($mS\ m^{-1}$)		1200	140	60	45	15

Salts used for micronutrients:

Fe-citrate
$MnCl_2$;
H_3BO_3
$CuCl_2$
$ZnCl_2$
$NaMoO_4$

Table 2. Optimum mass proportions of mineral nutrients (N=100) (from Ingestad 1979).

SPECIES	N	K	P	Ca	Mg	S
Betula verrucosa	100	65	13	7	8.5	9
Picea abies	100	50	16	5	5	9
Picea sitchensis	100	55	16	4	4	9
Pinus sylvestris	100	45	14	6	6	9
Tsuga heterophylla	100	70	16	8	5	9
Vaccinium sp.	100	50	13	9	8.5	9

All species:	Fe	Mn	B	Cu	Zn	Cl	Mo	Na
	0.7	0.4	0.2	0.03	0.03	0.03	0.007	0.003

Populus tremuloides seed was collected in May 1984 from five individual trees in the Edmonton area. Two trees were located at the Devonian Botanical Garden, two were located on the upper south bank of the North Saskatchewan River near the University of Alberta campus, and the final tree was located on a slope in the Whitemud Ravine. *Picea glauca* seed was from a bulk collection made in the Footner Lake region of northern Alberta.

Upon collection, *P. tremuloides* seed were extracted, air-dried, then stored at -17 C. Seed were then sown onto sand in the conetainers, kept moist during establishment, and thinned to one seedling per conetainer 21 days after sowing. *P. glauca* seed, in contrast, were germinated in petri dishes and transplanted to the conetainers approximately one week after germination. Seedlings were randomly assigned to CO_2 regimes at the time of sowing or transplanting. Both species were fertilized periodically with solution #5 for 25 days during establishment. At that time the nutrient treatments were begun; they were continued for 33 days with *P. tremuloides* and 55 days with *P. glauca*. All seedlings were irrigated with 50 ml of the appropriate solution 3X per week with a repipet and were flushed with distilled H_2O immediately prior to fertilization to prevent any salt accumulation.

Oven-dry (70 C for 24 hours) mass of roots, stems, and leaves was measured on a random sample of seedlings at the beginning of the nutrient treatments and on all surviving seedlings at its termination. In addition, height and leaf area were determined on freshly harvested _P. tremuloides_; needle numbers were counted on _P. glauca_ seedlings.

RESULTS

Final growth data was affected by at least one problem resulting from the culture procedure. Approximately 4 weeks into the experimental period, a substantial number of _P. tremuloides_ seedlings, fertilized with solution #2 ("optimum"), died or were severely damaged. Most damage was confined to the CO_2-enriched plants, although some occurred in control plants as well. The damage seemed to result from severe water stress, which likely developed through a combination of large leaf area in the plants fertilized with solution #2, accelerated plant growth under CO_2 enrichment, and, possibly, a slightly higher vapor pressure deficit in the high CO_2 room. Seedlings from each seed source were damaged, with the greatest mortality occurring in seedlings from the Whitemud (WM) seed source. Thus, the final data from WM is not presented here; data from the final harvest of the other seed sources fertilized with solution #2 may not accurately reflect growth responses occurring if water stress had not been imposed.

All P. tremuloides seedlings fertilized with solution #1 died within a week of the initial fertilization. Some mortality also occurred in P. glauca seedlings; of those surviving, most had stunted needle and shoot growth. Interestingly, P. glauca seedlings which survived the solution #1 fertilization had similar R:S ratios as did those fertilized with the "optimum" solution, solution #2 (Table 3).

Within a given seed source and CO_2 regime, P. tremuloides seedlings fertilized with solution 5 (all elements reduced 10-fold from optimum) may have had slightly greater leaf and total dry mass than did seedlings fertilized with solution 3 (N only reduced 10-fold from optimum) (Tables 4, 5); if so, the difference was most pronounced in seedlings from RB-5 (riverbank). In contrast, P. glauca seedlings fertilized with solution 3 may have been slightly larger than those fertilized with solution #5 (Table 3).

When N availability only was altered (solutions 2,3,4), the absolute gain in biomass with increasing supply of N was greater in P. tremuloides than in P. glauca, within a given CO_2 regime (Table 6). The degree of change in R:S, however, appeared similar between the two species. The responses of different seed sources of P. tremuloides to an increasing supply of N appeared similar as well (Tables 3,4).

Table 3. Growth responses of *Picea glauca* seedlings to CO_2 enrichment and nutrient regime. Nutrient regimes are as outlined in Table 1 and text (mean ± sd; n=6 for Nutr. 1, 350 ul l^{-1} treatment; n=7 for Nutr. 1, 750 ul l^{-1} treatment; n=9 for all other treatments). The experiment lasted 55 days. Abbreviations: RM=dry root mass; LM=dry leaf mass; TM= total dry mass; LN=needle number; R:S=root:shoot ratio.

TREATMENT	RM(g)	LM(g)	TM(g)	LN	R:S
350 ul l^{-1} CO_2					
Nutr. 1	0.04(.02)	0.09(.04)	0.14(.06)	206 (57)	0.38(.08)
2	0.14(.03)	0.30(.11)	0.49(.16)	508(160)	0.43(.11)
3	0.09(.02)	0.17(.04)	0.28(.07)	318 (62)	0.46(.06)
4	0.03(.01)	0.04(.01)	0.08(.02)	86 (23)	0.79(.15)
5	0.07(.04)	0.10(.06)	0.18(.11)	206 (88)	0.57(.10)
750 ul l^{-1}					
Nutr. 1	0.07(.05)	0.13(.07)	0.22(.13)	325(134)	0.44(.17)
2	0.26(.06)	0.52(.11)	0.86(.17)	993(229)	0.45(.08)
3	0.16(.06)	0.23(.08)	0.42(.16)	426(117)	0.62(.07)
4	0.05(.02)	0.03(.02)	0.09(.04)	72 (34)	1.27(.25)
5	0.13(.08)	0.19(.11)	0.34(.20)	322(142)	0.64(.14)

TREATMENT	SEED SOURCE	n	RM(g)	LM(g)	TM(g)	LA (cm^2)	SHT(cm)	R:S
350 ul l^{-1}								
Nutr. 2	Dev-1	2	0.36(.10)	0.66(.17)	1.16(.29)	173(40)	14.4(3.6)	0.46(.12)
	Dev-2	4	0.24(.05)	0.42(.27)	0.76(.45)	122(61)	11.7(4.3)	0.43(.17)
	RB-4	2	0.38(.05)	0.79(.10)	1.37(.15)	209(34)	17.8(1.9)	0.39(.02)
	RB-5	8	0.37(.09)	0.59(.14)	1.13(.27)	166(32)	17.1(3.0)	0.50(.05)
Nutr. 3	Dev-1	9	0.22(.02)	0.27(.04)	0.54(.07)	59 (7)	9.3(1.8)	0.70(.11)
	Dev-2	9	0.21(.04)	0.25(.03)	0.52(.07)	68 (5)	9.5(1.9)	0.68(.13)
	RB-4	9	0.23(.03)	0.24(.04)	0.53(.08)	59(11)	9.7(1.7)	0.77(.10)
	RB-5	9	0.16(.06)	0.17(.06)	0.37(.14)	42(13)	7.4(2.0)	0.80(.11)
Nutr. 4	Dev-1	9	0.06(.01)	0.06(.01)	0.12(.03)	12 (2)	2.9(0.7)	0.89(.14)
	Dev-2	9	0.05(.01)	0.04(.01)	0.10(.02)	13 (1)	3.4(1.1)	0.97(.22)
	RB-4	9	0.06(.01)	0.06(.02)	0.14(.02)	14 (3)	2.9(0.5)	0.88(.20)
	RB-5	9	0.04(.01)	0.04(.01)	0.09(.02)	12 (2)	3.6(0.5)	0.92(.11)
Nutr. 5	Dev-1	9	0.22(.03)	0.30(.06)	0.58(.09)	69(11)	9.4(1.5)	0.63(.11)
	Dev-2	9	0.22(.04)	0.30(.06)	0.59(.10)	72(11)	11.1(1.9)	0.59(.05)
	RB-4	9	0.25(.07)	0.31(.06)	0.82(.14)	61(12)	9.9(1.9)	0.65(.12)
	RB-5	9	0.22(.04)	0.23(.02)	0.51(.06)	66 (7)	10.3(1.5)	0.74(.10)

Table 4. Growth responses of _Populus_ _tremuloides_ seedlings to nutrient regime (described in text and Table 1) at 350 ul l^{-1} atmospheric CO_2 (mean $\pm$ sd). The experiment lasted 33 days. Abbreviations: RM=root mass; LM=leaf mass; TM=total mass; LA=leaf area; SHT=shoot height; R:S=root:shoot ratio.

TREATMENT	SEED SOURCE	n	RM(g)	LM(g)	TM(g)	LA(cm^2)	SHT(cm)	R:S
750 ul l^{-1}								
Nutr. 2	Dev-1	4	0.58(.15)	1.13(.17)	2.01(.36)	235(28)	21.4(2.1)	0.40(.08)
	Dev-2	2	0.62(.27)	0.98(.18)	1.87(.50)	237(24)	19.0(0.7)	0.48(.13)
	RB-4	5	0.67(.06)	1.17(.05)	2.15(.13)	248(23)	19.6(3.2)	0.46(.04)
	RB-5	3	0.53(.02)	0.93(.12)	1.72(.12)	213(59)	19.5(2.5)	0.45(.06)
Nutr. 3	Dev-1	9	0.30(.08)	0.30(.07)	0.67(.17)	67(10)	11.7(2.0)	0.79(.10)
	Dev-2	8	0.26(.09)	0.30(.13)	0.63(.15)	57(16)	9.9(2.5)	0.76(.12)
	RB-4	9	0.33(.04)	0.34(.04)	0.75(.07)	70 (9)	12.2(2.0)	0.77(.10)
	RB-5	9	0.31(.06)	0.23(.05)	0.61(.12)	51 (8)	10.2(1.6)	1.05(.14)
Nutr. 4	Dev-1	9	0.09(.01)	0.08(.01)	0.18(.02)	16 (2)	3.4(.06)	0.93(.11)
	Dev-2	8	0.06(.02)	0.05(.02)	0.11(.04)	12 (3)	3.3(.05)	1.13(.16)
	RB-4	8	0.07(.01)	0.07(.02)	0.07(.02)	14 (1)	3.6(.06)	0.88(.11)
	RB-5	8	0.06(.02)	0.05(.05)	0.12(.03)	11 (2)	3.3(.05)	1.08(.13)
Nutr. 5	Dev-1	9	0.29(.04)	0.36(.04)	0.74(.06)	69 (5)	11.2(1.4)	0.66(.11)
	Dev-2	9	0.33(.04)	0.37(.05)	0.80(.08)	69(10)	12.1(1.5)	0.72(.12)
	RB-4	8	0.34(.08)	0.35(.06)	0.78(.14)	63 (7)	11.3(1.2)	0.77(.18)
	RB-5	8	0.36(.07)	0.35(.04)	0.81(.12)	66 (7)	11.3(1.2)	0.80(.10)

Table 5. Growth responses of *Populus tremuloides* seedlings to nutrient regime (described in text and Table 1) at 750 ul l^{-1} atmospheric CO_2 (mean ± sd). The experiment lasted 33 days. Abbreviations: RM=root mass; LM=leaf mass; TM=total mass; LA=leaf area; SHT=shoot height; R:S=root:shoot ratio.

The absolute effect of CO_2 enrichment on total dry mass increased with increasing availability of N in both species (Table 6, Figure 1), a trend also shown for leaf area of *P. tremuloides* and needle mass of *P. glauca* (Table 6). The relative effect of CO_2 enrichment on *P. tremuloides* did not consistently increase with availability of N, however. For example, CO_2 enrichment had similar relative effects on root mass of the Dev-1 seedlings at all three levels of N supply, but had greater effects on root mass of the RB-5 seedlings in the medium-N (solution 3) treatment (Tables 4 and 5). CO_2 enrichment had the greatest relative effect on leaf mass and leaf area of seedlings of both seed sources at the optimum N level (solution 2). Relative effects of CO_2 enrichment on mass and needle numbers of *P. glauca* increased with availability of N (Table 6).

Table 6. Relative and absolute effects of N supply and CO_2 enrichment on total dry mass (TM) and leaf area (LA) of *Populus tremuloides* (*P. trem.*) and total and leaf mass (LM) of *Picea glauca*. Seedlings were grown at atmospheric CO_2 levels of 350 or 750 ul l^{-1}.

	Relative effect				Absolute effect			
P. trem.	Dev-1	Dev-2	RB-4	RB-5	Dev-1	Dev-2	RB-4	RB-5
N effect[1/]								
350								
TM(g)	9.4x	7.6x	10.1x	12.0x	1.04	0.66	1.23	1.03
LA(cm^2)	4.4x	9.4x	14.9x	13.8x	161	109	195	154
750								
TM	11.2x	16.4x	13.9x	14.4x	1.83	1.76	1.99	1.60
LA	14.7x	20.0x	17.7x	19.4x	219	225	234	202
CO_2 effect								
15.5mM-N								
TM	1.7x	2.5x	1.6x	1.5x	0.86	1.11	0.78	0.60
LA	1.4x	1.9x	1.2x	1.4x	62	115	39	47
1.55mM-N								
TM	1.2x	1.2x	1.4x	1.6x	0.13	0.11	0.23	0.24
LA	1.1x	0.8x	1.2x	1.2x	8	-9	11	9
0.155mM-N								
TM	1.5x	1.1x	1.1x	1.3x	0.06	0.01	0.02	0.03
LA	1.3x	0.9x	1.0x	0.9x	4	-1	0	-1
Picea glauca								
N effect								
350 ul l^{-1}								
TM	6.4x				0.41			
LM	7.8x				0.26			
750								
TM	10.0x				0.78			
LA	15.7x				0.49			
CO_2 effect								
15.5 mM-N								
TM	1.8x				0.38			
LM	1.7x				0.22			
1.55 mM-N								
TM	1.5x				0.14			
LM	1.3x				0.06			
0.155 mM-N								
TM	1.1x				0.01			
LA	0.9x				-0.01			

[1/] Difference between seedlings grown in solutions 2 and 4

DISCUSSION

These results indicate that the original premise of the study is sound; the two species do show somewhat different responses to increasing N supply and the effect of CO_2 enrichment is modified by availability of N. The effect of CO_2 enrichment on rate of biomass accumulation was greater in P. tremuloides than in P. glauca, at least when seedlings were fertilized with solution #2, supporting one of the original hypotheses of the study.

Additionally, the range of N levels used in solutions 2,3, and 4 seems appropriate for the main studies. However, the rapid growth of the Populus seedlings, particularly when fertilized with solutions 2 and 3, necessitates the use of larger containers in order to avoid problems with moisture and nutrient stress later in the study. Thus, the approach to be used in the subsequent longer-term growth experiment (Chapter 2) will be to grow P.tremuloides seedlings in large diameter (5 cm) PVC pipe. The approach seems reasonable if the container surfaces are at the same distance from the room lights as are the conetainer surfaces and if the volume of nutrient solution added to the PVC containers is increased proportionate to increased volume of sand. In order to minimize the possibility of short-term moisture stress, nutrient solutions will be added at half the volume, but 2X the frequency as used in this study.

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Appendix 3. Total surface area of <u>Picea</u> <u>glauca</u> needles as predicted from needle length.

METHOD

Needles were sampled from 3-4 seedlings in each treatment, with an objective of sampling the range of needle lengths found in the particular treatment. After measuring needle length, each needle was coated in dilute rubber cement, which was then allowed to dry for several minutes. The needle was weighed, then coated with a monolayer of Ballitoni glass beads, checked for evenness of coating with a dissecting microscope,, and reweighed. Needle surface area was then calculated, assuming a conversion factor of bead mass to coated surface area of 18.15 mg cm^{-2}. Regressions of needle area on length were developed for each treatment, for pooled data within each N and CO_2 regime, and for pooled data from all treatments.

RESULTS AND CONCLUSIONS

Pooling of data from different treatments did not improve the fit of the regressions and in two cases, substantially worsened the fit (Table 1). Thus, separate regressions were used for estimating leaf area in each treatment of experiments 1 and 2 (chapters 2 and 4). Regressions developed for the 15.5 mM-N and 0.155 mM-N treatments were used to estimate leaf areas in the "high-N" and "low-N" regimes, respectively, of experiment 2.

Table 1. Total surface area (A) of *Picea glauca* needles as predicted from needle length (l), based on area estimates using the Ballitoni glass bead technique (Thompson and Leyton 1971). Conversion factor of glass bead mass to surface area coated, assuming a monolayer coating of beads, was 18.15mg/cm^2.

TREATMENT	REGRESS.	N	R^2	RANGE,L (cm)	SEE 1/	95% CI (slope)	95% CI (int.)
350 ul l^{-1}							
15.5mM-N	A=.20L-.05	27	0.98	0.4-2.2	.0171	±.01	±.02
1.55mM-N	A=.22L-.06	20	0.97	0.4-1.9	.0204	±.02	±.02
0.155mM-N	A=.20L-.03	20	0.96	0.3-1.8	.0185	±.02	±.02
750							
15.5mM-N	A=.22L-.05	19	0.92	0.3-2.4	.0457	±.04	±.05
1.55mM-N	A=.23L-.06	22	0.93	0.3-2.0	.0332	±.03	±.04
0.155mM-N	A=.23L-.04	20	0.96	0.2-1.8	.0228	±.02	±.02
Pooled Data							
350	A=.26L-.09	67	0.38	0.3-2.2	.1774	±.08	±.11
750	A=.23L-.05	61	0.94	0.2-2.4	.0340	±.02	±.02
15.5mM-N	A=.26L-.08	46	0.35	0.3-2.4	.2160	±.06	±.16
1.55mM-N	A=.23L-.06	42	0.95	0.3-2.0	.0276	±.02	±.02
0.155mM-N	A=.21L-.04	40	0.95	0.2-1.8	.0227	±.02	±.02
All data	A=.24L-.06	128	0.51	0.2-2.4	.1299	±.04	±.05

1/ SEE=standard error of the estimate for the linear regression