

Photosynthesis, Nitrogen, Their Adjustment and its Effects on Ecosystem Carbon Gain at
Elevated CO₂ I. A comparison of loblolly and ponderosa pines

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Introduction

A functional understanding of terrestrial ecosystem carbon processes is essential for two reasons. First, carbon flow is a most fundamental aspects of ecosystem function as it mediates most of the energy flow in these systems. Second, carbon flow also mediates the majority of energy flow in the global economy and will do for the foreseeable future. The increased atmospheric carbon dioxide and its inevitable flow through global ecosystems will influence ecosystem processes. There is, of course, great interest in the potential of ecosystems to sequester some of the carbon being loaded into the atmosphere by economic activity.

Forest trees and forested ecosystems, upon which these studies have focused, play a large role in the global carbon cycle as well as in the forest products industry being as significant carbon based economic activity. Forests cover approximately 4.1×10^9 ha of the total land surface area of the earth, containing an estimated 1146 Pg of C in vegetation and soils (Dixon et al. 1994). This 1146 Pg of C accounts for as much as 80% of the total above-ground terrestrial carbon (Olson et al. 1983, Houghton 1993). It has been suggested that mid-latitude forests could act as a significant carbon sink and that re-growth in previously disturbed forests can act to sequester carbon (Tans, Fung & Takahashi 1990, Vitousek 1991, Taylor & Lloyd 1992, Houghton 1993, Dixon et al. 1994). Carbon fixation by forest trees may account for as much as 70% the global exchange of carbon between these pools (Waring & Schlesinger 1985). Clearly, forests serve as a major link between the atmospheric and terrestrial carbon

pools. Molecular biology, biochemistry, physiology and ecology have all made significant contributions to our understanding of ecosystem carbon gain, yet our ability to predict the magnitude, location and relative importance of current carbon fluxes and pools still limits our understanding of the global carbon cycle as a whole (Tans et al., 1990).

Understanding of ecosystem carbon exchange must begin with a functional understanding of photosynthesis and interaction with environmental resource availability. The partial pressure of CO₂ in today's atmosphere (pCO₂, Ca. 36 Pa, Keeling et al., 1995) is generally limiting to C₃ photosynthesis. Human induced changes in the global carbon cycle are increasing the global average pCO₂ by approximately 0.15 Pa annually (Keeling et al. 1989). Photosynthetic responses to elevated pCO₂ are complex. Regulation of photosynthesis at any scale is not so simple that one can predict sustained increases in carbon fixation resulting from the increased availability of a just one (the carbon) substrate in the C fixation processes. The nature, magnitude and mechanisms of photosynthetic adjustments in forest trees, induced by changes in atmospheric CO₂ have received much study and discussion (Eamus & Jarvis 1989, Bazzaz 1990, Gunderson & Wullschleger 1994). Ultimately, it is the interactions with plant resources, primarily nitrogen, water and light (Gunderson & Wullschleger 1994, Sage 1994) that will regulate net leaf and ecosystem carbon gain (Thomas *et al* this volume, Dickson 1989, Mooney and Koch 1994).

Field and Mooney (1986) demonstrated a strong general relationship between maximum photosynthesis (A_{max}) and leaf nitrogen concentration using a wide range of C₃ species and a wide range of environments. Although it was developed in an autecological context it has been logical and tempting, from simple relations of nitrogen investment in physiological processes (Evans, 1989; Evans and Seemann, 1989), to consider that the A_{max}/N relationship can be used to predict the adjustment of leaves to varying environmental

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conditions. Taken at the level of photosynthetic physiology, a linear A_{max}/N relationship suggests that nitrogen is flexibly and optimally distributed between the carbon fixation and the RuBP regeneration functions of photosynthesis. Should such a relationship hold up to the fullest logical extent, the A_{max}/N could be used to define the nature and extent of leaf level adjustment to elevated CO_2 . Re-examining the photosynthetic response curves of more than 40 different species, Wullschlegel (1991) concluded that this notion of the photosynthetic apparatus as an optimized system has merit. Developing this simple idea, one would suggest that ecosystem nitrogen availability ultimately determines the photosynthetic capacity of a system, and defines the limits of photosynthetic adjustment to elevated pCO_2 . Although clearly simplistic an idea such as this is a useful point from which to begin focused thinking.

In reality, adjustments of ecosystem photosynthesis in elevated CO_2 occur at various hierarchical levels, including adjustments of (1) Rubisco content and activity; (2) nitrogen redistribution among enzymes for light harvest, electron transport, carboxylation; (3) photosynthetic apparatus per area; and (4) canopy leaf area. All of these variables do involve the distribution of nitrogen. Rubisco content has been found to decrease in most CO_2 experiments. Elevated CO_2 , for example, reduced Rubisco content by 15-50% for species Solanum tuberosum, Chenopodium album, Phaseolus vulgaris, and Brassica oleracea (Sage et al. 1989). Rubisco activation status increased for some species (e.g. Pinus teada; Tissue et al., 1994) and decreased for others (e.g. Brassica oleracea Sage et al. 1989). When plants are exposed to elevated CO_2 , one initial effect is that photosynthesis is more likely to be limited by the capacity to regenerate RuBP. Over a few days, the amount and activity of Rubisco may be regulated downward to balance the limitation in the rate of RuBP regeneration (Sage 1990). Thus, plants grown in elevated CO_2 usually redistribute leaf nitrogen as to reduce the portion for carboxylation and to increase the portion for light harvest and electron transport (Sage, 1990; 1994). In addition to biochemical adjustments, elevated CO_2 may induce a variety of

morphological changes, including stimulated leaf mesophyll growth. Vu et al. (1989), for example, observed an extra layer of mesophyll tissue for Glycine max grown in elevated CO₂ in comparison to ambient CO₂. After surveyed changes in leaf weight per unit area from 35 species in 39 published studies, Luo et al. (1994) concluded that this variable almost always increases in elevated CO₂. Increased mesophyll enhances photosynthetic capacity in most cases.

One of the key determinants of carbon fixation by ecosystems is the amount of photosynthetic machinery per unit area of land. Although elevated CO₂ has been speculated to stimulate canopy development, leading to greater leaf area index (LAI), field data are scant. An experiment in artificial tropical ecosystems indicated that elevated CO₂ did not change canopy structure and LAI (Korner & Amone, 1992). Experimental studies in natural ecosystems, however, strongly suggest that LAI may be increased in elevated CO₂, especially in early stages of canopy development. Elevated CO₂ will increase carbohydrate availability in leaves, leading to increased rate of leaf expansion and large leaf area per plant. Ontogeny of plants may be also accelerated in elevated CO₂, resulting in faster development of the canopy. Leaf area ratio (leaf weight per plant weight) may decrease but total leaf area usually increases. CO₂-induced changes in branching patterns also potentially affect canopy development. Because plants in elevated CO₂ are able to gain more carbon under low irradiances and keep more leaves in shade, elevated CO₂ may also lead to larger LAIs in mature stands. These speculations, however, still need to be tested in fields. If growth in elevated CO₂ leads to higher leaf area in canopy, ecosystem photosynthetic capacity is expected to increase in elevated CO₂ (Long & Drake, 1993).

While we recognize that simple ideas such as capacities of various components of the photosynthetic biochemistry being well balanced and rates of carbon gain being proportional to

ecosystem nitrogen availability are from the chloroplast to the globe, resources, and especially nitrogen are central to the regulation of photosynthesis. Only by critical examination over scales of space and time can we build from an idea that is presently understandable toward increasingly accurate approximations to the truth. The studies summarized and synthesized here do not, in fact, support a simple conclusion. They do, however, both provide some important data on the comparative responses of two important species, and point in the direction of some new questions that have potential for further illumination.

2. Methods

2.1 OVERALL SCOPE.

The scheme of the overall experiment and details of the experimental layouts at the Duke and Placerville field sites has been described earlier in this volume (Strain *et al*). Gas and N levels etc. to be explained there if necessary.

2.2 LEAF-LEVEL GAS EXCHANGE.

At Duke, gas exchange measurements were made with a LiCor 6200 system. This system was modified so that the atmosphere in the cuvette could be brought up to a desired CO₂ level immediately after the leaves had been sealed in. At Placerville, the leaf level gas exchange was done primarily with a Campbell Scientific MPH 1000. This system was modified so that mass flow controller that normally supplies moist air supplied O₂ instead. The "dry air" flow controller supplied N₂ so that by mixing nitrogen and oxygen air of either 2% or 20.9% O₂ could be made. The airstream was humidified with either a condensing-dewpoint generator of our own design (see Field *et al* 1988) or the commercial equivalent, Li-Cor 610.

In Placerville 8 to 16 needles were placed in the gas exchange cuvette for each measurement. From 2 to 4 such sets were measured from different positions on a tree and on 3 to 4 trees per chamber within each of three chamber replicates per treatment. Together each gas exchange point reported represents data from between 12 and 20 trees per CO₂ by N treatment. For statistical analysis data from each chamber was pooled so that the sample size is three. At Duke four to eight needles were placed in the gas exchange cuvette for each measurement with tree trees being measured per chamber. The samples from the nine trees per treatment were also pooled by chamber to yield N=3 per treatment.

2.3 WHOLE OPEN-TOP GAS EXCHANGE.

Whole open-top chamber gas exchange was conducted on ponderosa pine in Placerville in 1993 using the closed system approach (see Field *et al.* 1988 for a description of this approach). This method is advantageous when the leaf area in the chamber is quite low because one can wait for a detectable CO₂ depletion to develop so long as the chamber does not leak significantly and one is willing to tolerate a rise in chamber air temperature. We used an empty open-top chamber to develop and prove a repeatable chamber sealing system. These empty chamber experiments tested the leak rate by injecting CO₂ into the empty chamber at a rate consistent with that expected from apparent respiration, then verifying the integrity of the chamber seal by watching the signal associated with the injected CO₂ decay. Leak rates an order of magnitude less than what would have compromised our measurements were achieved. Whole system flux rates were then measured by sealing the chambers for a

four minute period, measuring the CO₂ drawdown (or nighttime increase), and calculating the flux rate given the known chamber volume.

From 1994 through the present we have employed the open-flow approach to whole open-top gas exchange. (See Field, *et al* 1988 and Ball, 1987 for full details on this method of gas exchange). The exact plumbing scheme to achieve the best measurements is still evolving and will be described in a future paper (Picone, Ross et al, in preparation). The reader should be aware that there are two problems that make application of the open -flow approach very challenging at the size scale of an open-top chamber and in the field. First, the open flow approach gas exchange requires that the flow of gas into the cuvette be accurately measured. For this experiment, we measured this gas flow rate by injecting a known amount of CO₂ into the air entering the Open-top chamber, and measure the resultant offset in CO₂ concentration after the air passed the injection site in the duct. The air flow rate may then be calculated simply. Second, and quite obviously, any variability in the ambient CO₂ concentration introduces noise into the system under study. To minimize this noise, long intake ducts were constructed allowing air to be drawn into the system from 30 meters upwind and 4 meters above ground level. In order to insure maximized signal to noise ratio, the flow rate of air into the system was reduced to our nighttime flow rate (66 m³min⁻¹). To minimize incursions of ambient air into the frustum opening, a low density 5 mil polyethylene cover with a 35 cm diameter opening with a second, 50 cm plastic sheet placed over that hole in the manner of a flapper valve, was installed to close the top of the chamber frustum. Four propeller fans were placed inside to stir the chamber.

2.4 GAS EXCHANGE AND THE PHOTOSYNTHESIS MODEL UNDERLIE SOME OF THE INTERCOMPARISONS MADE HERE.

While all of the gas exchange at the Duke site took place in air of normal oxygen concentration, the gas exchange in Placerville used both normal and 2% oxygen. Low oxygen A/C_i curves provide an unequivocal measure of *in vivo* rubisco activity as it is unencumbered by any variation in photorespiration (Seemann *et al*, 1981). Reasonable and reliable rubisco activity measurement have been obtained for loblolly pine, making it possible to relate enzyme levels and activity to gas exchange, nitrogen supply, and other environmental conditions. Attempts to obtain reliable biochemical rubisco assays for ponderosa pine have not been fully satisfactory. The gas exchange approach to understanding rubisco activity is satisfying because one measures directly what one wants to predict from the activity value. Because different approaches were taken at the two sites the Farquhar, von Caemmerer, Berry (1980) model was employed to rectify and compare the measurements. Both enzymological and gas exchange data are available for loblolly pine. Using that data set, we employed the model in both directions to make certain that comparisons between species would be valid.

3. Results

3.1 RATES OF LEAF-LEVEL PHOTOSYNTHESIS AT GROWTH CO_2 AND THEIR RELATIONSHIP TO POTENTIALLY LIMITING BIOCHEMICAL PROCESSES.

Leaf-level rates of net photosynthesis (A) are fundamental to understanding the actual values of plant and ecosystem carbon throughput and balance. Panels A and B of Figure 1 show, respectively, 1993 and 1994 growing season net photosynthetic rates of ponderosa and loblolly pines growth at three levels of atmospheric CO_2 . The ponderosa pines (abbreviated Pipo in the figure legends) received annual nitrogen fertilization as has been described by Strain *et al* and Johnson *et al* in this volume. The N 0, N 10, and N 20 labels in the figure legends signify zero, 10, or 20 grams nitrogen fertilizer per square meter of ground area per annum. The loblolly pine fertilization scheme was over-ridden by high mineralization rates from

disturbed soils as has been described in this volume. Thus the label HN, for high nitrogen, accompanies the *Pinus taeda* (Pita) labels in our figures.

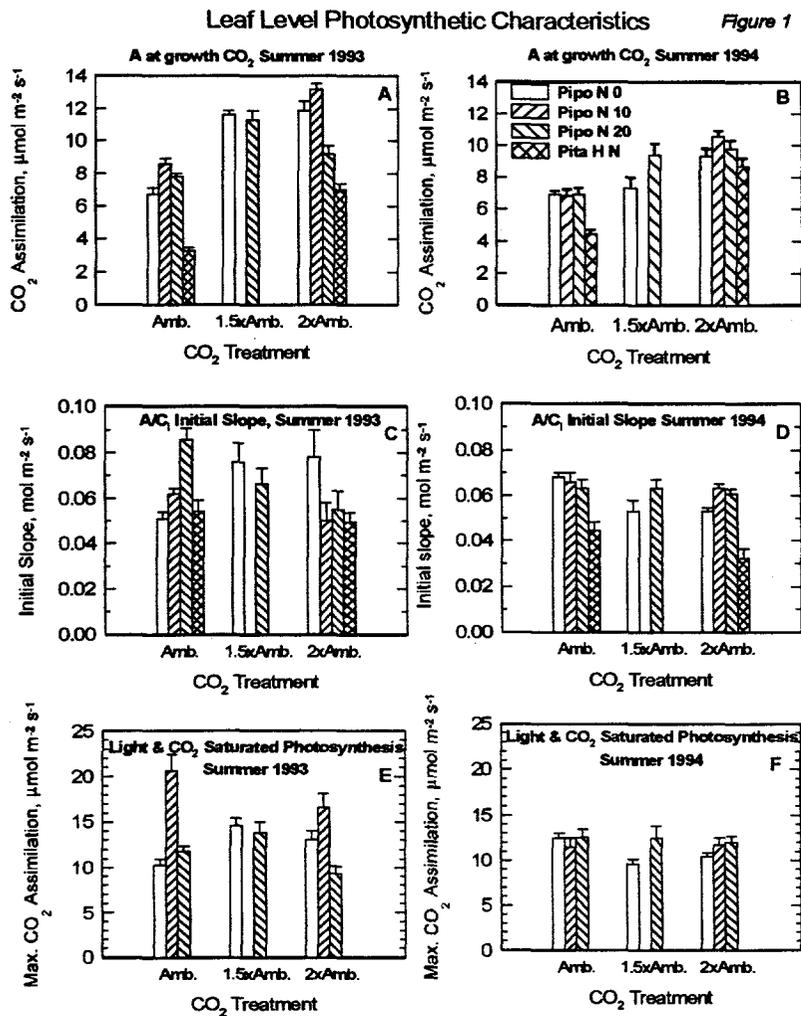
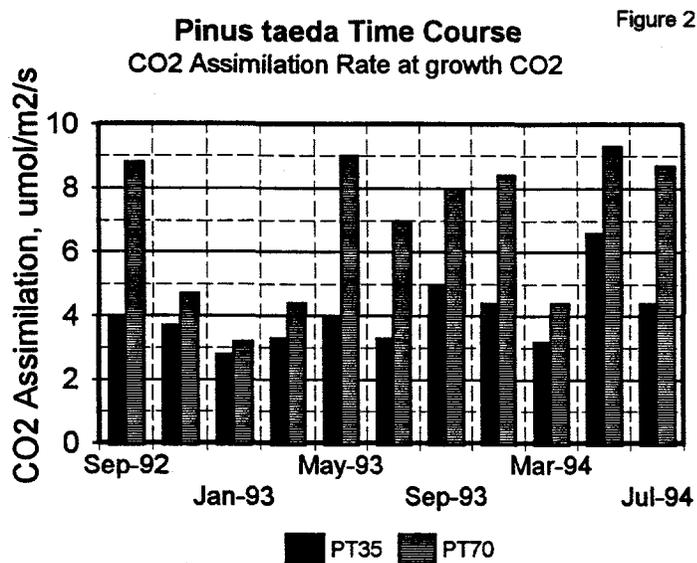


Figure 1

For both the 1993 and 1994 growing season (panels A and B) measurement at the growth level elevated partial pressure of CO₂ resulted in substantial higher rates of photosynthesis than control plants for both species. (CO₂ assimilation rates are expressed on a total needle surface area rather than on a projected area basis. Error bars denote one

standard error from the mean.) Nitrogen had much less influence on the leaf-level photosynthetic rates than did CO₂. It is interesting that 1993 season, ponderosa assimilation in the highest CO₂ treatment was significantly lower at the highest of N level relative to the other nitrogen levels at elevated CO₂. This was not a nitrogen toxicity problem since these trees were largest and had the most leaf area.

An eighteen month time course leaf photosynthesis was compiled for loblolly pine from the autumn of 1992 through mid-summer 1994 (Figure 2). Two time points for ponderosa at the high nitrogen level are included in this figure for comparison. The enhancement of photosynthesis by elevated CO₂ is much stronger during the warmer months of the year. Analysis of the gas exchange and enzyme data, although not included here as a figure, indicate that activity of rubisco has a strong seasonal pattern in loblolly (Tissue and Lewis, in press). On average through the time course, photosynthesis is 70% higher for elevated CO₂ than ambient grown loblolly trees. Across time and nitrogen treatments measured rates for ponderosa are 35% higher in the twice ambient CO₂ treatment. From Figure 1A and B, the photosynthetic rate for ambient-grown loblolly is roughly half that of ambient grown ponderosa. As the rate for double-CO₂-grown ponderosa relaxed in 1994, assimilation for the two species at high CO₂ became statistically indistinguishable.



The decline in photosynthetic rates from 1993 to 1994 for ponderosa pine is immediately noticeable in Figure 1. Data shown below reveal that many controlling aspects of the photosynthetic system converged to similar values across the ponderosa treatments in 1994. These trends toward convergence continued in 1995 (data not shown) and merit a discussion beyond the scope of this paper.

3.1.2 CONTROLS ON THE RATE OF NET PHOTOSYNTHESIS.

In trying to develop a predictive understanding of photosynthetic responses to different environmental conditions—that is, attempting to understand how photosynthetic rates are established—we use the interpretation of photosynthetic/CO₂ response curves (A/C_i curves) that arises from the Farquhar, von Caemmerer, Berry model (1980). Very briefly, the initial, linear, photosynthetic response to CO₂ (Figure 3) reflects a rate determining number of events where CO₂ is added to ribulose 1,5-bisphosphate (RuBP). That limitation is directly traceable to the number of active rubisco enzyme molecules relative to the CO₂ concentration within the

leaf. (Rubisco is the carboxylating enzyme Ribulose 1,5-bisphosphate carboxylase/oxygenase). More active rubisco molecules result in a steeper initial slope such as is reflected in the “355” versus the “700” panel of Figure 3. Above some leaf intercellular CO₂ concentration photosynthesis become very much less responsive to CO₂. In these “CO₂ saturated” conditions control of the photosynthetic rate shifts from rubisco itself to the complex of reactions involved in the regeneration of rubisco’s substrate RuBP. The rate of the “regeneration” reactions is generally limited by either the supply of ATP and reducing equivalents coming from the light capturing reactions of photosynthesis or by the supply of inorganic phosphate re-entering the chloroplast (and used in ATP production) after having being released from sugar phosphates in the cytosol. The decline in photosynthesis at higher CO₂ levels seen for the solid black circles in the “355” panel of Figure 3 is diagnostic of this inorganic phosphate limitation to RuBP regeneration (Sharkey, 1985).

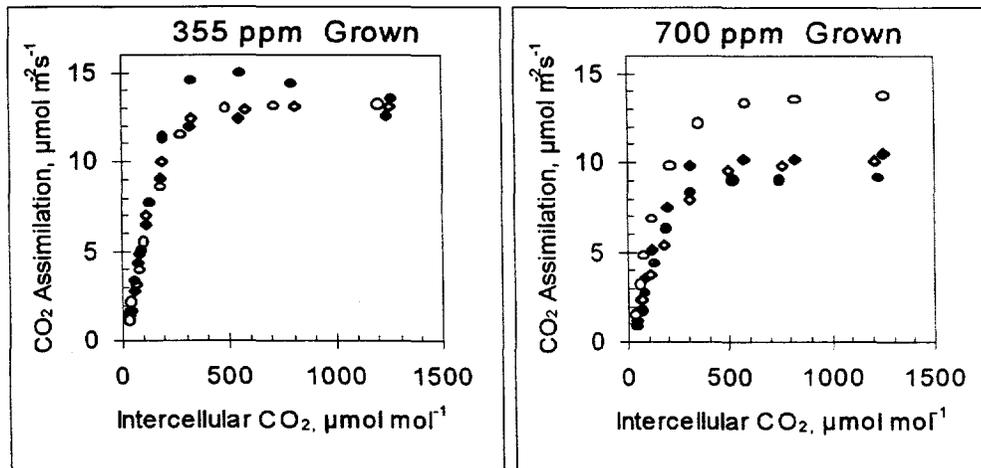


Figure 3.

(FIG. 3 NEEDS TO BE MODIFIED TO INCLUDE A SET OF LOBLOLLY CURVES .)

Therefore, the shape of A/c_i curves (as in Figure 3) — their initial slope, their slope at the higher CO₂ and the transition between the two — depend upon the balance between the

active quantity of rubisco and the capacity to re-supply RuBP. Both of these functions represent expensive nitrogen investments. Where a plant actually operates on these curves is influenced primarily by the leaf intercellular CO₂ partial pressure but also by environmental conditions such as light and temperature. Higher leaf intercellular CO₂ concentrations (c_i) in elevated CO₂ atmospheres gives plants the opportunity to operate farther to the right on the graphs shown in figure 3. However, in adjusting to growth at elevated CO₂, plants are often found to have lower activities of both rubisco and the complex of "machinery" responsible for regenerating RuBP (Sage, 1994; Luo *et al.* 1994)

Panels C and D in Figure 1 indicate the potential activity of rubisco, as reflected in initial A/c_i slopes in our two pine species when they were grown at different CO₂ and nitrogen levels. For loblolly pine in 1993, there was not a significant decline in rubisco activity across the CO₂ treatments. By 1994 the rubisco activity in the elevated CO₂ treatment was 27% lower than in the ambient treatment. For ponderosa pine at ambient CO₂ in 1993 there was a large increase in rubisco activity with increasing N fertilization that was not evident at other CO₂ concentrations. In the two elevated CO₂ treatments at background N there were similarly high rubisco activities. In the treatments where N was added there was a downward trend in rubisco activity with growth CO₂ treatment. For ponderosa in 1994 there was no longer a strong nitrogen effect on rubisco activity in any of the CO₂ treatments. In treatments where N was added, there were no differences among the responses of different CO₂ treatments with respect to initial slope. For the background N treatments, the mean initial slope was the same at the 1.5 and 2 times ambient CO₂ treatments. These means were significantly ($p < 0.05$) lower than that for the ambient CO₂, NO treatment. There was also a significant decline in rubisco activity from the ambient to the elevated CO₂ treatment in loblolly pine in 1994.

Panels E and F in Figure 1 indicate the potential activity of the RuBP regenerating functions in ponderosa pine grown at different CO₂ and nitrogen levels. Maximum A data were not obtained for loblolly pines because the gas exchange system available was not designed to obtain these measurements. We focus first on the 1993 data, panel E. The middle nitrogen treatment (N10) for ponderosa showed significant increases in A_{max} (in the ambient and twice ambient CO₂ treatments where there is such a treatment). For the ambient CO₂ treatment, the increase was highly significant. At twice ambient CO₂, the increase over the background N treatment was significant at the $p < 0.15$ level. Looking more generally at panel E, there was a decline in A_{max} in the highest CO₂ and N treatment that it was significantly below those in all but the ambient CO₂ and zero N treatment. For A_{max} in 1994 (panel F) the large middle N response of 1993 was no longer present. Significant declines in A_{max} at the two elevated CO₂ levels occurred only in the background nitrogen treatments.

3.1.3 OPERATIONAL PHOTOSYNTHESIS WITHIN THE BALANCE OF CO₂ AND ENERGY CAPTURE FUNCTIONS.

Rubisco activity, A_{max} and their balance dictate the shape of the A/c_i curve and primarily indicate the relative investment made in carbon fixation *versus* the light capture/electron transport functions within photosynthesis. Actual photosynthetic rates of leaves are the result of where c_i positions a leaf in the framework of that balance. Figure 4 captures some the important aspects of how fully illuminated ponderosa pine needles were positioned in that A/c_i framework. The graph shows measured photosynthetic rates at their growth CO₂ plotted against measured values of A_{max} . For reference, the 50%, 75%, and 100% lines indicate the region where points would fall if there is sufficient rubisco activity, given its operational c_i , to

position the leaf the respective percent of the way up the initial A/c_i slope toward A_{max} . The triangular points are the 1993 data and the circular points the 1994 data. Operation above the 75% line indicates a close balance in investment between the energy capture and carbon capture components of photosynthesis and is "expected". It is interesting that in 1993 the data are widely scattered across the diagonal while in 1994 they became much more clumped.

$A_{growth\ CO_2}$ related to A_{max}
Ponderosa pine, Placerville
Summer 1993 & 1994

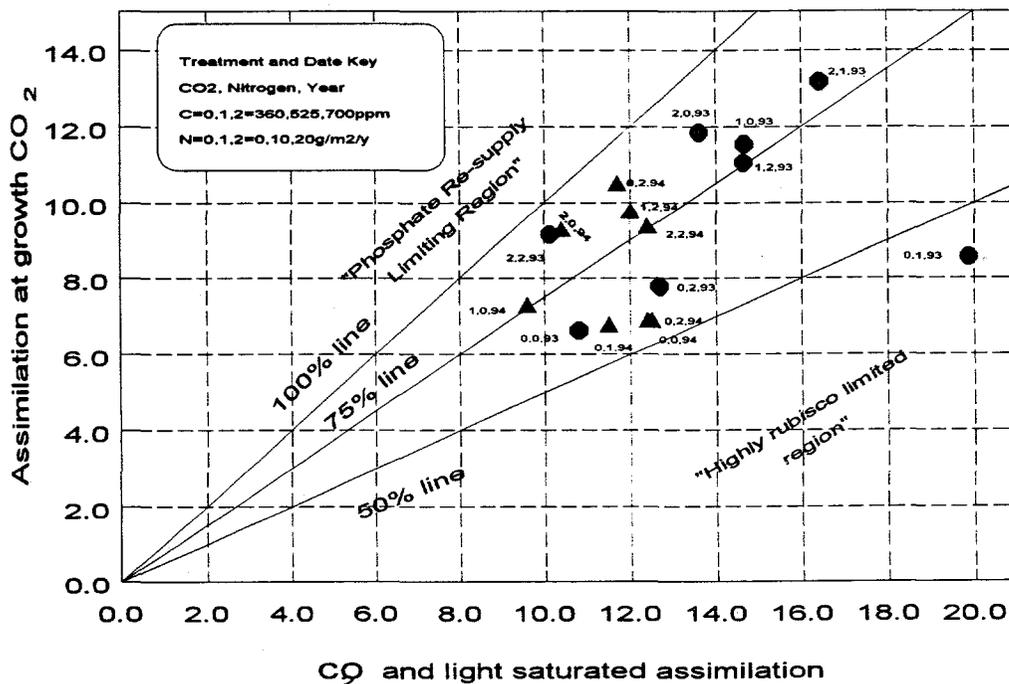


FIG 4

3.1.4. APPARENT UTILIZATION OF RUBISCO.

In 1993 total rubisco content of ponderosa needles was measured in each treatment using the labeled carboxyarbinitol 1,5-bisphosphate trap method (Collatz and Badger, 1977). This was done at two points in time with virtually identical results. Data from a late August sampling were used in the generation data shown here because the samples were either the actual needles used in gas exchange measurement or immediately adjacent needles. Dividing the calculated rubisco activity (indicated by the initial A/c_i slope in 2% O_2) by the measure total rubisco, one obtains the apparent activation state of the enzyme (Figure 5). Overall these values are surprisingly low. Comparing data points in Figures 4 and 5 one can visualize the extent to which changes in the activation state of Rubisco could effect photosynthesis. The ambient CO_2 grown trees (at all three N levels) are operating around the 50% line in Figure 4 could gain substantially more carbon if their rubisco had been at a higher activation state. These plants had capacity for both functions in photosynthesis that appears to be unused. The elevated CO_2 grown plants operate at or above the 75% line in Figure 4 indicating that their investment in the energy capture/RuBP regeneration function is well matched to the their rubisco activation states. However, where both CO_2 and N were elevated there is a substantial pool of rubisco which would do little if it were activated. Only the two low nitrogen–elevated CO_2 treatments show activation states in the 0.8 to 1.0 range of Figure 5. (Similarly high activation states are found in loblolly pine (Tissue and Lewis, in press) and are typical of crop plants.) Those two treatments also operate in the “expected” region above the 75% line in Figure 4 (points 2,0,93 and 1,0,93) and achieve the second and third highest operational photosynthetic rates at the leaf level.

Proportion of Rubisco Needed for Initial Slope
Ponderosa Pine, Placerville Field Site
Summer 1993

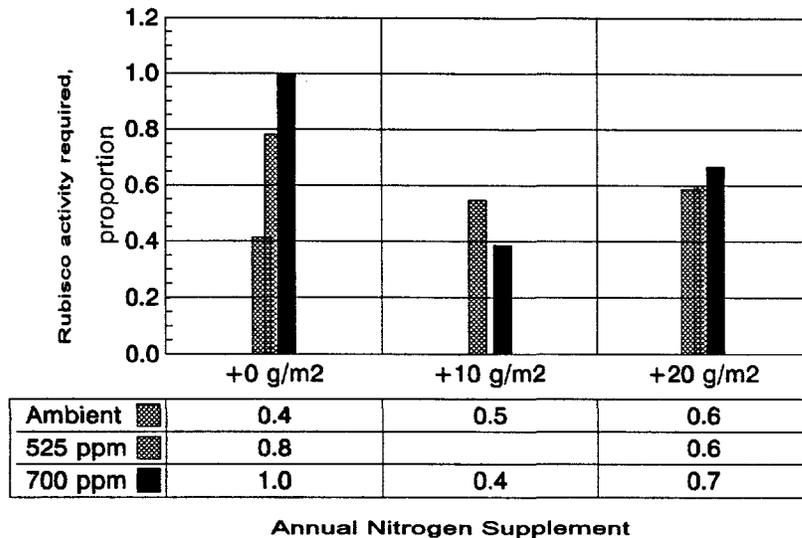


FIG.5.

3.2 Photosynthesis related to leaf nitrogen, weight, and area.

One step higher in scale is the investment of the nitrogen-rich photosynthetic machinery per unit area and per unit weight of leaf relative to the number of leaves. As with the balance of the carboxylation capacity with the RuBP regeneration capacity there are factors to be balanced as investment is made into the photosynthetic apparatus. Studies on rates of carbon return per unit nitrogen invested in new versus old leaves, in sun versus shade leaves, and under different environmental conditions are well known (Bjorkman, 1980, Field 1983, 1988, Evans 1989). Nonetheless, application or critical examination of these principles has not been widespread in CO₂ related studies. Luo *et al* (1994) have proposed a model that attempts to explain the leaf level adjustments in photosynthetic capacity in atmospheres of different CO₂ by taking into account whole plant carbon and nitrogen budgets. There should be as much, if not

more room to adjust rates of plant and ecosystem carbon gain in regard to how the machinery is deployed as there is in how it the machinery is poised within a leaf.

Nitrogen per unit weight (concentration) is often used as the index of how the photosynthetic machinery is deployed. Indeed the photosynthetic machinery generally accounts for the majority (though variable amount) of the N in leaves of the limited number of species that have been examined (Evans, 1989; Evans and Seemann, 1989). A general downward trend in leaf N per weight at elevated CO₂ is found across species. This trend has been widely discussed in the literature but has been understood less in terms of potential photosynthetic rates than in regard to decomposition of litter. Viewing changes in N on unit area as well as a unit weight basis is important to understanding photosynthetic responses to CO₂ (Luo *et al* , 1994).

Leaf Nitrogen, Weight and Area Relationships

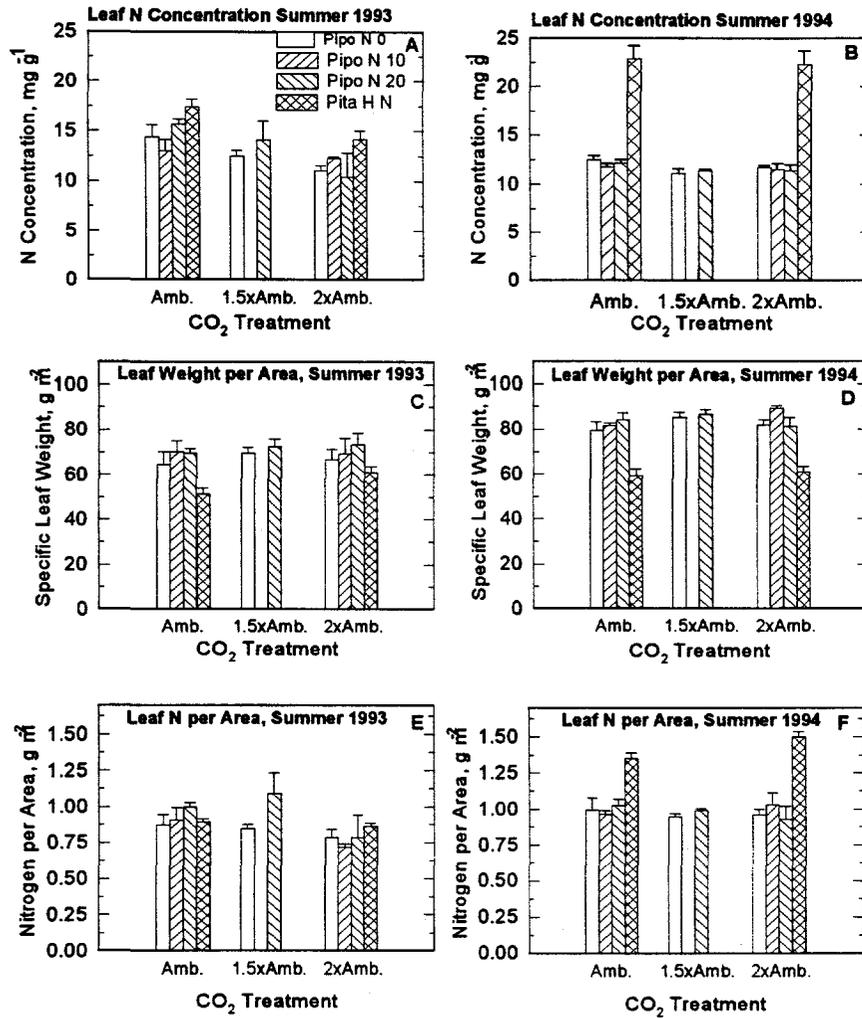


Figure 6.

Leaf Photosynthesis Related to Nitrogen

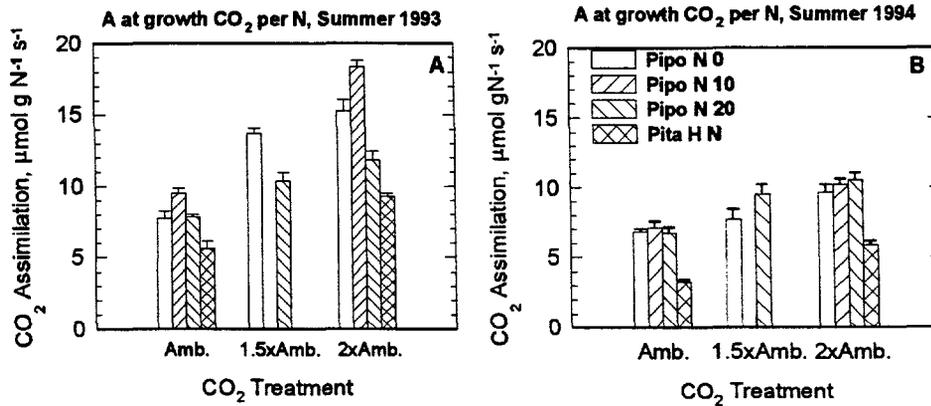


FIGURE 7.

Panels A and B in Figure 6 show the leaf nitrogen concentration (mg N per g tissue) across the treatments for both ponderosa and loblolly pines. In the 1993 data there is a general downward trend in leaf nitrogen concentration from ambient up to the highest CO₂ concentration. Importantly, the N treatments in ponderosa pine did not significantly influence the N concentration of leaves. By 1994 the nitrogen concentrations were virtually the same across all treatments of ponderosa. The N concentration was also statistically the same across CO₂ treatments in loblolly but at much higher levels than the previous year and than in ponderosa.

From Panels C and D in Figure 6, there was a statistically significant upward trend in leaf weight per unit area in ponderosa pine from 1993 to 1994. Within each year however, there were no differences between treatments. Loblolly pine on the other hand did not show any significant variation across either treatments or time. The differences in weight per area between loblolly and ponderosa are interesting in the ecological context. Combining N concentration and weight per area yields nitrogen per unit area, which is shown in panels E and F of Figure 6. There was a downward trend in nitrogen per area in 1993 for ponderosa but not

so for loblolly pine. Again, the ponderosa N treatments did not influence these leaf level values. In the 1994 data neither CO₂ nor N treatment appeared to influence the nitrogen per unit area.

3.3 CHANGES IN THE NITROGEN PHOTOSYNTHESIS RELATIONSHIP.

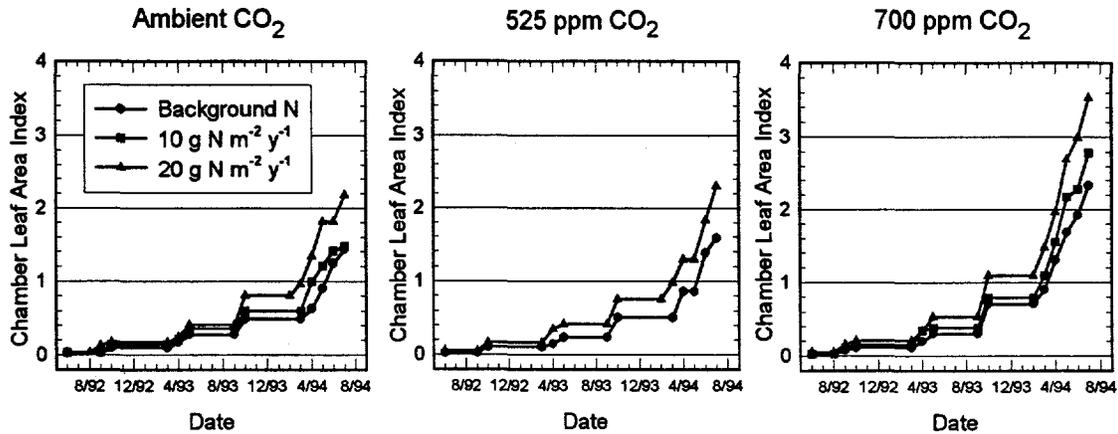
We mentioned, in regard to figure 6, that N concentration is often taken as an index of photosynthetic capacity. Also, the inverse of concentration is often regarded as "nitrogen use efficiency" with the presumption that the nitrogen in tissue has been responsible for building the tissue. Combining photosynthesis data with leaf nitrogen and area/weight relationships, one may calculate photosynthesis per unit nitrogen (Figure 7). Within a N treatment and across the CO₂ treatments (in both species and in both years) the increased A/N is almost completely due to higher leaf intercellular CO₂ concentration. In general, there were not changes in the way nitrogen was deployed in the photosynthetic system that would make the systems intrinsically more efficient at elevated CO₂. Only at the middle N treatments in the 1993 ponderosa data did the rubisco activity show a relative drop while A_{max} increased at 2x ambient CO₂ (refer to Figure 1). In both species the higher A/N with elevated CO₂ had substantially collapsed in 1994. Surprisingly, this was the result of much increased N leaf concentrations with some decline in CO₂ assimilation rate.

3.4 LEAF AREA DEVELOPMENT IN PONDEROSA PINE.

Figure 8 shows the time course of leaf area index development. These time courses were calculated from a regression of harvested leaf area on the three-halves power of the stem height-diameter product developed as described by Tingey *et al* (this volume) and as verified at

several points by a Licor Canopy Analyzer and retrodiction to a previous harvest. The supply of N had a dominating positive influence on the rate of leaf area development. One will recall that nitrogen supply in the range available to the trees in these experiments had little if any positive influence on of leaf level photosynthetic rates. As early as six months after seed germination there was roughly twice as much leaf area supported on high N seedlings as compared to the low N plants at all three CO₂ levels. Through the 1992 to 1994 growing seasons high N plants averaged about 1.6 times more leaf area than the non-fertilized counterparts across the CO₂ treatments. The effect of doubling CO₂ on leaf area development increased through time, consistently across of the N treatments, from a factor of approximately 1.15 in 1992 to approximately 1.6 times more leaf area in 1994. Seasonal initiation of shoot development has not appeared to be changed as a result growth at high CO₂ or nitrogen. However, timing of leaf area development has appeared to be shifted slightly forward at elevated CO₂ but end of season ratios of leaf areas indices across N treatments have been very similar. It is worth noting that ponderosa pine produces two (and in an occasional tree three) flushes of growth under the conditions of our experiment. This contrasts with the single flush per year that one observes in nature. The loblolly pine have flushed three times per year as is observed in the field in favorable years. It is also worth noting that while growth is greatly accelerated in both loblolly and ponderosa pines by the CO₂ and N treatments, the allometric relationships between height, diameter, and leaf area are unchanged and remarkably similar between the two species (See Thomas *et al.*, this volume).

Calculated Average Leaf Area Index, Ponderosa, Placerville

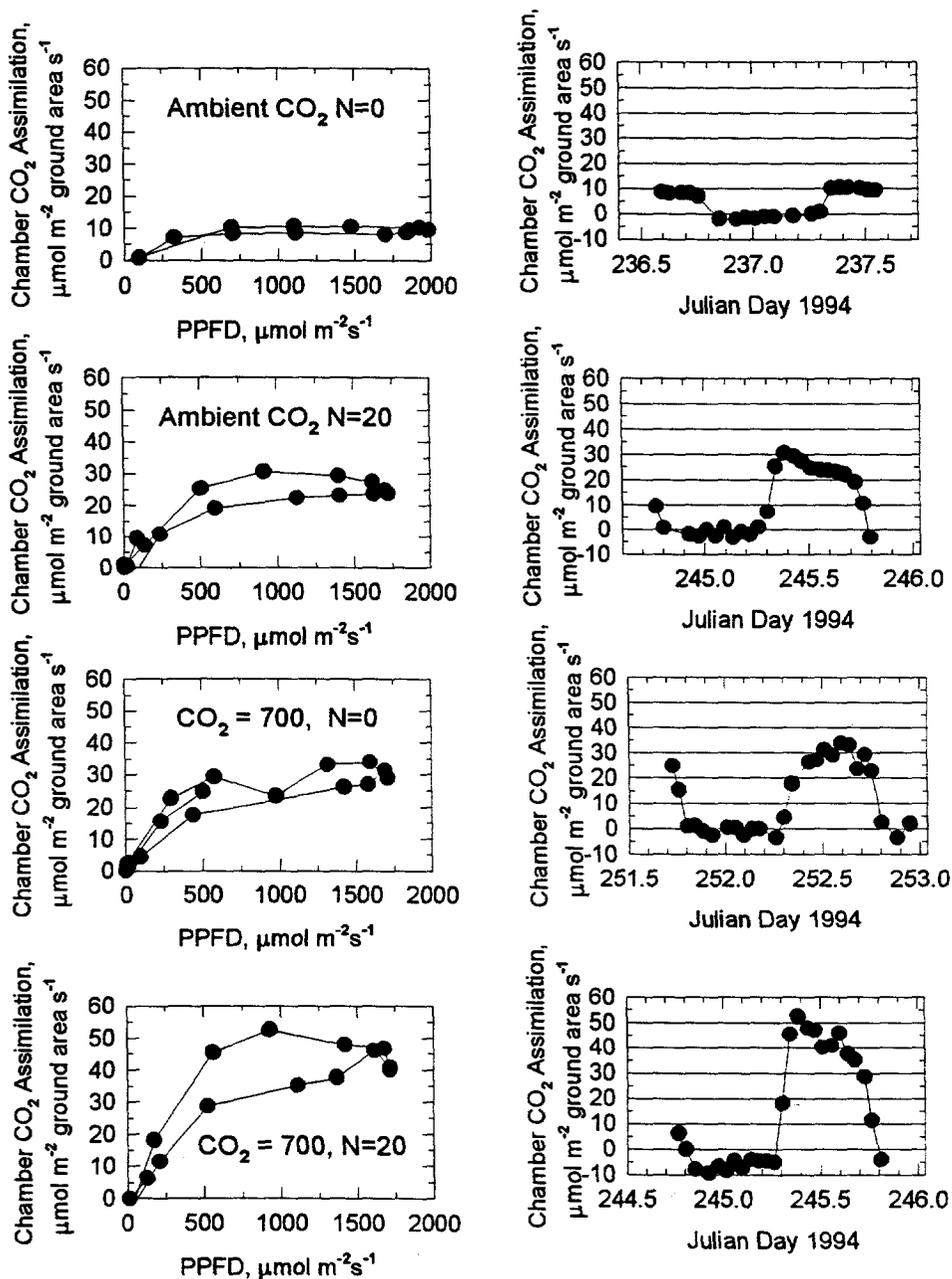


(Fig. 8)

3.5 WHOLE-SYSTEM GAS EXCHANGE, CARBON BALANCE AND GROWTH RESPONSES.

Development of whole open-top chamber gas exchange measurement capabilities has challenged us to bring together our understanding of carbon and nitrogen allocation as it has been influenced by nitrogen and CO₂ supply. In particular we are challenged to understand the influences of C and N supply on whole system input, output and storage of carbon. Figure 9 shows diurnal courses of photosynthesis and light response curves derived from them those courses for four chambers on the ponderosa field site in the summer of 1994. These four chambers represent the four corners of the experimental matrix of three levels of CO₂ by three levels of N fertilization. Similar data have been collected in 1993 and 1995 for ponderosa and briefly in 1994 for loblolly pine. Consideration of the influence of varying environmental conditions, different leaf areas and root biomass per chamber, and the placement of measurement point during the day, as well as marginal resolution of night fluxes and all fluxes for chambers in the early stages of leaf area development, etc. make full analysis of these data

well beyond the scope of a summary paper. Certainly absolute rates from the sample of data presented here are inappropriate for scaling to real world conditions.



(Figs. 9)

The daily integrated uptake of CO₂ has scaled at close to the leaf area index times the leaf level assimilation rate at the respective CO₂. The relative enhancement of carbon gain at elevated CO₂ average from 2.1 in sparser canopies to 2.36 in the denser canopies. In general, leaf area development is more significant than is the leaf level rates of photosynthesis, in determining the relative values of whole system CO₂ uptake. It is interesting, if not important, that as differences in leaf-level photosynthetic rates (between ambient and elevated CO₂ grown trees) have been reduced over time the leaf area differences have continued to expand. Equally interesting is that the daily loss of carbon have scaled in very similarly proportion to the increased inputs. That is, elevated CO₂ chambers which gained twice as much carbon had approximately twice as much carbon loss. The fact that the change in C uptake is largely driven by increased tissue quantity helps to explain the proportionality. Overall, we estimate that daily net carbon losses may be as high as 30 to 35% of daily net fixation. It is difficult to know exactly how to scale all of the carbon losses because our open-top chambers enclose the surface for only half of the soil volume the trees roots are allowed to explore. We measure soil CO₂ efflux into inverted caps and scale that evenly for the whole area that the roots can occupy, but heterogeneity is significant. Also, the numbers we report here are only summer season values and there may be significant deviation from these patterns through the year. For example, in the autumn and early winter when air temperatures are low but the soil is still warm there could be a much greater proportion of daily photosynthesis than we have yet seen. We are working at this time to develop a representative annual course of diurnal courses from the corner treatments on our experimental matrix.

Although there are many caveats to the interpretation of the whole ecosystem gas exchange we feel that these measurements point out how little we understand about system carbon budgets. Figure 10 shows of the relative stem "volume" response of ponderosa pine to CO₂ at the three nitrogen treatments in our experiment from April 1995. Stem "volume" is

calculated as the square of the radius of the tree (above the root collar) times π times height.

We have found volume measurement at harvests to be proportional to harvestable course roots and to total biomass. If biomass accumulation were proportional to the net ecosystem gas exchange as we have measured it, we would have expected more than a doubling of growth at elevated CO_2 where we see only a 40 to 60% stimulation. Although we know carbon is being put into the soil organic matter, we are unable to identify the difference between what we think remains in the system and what we harvest plus what we find accumulating in the soil.

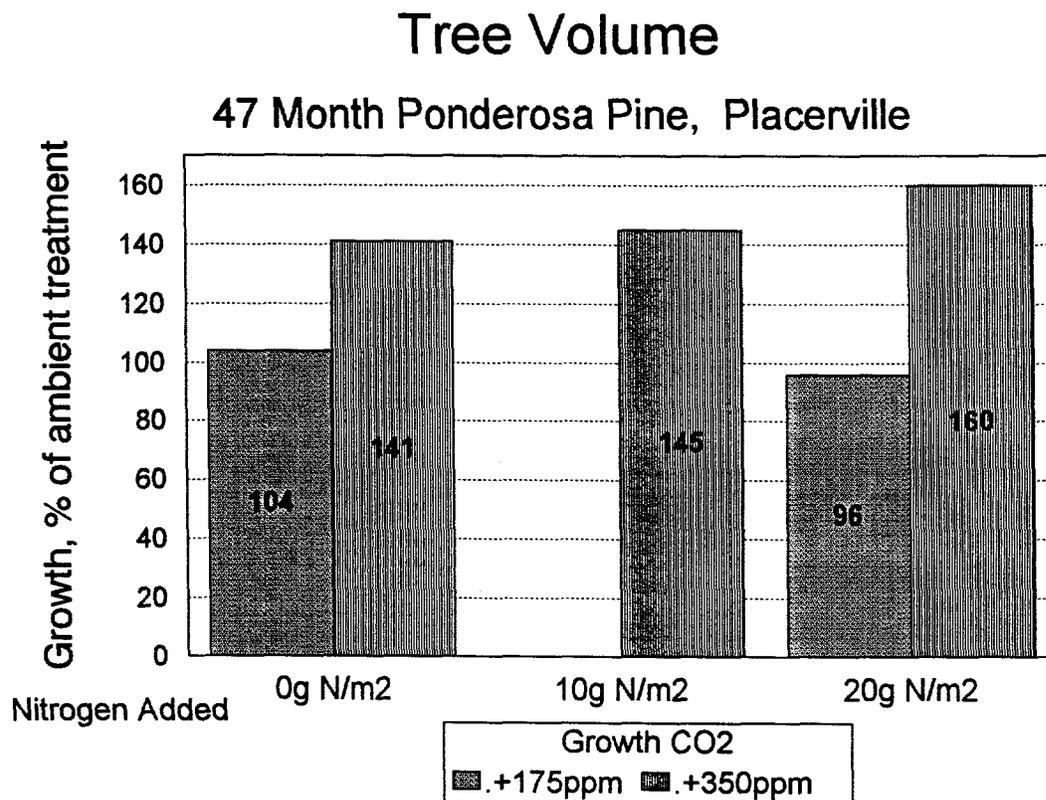


Fig. 10

4. Discussion

4.1 THE PHOTOSYNTHESIS - NITROGEN RELATIONSHIP AS ATMOSPHERIC CO_2 RISES.

At the level of the leaf, there are a number of important differences in the way that ponderosa and loblolly operate their photosynthetic systems and in how they adjust to elevated CO_2 . The leaves of ponderosa pine are considerably heavier and capable of more photosynthesis per unit area than are loblolly pines. In our experiment, when ponderosa was grown and photosynthesis was measured at present ambient levels of CO_2 , c_i resulted in operation at only about 50% of the maximum photosynthetic rate. This is well below where most plants, including loblolly pine (Figure 3 [loblolly graph to be added], also see Tissue and Lewis, Global Change Biology in press), operate on their A/c_i curves. The higher weight and photosynthetic rate per unit surface area plus operation low on the initial A/c_i slope may be adaptations to the more water limited environment where ponderosa pine grows. All else being equal operation low on the A/c_i curve would make a plant less photosynthetically nitrogen use efficient (less photosynthesis per unit area). According to Figure 7 loblolly has a lower A/N ratio than does ponderosa. This is all the more surprising given that much of the rubisco in ponderosa pine is apparently not activated and thus not contributing to photosynthesis (Figure 5).

With respect to the adjustment of photosynthesis and the notion that it is driven by optimization of N use, the following considerations are relevant. Plants operating well down on their A/c_i curve "should not" down regulate rubisco capacity. Such plants might be expected to up-regulate rubisco because it can be used more effectively. Ponderosa, in fact, down regulated the activity of rubisco as many other plant do resulting in only about a 35% stimulation on average across the study. Logically, one might suggest that selection for water conserving characteristics is overriding, limiting up regulation of photosynthetic capacity. For loblolly, on the other hand, even though rubisco was down-regulated the photosynthetic rate was more than 70% higher at elevated CO_2 . Homeostatic adjustment of an optimized system would have down regulated rubisco and light harvesting to a larger extent in that species.

Even more puzzling is the fact that leaves of both species in 1994 had more N but lower A than in 1993. Clearly the nitrogen metabolism and dynamics of these two species do not fit into a simple linear nitrogen-photosynthesis paradigm. Nor does understanding the shifts in enzyme capacity in photosynthesis lead directly to a simple prediction of photosynthetic adjustment to elevated CO_2 .

4.2 LONGER-TERM PROSPECTS FOR C:N RELATIONSHIP.

We speculate that the serious deviations from a linear A_{max}/N relationship may be the result of these two species, accumulating N while it is available in the environment. Only by taking these experiments into a low-light/closed-canopy situation with a closed and limiting nitrogen cycle will we know if these apparent nitrogen stores play-out to support additional growth and carbon storage in elevated CO_2 . It is interesting that while having little influence on leaf N concentration and photosynthetic rate, nitrogen fertilization has stimulated canopy development more or less evenly across the CO_2 treatments and across time. Concomitantly, the effect of elevated CO_2 on leaf area development seems to be accumulating through time.

5. CONCLUSIONS

The objective of our studies has been to explore the nitrogen-photosynthesis relationship in two pines that are genetically close but are ecologically quite different. Our simple hypothesis has been that nitrogen supply would be the dominate controller of photosynthetic rates across all CO_2 levels and across all scales of space and time. Although going into this study we might have guessed that our simple working hypothesis would need to be modified in some ways, we have been surprised at how much there is to learn about the dynamics of leaf nitrogen. It is in no way clear to us that either species is optimizing the use of nitrogen at least for the short-term. Optimization hypotheses are, as we said in the

introduction, useful points to begin focused thinking. In the longer term however, we will want to know mechanistically how the system is regulated. At this point we can benefit from both trying to understand both in biochemical and ecological terms just how nitrogen is being used in these pines.

We commented on our poor understanding of the flows in ecosystem carbon budgets in the results section. Our limiting understanding of nitrogen allocation is a related problem. Development of whole open-top chamber gas exchange measurement capabilities has challenged us to put changes in nitrogen allocation, photosynthesis, and rates of leaf area development into a larger perspective of whole system carbon input, output, and storage. Those issues should be attacked together, as these processes seem almost certain to be linked. We need insights into the mechanisms of these linkages. We have almost completely ignored respiration in this consideration of carbon metabolism. There is little hope of building a predictive understanding of ecosystem carbon budgets without a much improved understanding of the functions which release carbon back to the atmosphere. Here again, a mechanistic understanding of how nitrogen is used in plants and ecosystems should provide a vital link.

