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Allocation of Carbon and Nitrogen in the Growth of Ponderosa Pine Seedlings Supplied Different Levels of CO_2 and Soil Nitrogen

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***Key words*:** carbon and nitrogen allocation, carbon dioxide, global change, *Pinus ponderosa*, plant growth, resource availability.

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Abstract

A long standing challenge has been understanding how plants and ecosystems respond to shifts in the balance of resource availabilities. The continuing rise in atmospheric CO_2 will induce changes in the availability and use of several terrestrial ecosystem resources. We report on the acquisition and allocation of carbon and nitrogen in *Pinus ponderosa* Laws. seedlings grown at three levels of atmospheric carbon dioxide (370, 525, and 700 μmol mol^{-1}) and three levels of soil nitrogen supply in a controlled environment experiment. Nitrogen was applied (0, 100, and 200 μg N g $soil^{-1}$) at planting and again at week 26 of a 58-week, 4-harvest experiment. At the final harvest, plants grown with very low available soil nitrogen showed no significant response to atmospheric CO_2. Plants at higher N levels responded positively to CO_2 with the highest biomass at the middle CO_2 level. Plants growing at the lowest N levels immediately allocated a relatively large portion of their nitrogen and biomass to roots. Plants growing at near present ambient CO_2 levels allocated relatively little material to roots when N was abundant but moved both carbon and nitrogen below-ground when N was withheld. Plants growing at higher CO_2 levels, allocated more C and N to roots even when N was abundant, and made only small shifts in allocation patterns when N was no longer supplied. In general, allocation of C and N to roots tended to increase when N supply was restricted and also with increasing atmospheric CO_2 level. These allocation responses were consistent with patterns suggesting a functional balance in the acquisition of above-ground versus below-ground resources. In particular, variation in whole tree average nitrogen concentration can explain 68% of the variation ratio of root biomass to shoot biomass across the harvests. The capability to respond to temporal variation in nutrient conditions, the dynamics of nutrient uptake, and the dynamics of nutrient use were all seen to be influenced by the interplay between previous N supply, previous C supply, and the concentration of CO_2 in the atmosphere. The data suggest that in an elevated CO_2 atmosphere ponderosa pine seedlings

will have higher root biomass and be likely to capture more N compared to seedlings today. Further, the combined growth and allocation responses of Ponderosa pine at elevated CO_2 resulted in higher growth per unit N (nitrogen productivity) and lower N per gram of tissue (all tissues not just leaves) when nitrogen was not in abundant supply.

INTRODUCTION

Atmospheric CO_2 concentrations continue to rise and are expected to reach approximately 700 μmol mol^{-1} in the middle of next century (Houghton *et al.*, 1990). As the prospect of such high CO_2 levels becomes more certain, the potential for changes in the function of organisms and ecosystems has become a question of interest to people ranging from policy makers to molecular biologists. The potential for ecosystems to sequester some additional C from the atmosphere has become a question of practical importance (Dixon *et al.*, 1994; Houghton *et al.*, 1990; Vitousek, 1991). At virtually every level of biology, the potential to process or hold additional C within systems interacts with the availability of other resources (Mellilo *et al.*, 1989; Mooney and Koch, 1994; Peterson and Mellilo, 1985; Shaver *et al.*, 1992; Sheen, 1994; Krapp and Stitt, 1995). A long standing challenge in many biological disciplines has been to understand how organismic and ecosystem processes adjust to shifts in the balance of resources. In fact, these questions are at the core of our understanding of how natural and managed ecosystems, as well as organisms, interact with the physical environment. Rising atmospheric CO_2 makes the study of the resource relationships in the terrestrial C cycle a matter of widely recognized relevance.

Carbon, being both the stable carrier of biochemical energy and (by weight) the largest component of biomass (roughly 45%), is among the four resources that limit plant growth most often. The ability to capture C is the standard by which other limitations are judged. The three other dominating resources, nitrogen, water, and light energy are used mainly in the capture of C. Phosphorus, which primarily carries readily exchangeable energy, is also a limiting resource in many locations around the world and has important interactions with CO_2 (Conroy *et al.*, 1990a,b). Thus changing concentrations of CO_2 the atmosphere will inevitably change the functional relationships that control the flow and storage of the dominant resources in plants and subsequently in ecosystems. Consistent interrelationships between flows of these commodities is an integral part of the stability of ecosystems. The consistency results primarily from plant morphological and physiological adjustments to variable environmental conditions working together toward maintaining plant function. Many experiments document changes in process rates with plant growth at elevated CO_2, yet a predictive understanding of plant growth or resource-use responses, not to mention ecosystem responses, is not an immediate prospect. The difficulty is understanding just how functional relationships change with resource availabilities and then translate into plant and ecosystem function.

At the plant level we expect that consistent and predictable relationships are to be found from operational analysis (Hillier and Leiberman, 1980) (i.e., related rates, function costs, rates of return etc.) of C, N, and water gain and deployment. While ecosystems are not directly constrained toward optimization by natural selection as individuals are, predictable operational relationships are also present at the ecosystem level. Recent progress in understanding some important basic processes (e.g. photosynthetic biochemistry, the role of tissue chemistry in decomposition and N mineralization, etc.) add to the potential to investigate and understand the interrelationship of many plant and ecosystem processes in an operational sense. The application of mathematical frameworks (models, linear and differential analyses) are important tools for identifying quantitative relationships within systems as complicated as those in biology. In this paper we discuss quantitative differences in C and N allocation among ponderosa pine seedlings grown at different CO_2 levels and provided different fixed quantities of N. Especially interesting are growth rates and changes in C and N allocation as plants grow on fixed N supplies. The most widely used operational concept for allocation is the "functional balance concept." In essence, this concept recognizes that because plants are systems, the above and below ground functions must in some way be equalized (e.g. Brouwer, 1962, 1983; Johnson and Thornley, 1987; Reynolds and Thornley, 1982.). Thus there is likely to be some mechanism to, in a relative sense, shift the rate of C invested toward the function providing the lowest return relative to the need for that resource. Considerable qualitative evidence to support this idea has been amassed beginning with Brouwer's (1962) own work. Quantitative predictions and support for the concept have been more difficult because we have not had a basis to specify an absolute value for the ratio of above- to below-ground activities or resource needs. Regarding the distribution of N, Field (1983) and Field and Mooney (1986) showed that within canopies N was distributed in relation to the potential for a return of C on that investment as allowed by light penetration. Ingestad and co-workers (1979, 1980, 1981, 1985) have shown that growth rate is proportional to N content and that relative growth rate of young plants is proportional to plant N concentration. This relationship comes apparently from both the N requirement for photosynthesis and the protein costs for all cellular functions. Whole plant N concentration is an indication of the balance of root activity (N gain) and shoot activity (C gain). A well balanced root/shoot system would therefore yield a constrained or conservative plant N concentration. This has lead to conceptual (Troughton, 1977) and mathematical (e.g. Agren and Ingestad, 1987; and Levin *et al.,* 1989; Reynolds and Thornley, 1982) arguments hypothesizing that the allocation of C between roots and shoots should vary approximately linearly with plant N concentration. The above analyses (and many similar to these) have focused either on the distribution of C between roots and shoots or on the distribution of N within canopies. Hilbert and co-workers (1990,1991a,b) point out that N must be committed to roots as well as to leaves. This is because there is a N cost for root specific activity and N gain as well as for C gain. Their analysis shows that in a manner analogous to C, N allocation to roots versus shoots is expected to increase approximately linearly as plant N concentration falls.

In the experiment reported here, we allowed the soil N to be depleted, re-supplied N, and then allowed the trees to grow at a very low N concentration after the soil was again depleted. We observed a wide range of biomass and N allocation patterns as root and shoot activities changed in the course of the experiment. Two of the questions that asked were: (1) Does elevated CO_2 result in changes in C allocation that are consistent with patterns attributed to a functional balance between above-ground and below-ground resource capture? (2) Is there evidence of increased biomass accumulation and increased growth rate per unit N (N productivity) at elevated CO_2?

METHODS AND MATERIALS

Pinus ponderosa. Laws. was grown from seed in 1.1 liter Rootrainer pots (Spenser-Lemaire Industries) in an artificial, mixed soil to allow control and variation in nutrient availability. The experimental variables were atmospheric CO_2, soil N and phosphorus with three levels each in a complete factorial design. The soil mixture consisted of 60% sand, 20% peat, and 20% soil from the hydrothermally altered area at the Desert Research Institute – Dandini Campus (a Zephan very gravely sand loam, a Xerollic Haplargid). Before potting, the soil mixture was sieved (2 mm), homogenized, and mixed with $(NH_4)SO_4$ and K_2HPO_4 to achieve prescribed levels of N (0, 100, or 200 $\mu g\ g^{-1}$) and P (0, 100, 200 $\mu g\ g^{-1}$). We failed to achieve P deficiency with this soils mix despite a five-fold dilution of the hydrothermally altered soil even though it was reputed to be low in P (Johnson *et al.,* 1994). Growth responses among the P treatments were either not statistically distinguishable or inconsistent. The results reported here are from the low P (no added P) treatments.

We carried out four harvests in the course of a 58 week experiment. The first harvest, at four weeks after sowing, thinned the pots from three or four seedlings down to one. The seedlings that had been removed were dried, partitioned into root and shoot, then weighed. At the subsequent main harvests three to six pots from each treatment were destructively sampled. Dry mass of roots, stems, and needles were determined and the tissues were then ground for chemical analyses. Total N was determined with a Perkin-Elmer 2400 CHN Analyzer at the University of Nevada Reno. Other tissue nutrients were determined at the Oregon State University Soil and Plant Testing Laboratory and these data are published in Johnson *et al.,* (1994).

Soil analysis at the week 18 harvest showed that mineral N had been depleted to the extent that soils in all treatments were approaching the N concentrations of the control pots (Johnson *et al.*, 1994). Thus, the same quantities of N that had initially been added to each pot were re-added as a top dressing at week 26. Thereafter, no additional N was supplied. A second harvest was taken at week 36 and the final harvest at 58 weeks. Because we provided a very wide range of N fertilization and did so at distinct times over the course of the experiment (rather than continuously), we observed changes in C and N allocation with time. This fertilization strategy also allowed us to look for influences of atmospheric CO_2 concentration on growth and N use as the seedlings approached the “nitrogen:carbon stochiometric limit” (N concentration at which growth ceases).

Statistical analyses included two-way analysis of variance with treatment effects considered significant at the $p \leq 0.10$ using Tukey's HSD procedure (SYSTAT© Inc.).

The trees were grown in controlled environment chambers that were designed and built by two of us (PDR and JTB). The chambers have a 1m x 1m growth area and are enclosed on three sides and on the top by glass panels allowing high levels of natural light (if desired). The chambers are trace gas (in this case for CO_2), as well as temperature, and humidity controlled. Temperature control is based on a continuously-pumped chilled-water fan-coil unit operated with a small temperature gradient and high air speed across the coil so that condensation does not occur. Air flow (wind speed) is adjustable and was directed up from below the pots. Humidity is controlled (in this case to 60% RH) by introducing drier outside air into the unit as needed, via a small squirrel cage fan. An inexpensive CO_2 analyzer was built into each unit to monitor CO_2 concentration using an electronic data acquisition/control board. This board allowed rapid correction of divergence from set point through pulse-train modulation of the CO_2 supply introduced into the make-up air stream. During this experiment CO_2 levels were set to 700, 525, and 370 (slightly above ambient) μmol mol^{-1} air; lighting was artificial, set at 600 μmol quanta m^{-2} s^{-1} in the PAR and provided by pairs of 1000 W multi-vapor HID lamps for 16 hours per day; air temperature was 25 °C.

RESULTS

Biomass, Nitrogen Content and Concentration

Plants from the highest CO_2 treatment were statistically larger than the ambient treatments in both the lowest and highest N regimes at the 18 week harvest (Figure 1 Panels A, B, and C, note the different ordinate ranges among the panels). Middle CO_2 treatments were not statistically different from either the ambient or double CO_2 in any of the N treatments at week 18 (Johnson *et al.*, 1994). Plant biomass, N content, and N concentration are shown for leaves, stems, and roots in Table 1.

At the week 36 harvest, plants at the highest N level were approximately twice as large as plants in the mid-N treatment and five times the size of those in the zero N treatment. There was, however, no CO_2 effect evident within any N treatments at the week 36 harvest. Recall that N fertilizer was applied a second time at week 26.

At the final harvest, there was not a statistically significant CO_2 response in the zero N treatment (Figure 1 Panel C). Although trees in the low N treatment was severely N limited, they continued to grow, increasing 50% in biomass, in the 36 - 58 week interval. Carbon dioxide did have a positive effect in the two N-fertilized treatments at the 58 week harvest. The middle CO_2 treatments were the most responsive — growing to twice the size of trees in present ambient CO_2 levels. It is not completely clear to us why biomass in the twice ambient CO_2 treatments was lower than in the middle CO_2 treatments. Several of the plants in the high CO_2, high N treatment shed some primary needles near the end of the experiment. Most treatments apparently lost some roots during this period (see Table 1). Just as we do not include shed roots in the biomass numbers we do not include shed leaves. Thus, tissues alive at the final harvest somewhat under-represent total productivity. Our height and diameter record of the seedlings (not shown) indicates that the middle CO_2 treatments were already larger than the highest CO_2 treatments when the leaf shedding occurred.

Whole seedling N content was related to N supply and not tightly coupled to plant C content (Panels D, E, and F of Figure 1, note different ordinate ranges among these panels). This was also true for plant parts (Table 1, see also Figure 3 below). At the 18 week harvest in the middle N treatment (where biomass was not significantly influenced by CO_2) the low CO_2 treatment had almost 50% more N than the two elevated CO_2 treatments. At the same (18 week) harvest in the high N treatment, the 525 ppm CO_2 plants had the greatest N content — at almost twice that of the ambient CO_2 treatment and 40% more than the 700 ppm plants — but was only intermediate in biomass. At week 36 there were no significant differences in N content driven by CO_2, within any N treatment. Between weeks 36 and 58 all plants lost some N, primarily a result of root turn-over (Table 1). As was mentioned, the high N/high CO_2 plants did shed some leaves during this period. At the 58 week harvest in the high N treatment, the high CO_2 plants had the lowest N content, while ambient CO_2 plants were intermediate, and the middle CO_2 treatment had the highest N content. In the middle N treatment there were not statistically significant differences in N content among the CO_2. treatments.

Whole seedling N concentration (total N content divided by total biomass) is shown in Panels G, H, and I of Figure 1. At weeks 18 and 58, N concentration in tissues generally declined at higher growth CO_2 levels. At week 36 there were no differences in concentration in the low and middle N treatments, while in the high N treatment there was actually increased N concentration at elevated CO_2. We associate the week 36 situation with the fertilization at week 26 and note that those plants that had the highest growth rates at week 18 tended to have the highest N content at week 36. Only a small proportion of the N taken up in any treatment during the middle growth interval went to roots. Instead the N went primarily to leaves with both content and concentration of N in those tissues increasing (Table 1). Data in Johnson *et al.* (1994) show that available soil N was down to control levels at the time of the 36 week harvest.

Growth after week 26 occurred without additional fertilization so that the N concentrations in all tissues declined in the week 36 to 58 interval. We assume that the lower N concentrations (3 to 3.5 mg g^{-1}) seen in the zero N treatment seedlings must approximate the "stoichiometric N:C limit" for ponderosa pine (the N concentration at which growth ceases). Interestingly, the high N, ambient CO_2 treatment was growing only very slowly during the last interval (see Figure 2) and probably had not yet reached its stoichiometric limit. The reason for this slower growth becomes clear below.

In very low N status situations at the end of the experiment, N concentrations in leaves were about twice that in roots and stems (Table 1) across all treatments. This may represent a baseline difference in N concentrations between leaves and non-photosynthetic tissue. The roots and stems of some higher CO_2 treatments had surprisingly high N concentrations that did not always coincide with high concentrations in leaves.

The upper range for both foliage and whole seedling concentrations seen in this experiment was 15 – 18 mg N g^{-1} (Figure 1 Panels G – I, Table 1). Putting these tissue N concentrations into perspective, we are finding foliage (not whole seedling) values for well fertilized plants in the field to be in a similar range (data not shown). Typical foliar N concentrations for productive stands of ponderosa pine in nature are reported to be in the range of 9.5 mg g^{-1} (Blinn and Buckner, 1989). At a site in the San Bernadino Mountains of southern California that receives very high annual N inputs (35-45 kg ha^{-1}), Ponderosa pine needle N concentrations reach 12.9 mg N g^{-1} (Fenn *et al.*, 1996) At a site in these mountains that receives little N input from air pollution, needle N concentrations were 9 mg g^{-1}. In a dense young stand of ponderosa near Flagstaff AZ needle N is reported to have been 10 mg N g^{-1} (Klemmedson, 1975)

Nitrogen and Carbon Uptake

Seedling N and biomass accumulation patterns are depicted in Figure 2. The large uptake of N following fertilization at week 26 is seen in Panel A of this figure. Comparing uptake rates for different N treatments, one sees that uptake was roughly proportional to the supply. Although the amount of N that was put on each pot at week 26 was the same as had been applied at the beginning of the experiment, uptake after week 26 was much greater then at early growth. Presumably the younger seedlings were not able to capture the initial N dose and much of it leached away. The loss of N by all plants between week 36 and 58 is seen in the negative uptake values in Panel A during the final growth interval.

Seedling growth rate (Panel B) in the first two intervals of the experiment was nearly proportional to the quantity of N supplied with only a small influence by CO_2. It was in the third interval, where growth relied on internal N, that the effect of CO_2 on growth became dominant. In both added N treatments the two elevated CO_2 groups increased or in the case of the C 700, N 100 maintained growth rates in the final period. Both ambient CO_2 treatments that had been given N experienced decreased growth rates — the highest N plants to nearly zero (Figure 2 Panel B). These growth rate changes are connected to aspects of shifting C and C allocation.

There appeared to be a consistent relationship between growth rate and N content among many of the data points through the week 36 harvests but significant divergence occurred after that time (Figure 2 Panel C). However, plotting the growth rate per unit N (N productivity, Panel D) reveals substantial differences in N use. The zero N treatments generally had the highest N productivity but this was falling away in two of the three CO_2 levels in the final interval. In the first growth interval lower N treatments at a given CO_2 had the highest N productivity. Within a N treatment elevated CO_2 tended to yield higher N productivity. The high N, ambient and middle CO_2 treatments were, however, nearly identical to the ambient CO_2, mid-N. In the second growth interval the N productivity of the elevated CO_2 treatment, which had been high, dropped in conjunction with their large N uptake following fertilization. Values for the elevated CO_2 treatments rose again in the final growth interval. The two +N, ambient CO_2 treatments were constant and quite similar in N productivity until the final interval during which the growth of the high N plants went almost to zero.

Patterns of Carbon and Nitrogen Investments in Above- *versus* Below-ground Tissues.

Ambient CO_2 grown plants had a strong tendency to commit a lower proportion of their N to roots than did elevated CO_2 grown plants (Figure 3 Panels A, B, C). The significant exception to this is the N 200/C 700 treatment at week 58, where a large proportion of total N had been lost in shed tissues (see Table 1). Recall that net N uptake was near or below zero for all treatments in the 36 to 58 week interval (Figure 2A). From that and distribution of N and C in Figure 3 Panels B and C it can be concluded that N that had been in leaves at week 36 had moved into the stems and roots by week 58. The proportion of biomass committed to roots generally increased with CO_2 treatment across each N treatment and also increased with time (Figure 3 Panels D, E, F). While the trends in the partitioning of C and N between tissues were similar across time and treatment, it is clear from Figure 3 that C and N are not always committed to tissues together (i.e. not proportionally).

The dry weight of root tissue versus shoot tissue for each treatment is plotted in Figure 4 Panel A. The inserted panel magnifies the lower portion of the curves. All curves begin with values from the thinning harvest, one month after germination. Points farther up the diagonal are older plants with the exception of the C 525, N 0 plants. In the insert it can be seen that this treatment lost weight before the final harvest. This figure shows that as the experiment progressed from week 4, through week 18, to week 36 all treatments had nearly straight line relationships between root biomass and shoot biomass. There was a clear increase in slope from the higher to the lower N treatments. Within each N treatment the elevated CO_2 treatments always had steeper slopes than the ambient CO_2 treatments. When N was withheld after the week 36 harvest, the +N treatments all began to shift a greater proportion of biomass to roots. The shift in the allocation to roots was small for the higher CO_2 plants. The shift in allocation by the ambient CO_2 trees was marked. The C 375, N 200 treatment plants had a near zero growth rate in the final interval of the experiment (Figure 2). However, Figure 4 makes clear that some C was gained by these plants and root biomass increased while shoot biomass dropped in the final interval (see also Table 1). Growth of the C 375, N 100 treatment, did not drop as precipitously (Figure 2), but also went almost entirely into roots after week 36.

Figure 5, is analogous to Figure 4 but shows root versus shoot N content rather than biomass. The graph indicates that until the last harvest, 60% or more of the N in all plants was in shoots. Between week 36 and 58 many treatments showed some loss of N (Fig. 2). Importantly, Figure 4 shows that a considerable amount of the N that had been in shoots up to week 36 was moved into the roots by week 58. As the trees continued to grow without additional N both C and N were increasingly allocated to roots.

Several models have used variation in N concentration to allocate C between roots and shoots (e.g. Agren and Ingestad, 1987; Levin *et al.,* 1989; Reynolds and Thornley; 1982). Panels A and B in Figure 6 show, respectively, the root to shoot ratios for biomass and for N plotted as a function of total tree N concentration. Both ratios increased as N concentrations in tissues declined and C uptake continued. Sixty-eight percent of the variation in the root/shoot ratio for biomass and 38% of the variation in the N root/shoot ratio may be related to changes in whole tree N concentration. It is important to understand that data in this figure do not represent static, equilibrated values for root/shoot ratios as a function of N concentrations. There is clear hysteresis in the data. At the week 18 and 58 harvests N concentrations were clearly declining and roots were an increasing priority in the allocation of C and N. The week 36 harvest data are complicated by the fertilization 10 weeks earlier. Some of the data points from that harvest shifted rightward on the graph indicating increased N concentration with little change from the 18 week allocation pattern. Other treatments showed little change in N concentration but the ordinate values moved up indicating allocation to roots. More consistent relationships between N concentration and partitioning of C or N would likely be found if various tissue N concentration could be imposed and held.

DISCUSSION

Studying C and N acquisition and allocation in very young tree seedlings growing in pots has been justifiably criticized (including by ourselves, Johnson and Ball, 1996) as being nearly irrelevant to field situations and especially to mature forests. Several aspects of this experiment counter these criticisms. We obtained a more satisfying estimate of root biomass in controlled conditions then we were able to obtain in field plots. In the field, the complete excavation of plots required for the best estimates of root properties is unacceptable in a multi-temporal sampling scheme. In controlled environments we are able to consider variables and ranges that are difficult to produce or obtain consistently in the field. Further, very early patterns of C and N allocation, which can reasonably be studied in pots, are probably important in establishing the founding resource capital for further growth. In this experiment we were able to provide seedlings with a very wide range of N nutrition delivered in discrete pulses (as is important in the field) in a range of atmospheric CO_2 levels. From a physiological perspective, atmospheric CO_2 levels are shown here to effectively manipulate C availability to probe responses of the system of allocation in seedlings.

Growth responses to CO_2.

At the first harvest there were statistically significant differences ($p \leq 0.90$) between the 370 and the 700 ppm CO_2 treatments in both the lowest and the highest N treatments (1.5 and 2.1 times more growth respectively). It is not clear why a CO_2 response was not present in the middle N treatment in the first harvest. At the second harvest growth responses appear to have been dominated by the fluctuation in available N.

At the final harvest there was no CO_2 effect on biomass in the zero N treatments. This is perhaps not surprising. In the limit, where N is spread as widely as possible to simply maintain function, one might expect that all possible adjustments in N use would have been made. Extra C gain might, in fact, make maintenance more difficult.

Where N was available CO_2 had a significant positive influence on growth. The relative effect of CO_2 on growth was almost identical in both of the +N treatments. Comparing the middle CO_2 treatments to the ambient, there was 2.3 and 2.1 times more biomass in the middle and high N treatments respectively. Comparing the 700 $\mu mol\ mol^{-1}$ to ambient CO_2 treatments, both +N treatments had 1.7 times more biomass. This similarity is surprising in light of: (1) the smaller response at 700 compared to 525 $\mu mol\ mol^{-1}$ CO_2; and (2) the fact that we allowed the trees to grow into very N limited states (which they reached at very different rates). Interestingly, a similar response: the 525 ppm CO_2 treatment growing fastest, occurred at all N levels for the first year of our ponderosa pine field experiment that followed this pilot study. This pattern has not been sustained however (data not shown).

Nitrogen and Growth

The pattern of variation in N content and concentration through time is a clear reminder that N uptake and C uptake can be quite asynchronous. Because of the seasonal pattern of N availability in many natural systems (e.g. Nadlehoffer *et al.*, 1984; Zak and Grigal, 1991) such asynchrony must regularly be the case in nature. Often, constant nutrient supply rates are used in controlled environment experiments which may lead to accumulation of nutrients when plants are small and depletion of reserves when plants are larger (Ingestad, 1982). Still, it is widely assumed, especially in elevated CO_2 experiments, that lower plant N concentration is indicative of greater N use efficiency (e.g. Coleman *et al.*, 1993). Unless, as Ingestad (1982) suggested, root N uptake activity and shoot C uptake activity are exactly matched, plant N concentration will vary. That variation may not be a good reflection of the manner in which or efficiency with which N is being used. An important point is that taking up N when it is available is efficient and an important buffer when the N replenishment rate is low (Rastetter and Shaver, 1992). Our results show that there are quite different impacts of CO_2 on growth under different total N and N supply rate conditions. We review four situations.

A. At the 36 week harvest, ten weeks after a pulse of N, N concentrations in roots, leaves and, in some cases, even stems of elevated CO_2 grown plants were higher (not lower) then their slower growing counterparts at ambient CO_2 (Table 1). How that additional N was actually being employed is a question unfortunately beyond the scope of this study.

B. Although the 700 ppm CO_2 treatments within the highest and lowest N treatments did have the lowest N concentrations at the final harvest, it is not clear to us that this will be a consistent or ecologically meaningful result. All tissues had similarly low N concentration at the final harvest (Table 1). We conclude that elevated CO_2 is unlikely to allow large increases in the C:N ratio for plants at the lowest survivable N concentrations. However, mere survival at the lowest possible N concentrations is not what most plants experience. Growth with N supply varying from something above the average rate of N incorporation into proteins to something below the average demand for N must be common for plants. In other words, some degree of fluctuation in N concentration must almost always be underway. There are times when trees store N (see review of Dickson, 1989) and there must also be times when N is moved out of some enzymes and into more critical proteins.

C. In the 4 to 18 week and the 36 to 58 week intervals, the fastest growing plants (see Figure 2) must have had lower concentrations than others in their respective N treatments for virtually the entire periods (compare Panels G, H, and I in Figure 1). Thus, N concentration itself does not establish growth rate or relative growth rate. Rather a higher growth rate (e.g. at elevated CO_2) on a fixed quantity of N will yield a lower N concentration as Agren (1994) pointed out. Furthermore, because net N uptake was negative for the final growth period we can also conclude that N uptake rate *per se* does not establish growth rate. There are also times when N content is not a good predictor of growth rate (Figure 2 Panels C and D).

D. In the high N treatment at week 36 neither the biomass nor the N content were statistically different between the 370 and 700 ppm grown seedlings (see Table 1). By the final harvest the 370 ppm plants had grown to 118% of their week 36 weight. In contrast, the 700 ppm plants had grown to 223% of their previous weight. In the first two growth intervals the ambient CO_2 grown plants at high N were in a situation of abundant N and, relatively limited C. The majority of the tissues constructed by those seedlings went above-ground — toward C gain. In contrast, elevated CO_2 grown plants, having a more substantial C supply, tended toward lower internal N concentrations early in growth. Relatively, the 700 ppm seedlings committed more of both N and C to their root systems throughout the experiment than seedlings in other CO_2 treatments. This effectively pre-positioned the elevated CO_2 grown plants in the configuration that all plants reached after N was depleted (Figure 4). When the N supply ran out, the ambient CO_2 grown plants substantially re-oriented the allocation of both C and N. Both C and N were taken out of needles and put into roots (Table 1, Figure 4). The cost of re-deploying material to the roots appears to have consumed most of the C that we presume was being fixed in the final time period. Thus ambient seedlings grew only 18% in the final interval in spite of beginning the final period with an N content that was not statistically below the other CO_2 treatments at the high N level. Less leaf and root mass to contribute to future growth would have been perhaps an even more important cost had the experiment continued. Consider that at the final harvest, the 370 ppm plants were approaching the same root:shoot ratio as the 700 ppm plants but with only 65% of the leaf mass and 57% the total mass of the 700 ppm grown plants. The 700 ppm CO_2 grown plants making only a small adjustment in allocation pattern during the final period more than doubled in biomass in that interval. Another indication of the benefit of not having to adjust allocation patterns when resources declined is that these 700 ppm grown plants had the second highest N productivity (behind the C 700, N 0 treatment) (Figure 2) in the final growth period.

These four cases illustrate that growth, ultimately, is more related to N content than to N concentration. This statement underlies the concept of N productivity — growth per unit N (Vitousek, 1982; Agren, 1985). While this concept has many advantages over other measures for judging the efficiency of N use, it like other measures must be used with discernment. Relevant to our experiment, N taken up but not yet fully deployed would be judged inefficient as we suspect the situation was at the week 36 harvest. Some N storage is probably very useful and efficient in the longer term. Also, when the stoichiometric limit is encountered N productivity falls to zero while the N is crucial to survival and, in an ultimate sense, efficient.

The productivity concept is valuable in pointing our attention toward the *interaction* of N and C in producing *new growth*. Workers with interest in photosynthesis often see productivity as simply a problem of C gain. At the same time workers oriented to below-ground processes see growth rate and final biomass as a question of N content. Our data make it clear that neither view in isolation is correct. A more useful perspective is, that while resource content (C and N here) is central to the addition of biomass, the manner in which new resources are deployed (to capture the next increments of resource) is also critical. This view is perhaps, in two aspects, beyond the way that the functional balance concept is often considered. The first aspect is that pointed out by Hilbert and co-workers (Hilbert, 1990; Hilbert *et al.,* 1991; Hilbert and Reynolds, 1991), all deployment and all capture of C and N should be evaluated together. In particular, these workers formally include the N cost of N gain with the three previously considered costs: C cost of C and N gain, and the N cost of C gain in their analysis. The second aspect is that functional balance is often cast in the context of balanced exponential growth. While this is mathematically convenient it is not a strict requirement. The real allocation problems that plants face must often balance resource income in situations of fluctuating external resource levels. What matters toward growth is capturing the next increment of needed resource. That may include re-allocation of material as external resource levels vary (e.g. Brouwer, 1983). The translocation of N from shoots to roots seen in Figure 5 is an indication of both the N cost of N gain and the fact that plants are capable of changing allocation patterns.

Oscillations in availability of external N (e.g. Birk and Vitousek, 1986; Nadlehoffer *et al.*, 1984; Zak and Grigal, 1991) and varying dependence on a mix of external and internal N sources for growth must, as we have said, be the norm for plants in nature. The fact that CO_2 is a resource at low concentration but of inexhaustible supply whereas N is a depletable, supply-rate limited resource (Rastetter and Shaver, 1992) makes a difference as to the optimal "foraging strategy" for these resources. We know little about the physiological underpinning of response to, or consequences of variation in N availability. Trees, in particular, store N in several forms, which may buffer against variations in external N availability (Dickson, 1989). More is known about carbohydrate stores and their use. The range and speed of the fluctuation in N availability in this experiment was greater than many plants experience in nature. The C (and possibly the N) cost of the reallocation response must be related to the size of the response and thus, the fluctuation so that our results should not be used as an absolute indication of increased growth costs in response to a fluctuation in N. Yet there can be no doubt that these seedlings were capable of reallocating their resources, but apparently at significant expense. These results suggest that reduced growth as N is depleted is more than just a matter of reduced C uptake rather it may also involve energy costs of material re-allocation. We are not aware that this has been considered previously. The results further suggest that plants growing at elevated CO_2 might face a much smaller need for readjustment when nutrient supplies fluctuate than do plants in today's atmosphere. Furthermore, plants growing at elevated CO_2 may have more carbohydrate stores so that C costs can be more easily borne (A. Peterson, personal communication). Costs of re-allocation seemed to be the largest factor in determining the difference between ambient and elevated CO_2 grown plants in at least the high N-treatment. Potential differences in the costs of tissue construction in higher than present CO_2 atmospheres has been found to be small Griffin *et al.*, 1995) That assessment is based on the assumption that beginning with glucose and N of known oxidation state does not apply to re-allocation situations. We can suggest that some indication of the cost of reallocation could be obtained from measuring whole plant gas exchange. We predict that increased respiration will accompany re-allocation processes, giving some indication of the C costs. Compared to the plants in today's atmosphere, growth at elevated CO_2 may involve significant differences in uptake, distribution, and use of C and N in plants.

Carbon and Nitrogen Allocation Changes Correlated with Plant N Concentration Changes.

The results reported here contrast with a study by Griffin *et al.* (1995), which is programatically related to the present study, that compared growth responses of ponderosa and loblolly pines in a common garden. Their study was a modified hydroponics experiment with CO_2 levels of 35 and 70 Pa and two N levels — sand flushed with nutrient solutions containing 1.0 and 3.5 mM NH_4^+ daily. The ponderosa pine used by Griffin *et al.* was from the same seed lot used here. They found root/shoot ratio to be approximately 1.2 across treatments without a detectable N or CO_2 influence. In our experiment we saw no root/shoot ratio greater than 0.9 and the highest values occurred only at very low N levels. In the Griffin *et al.* experiment the low N treatment resulted in tissue N concentrations that are at the upper range of our high N treatments: whole-tree average N concentrations of 12.5 and 16.8 mg g^{-1} and needle N concentrations of 21.5 and 18.8 mg g^{-1} for trees grown at 35 and 70 Pa CO_2 respectively. There are several possible reasons that the results of these two experiments differ. In the hydroponic experiment N was provided as NH_4^+ only. Also, the flux of nutrients past these roots was by design quite high. In the higher N treatments the hydroponic seedlings had roots which were noticeably thickened and unbranched (K. Griffin, personal communication).

Root responses to N have generally been defined in terms of external N concentration. As available soil N increases root growth decreases relative to shoot growth eventually stopping (Sattlemacher *et al.*, 1990; Boot and Mensink, 1990). At the same time it is well known that roots proliferate and have a more branched architecture in N-rich areas (Drew, 1975; Granato and Raper, 1989; Jackson and Caldwell, 1989; Fitter *et al.*, 1988). Experiments of Jackson and Bloom (1990) and Bloom *et al.*, 1993 show that there are distinct optima within moderate ranges of NH_4^+ and NO_3^- for root biomass, for length, and for branching.

The time course of plant N concentration is a practical metric of the relative activities of roots (N-uptake) and shoots (C-uptake) when these are the dominant below- and above-ground limiting resources. It has been hypothesized, as was mentioned in the introduction, that decreasing plant N concentrations should lead to allocation toward increasing root specific activity as opposed to shoot activity (e.g. Agren and Ingestad, 1987; Hilbert, 1990; Levin *et al*, 1989; Troughton, 1977; Reynolds and Thornley, 1982). Such adjustment of specific activities would stabilize whole plant N concentration. Thus experiments to test this hypothesis done with continuously available N have difficulty distinguishing cause from effect in the correlation between N concentration and partitioning between root and shoot activities. Our experiment involved periods of presumably rapid N uptake followed by clearly much longer periods of very low soil N availability. During these longer periods N for new growth was supplied from internal pools (Figure 2). Thus the correlation between tissue N concentration and root/shoot ratio (Figure 5) was not dependent upon continuous N uptake. This result is supportive of the hypothesis that N concentration is on the causal side of this correlation between N concentration and root/shoot ratio. This is not proof that N concentration is the signal that results in changes in allocation. Nor does this prove that plants always respond according to our present concepts of functional balance. The data from Griffin *et al.* (1995), for example, speak against both of these propositions. Root and shoot activities must be coordinated in some fashion but given the many resources that roots must acquire, and shifts among several potential limitations, it is not always clear how to appraise that coordination. Mechanistic connections between N concentration and the controls over the allocation of C and N are worthy of investigation. Our results lead us to expect that the supply of C, including modification by the level of atmospheric CO_2, will interact with local soil conditions to establish root growth patterns.

Assessing Responses to Elevated CO_2

Carbon and N portioning remains one of the most significant uncertainties in environmental plant physiology today. Potential impacts of rising atmospheric CO_2 on allocation and subsequent impacts on ecosystems heightens the importance of these issues. In assessing growth and allocation it has become common practice to plot logarithmic transforms of root, shoot, whole plant biomass or N content against each other. This is justified on the basis of an appropriate desire to compare plants of equal size but implicitly assumes logarithmic growth. In our experience, sustained logarithmic growth is uncommon. Furthermore, plants of equal size *are of equal size* and can very well be compared in absolute terms even if size does not increase linearly. Transformations that force approximately linear relationships will always hide potentially important detail. In our first consideration of the data reported here (Ball *et al.,* 1992) we undertook such transformations and the patterns reported here were undetectable. Even plotting the ratios of tissue weight can hide important detail (Brouwer, 1962).

The implications of this study for forest ecosystems function and sequestration of atmospheric CO_2 are important. Growth and growth rate per unit N are higher at elevated CO_2.

Although some of this elevated productivity may be the result of reduced rubisco and consequently greater internal N availability (Woodrow, 1994; Bowes, 1991), changes in C and N allocation in response to elevated CO_2 can also contribute a great deal to increased C gain and sequestration at elevated CO_2. The quantity of both N and C available for allocation to the photosynthetic apparatus is dependent on both supplies and the allocation toward other priorities within plants. One should notice that the most important driver of N content in these seedlings was the supply. N availability in forest soils is strongly seasonal and to the extent that elevated CO_2 induces fine root activity at higher soil N concentration and earlier in time, trees may be able to obtain more N from seasonal pulses. Outside of seasonal pulses, competition with bacteria for N must be a matter of having fine roots densely deployed. Opinions differ about the relative importance of several factors involved in N availability to plants: (1) competition for N between plants and bacteria, (2) the degree to which C limits N exploitation by roots, (3) the role of root exudation and fine root turn over in regulating bacterial N use, and (4) the role of newly acquired N versus N from internal pools for sustaining mid-season forest growth. These processes are interactive and are all likely to change for forests growing at elevated CO_2. Both the capacity for C uptake and for sequestration are impacted by the flux of N within ecosystems (Cole and Rapp, 1981). Thus these issues are pivotal and contribute to the uncertainty about ecosystem responses to global environmental change.

CONCLUSIONS

In forests, nutrient conditions are likely to be much more variable than steady through time. In this experiment we provided N in two discrete pulses as opposed to continuous supplies as is often done for physiological studies. Our results showed (1) that the capability to respond to temporal variation in nutrient conditions, (2) the dynamics of nutrient uptake, and (3) the dynamics of nutrient use are all influenced by the interplay between (a) previous N supply,

(b) previous C supply and (c) the atmospheric CO_2 level. Logically, we know that these same interactions are and will be important to the function of forest ecosystems.

We asked two questions in this paper with respect to ponderosa pine seedlings growing at elevated CO_2: (1) Does elevated CO_2 result in changes in C allocation that are consistent with patterns attributed to a functional balance between above-ground and below-ground resource capture? The answer to that question is yes. (2) Is there evidence of increased biomass accumulation and increased growth rate per unit N (N productivity) at elevated CO_2? The answer to this question is also affirmative. Furthermore, increases in productivity at elevated CO_2 came not just as a result of higher rates of C uptake, but also as a result of changes in allocation. Ponderosa pine does tend to operate with higher N productivity and with lower N per gram of tissue at elevated CO_2. The change in N concentration can occur in all tissues, not just in leaves, but only happens when N can not be taken up at a rate that matches C gain. *Lower tissue N concentrations do appear to be related to signals changing C and N* allocation to roots versus shoots. The largest effects of elevated CO_2 on plant growth occurred once plants began to draw heavily on internal N pools for growth.

The caveat underlined above is important to understand when interpreting experimental results. Low but continuos supplies of N can be equivalent to a large pulse of N. When N is available it seems to be taken up without regard to its immediate synchronization to C supply. Only under rather constant resource supplies would we expect to see root and shoot activities appearing to be well matched. In nature such constancy is likely to be rare, so that allocation between roots and shoots must be in a near constant state of readjustment.

We saw evidence that the cost for reallocating material when resource levels fluctuate can be substantial. We noted that elevated CO_2 essentially pre-positioned root/shoot ratios for low mineral nutrient conditions without suffering the penalty of reduced growth rates due to the need to re-orient C and N allocation. This could potentially lead to a tighter N cycle, an improvement of the competitive position for plant vis–à–vis bacteria for N, and an increase in C per unit N in ecosystems of an elevated CO_2 world.

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Figure Captions

Figure 1. Seedling biomass (Panels A, B, C), Seedling nitrogen content (Panels D, E, F), and Seedling nitrogen concentration (Panels G, H, I) at 18, 36, and 58 week harvest respectively. The three carbon dioxide treatments (L, M, H; solid, shaded, open bars) are grouped by nitrogen treatments (0, 1, 2). Nitrogen was added twice during the experiment at the levels indicated (0, 100, 200 µg N per g soil).

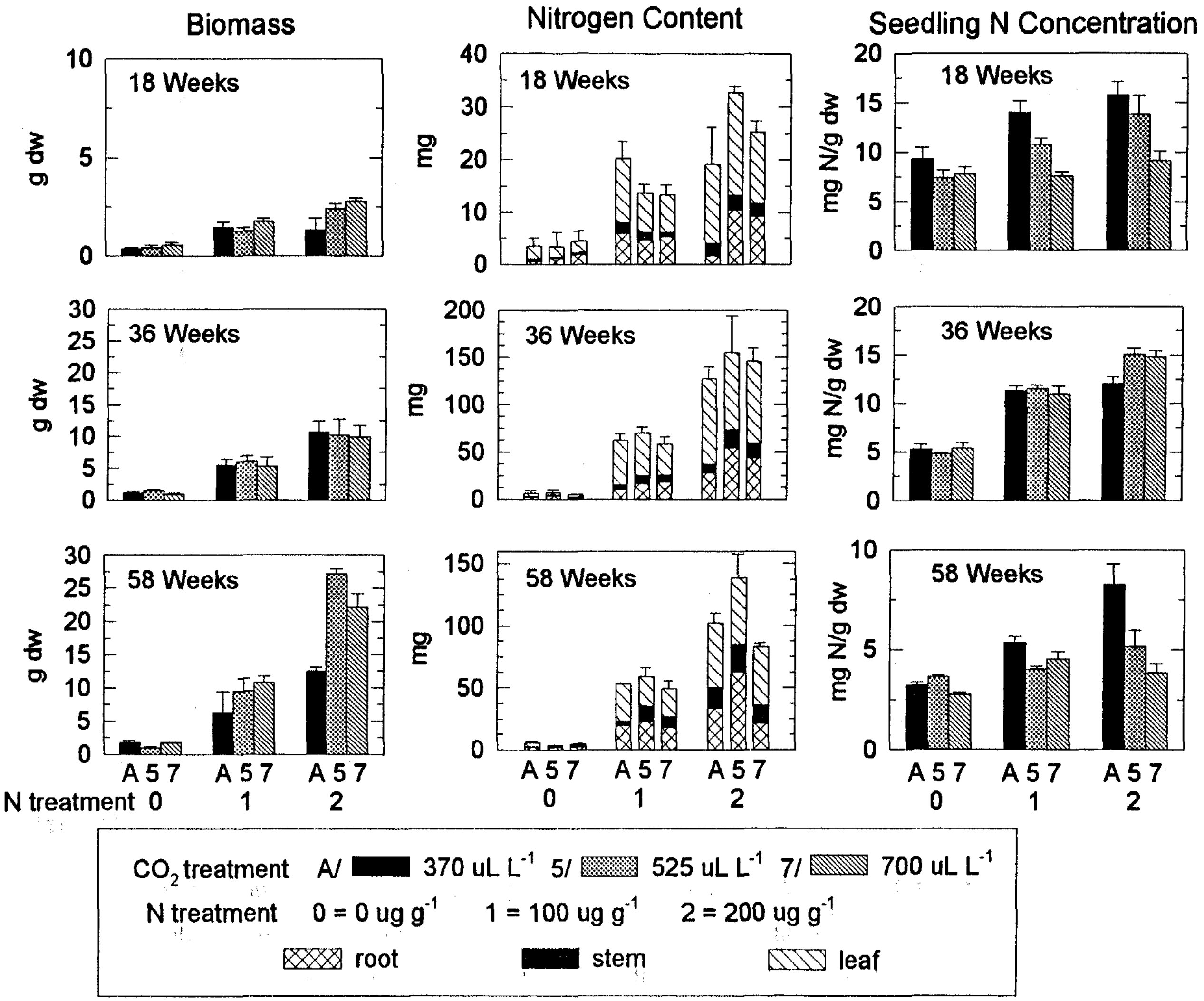
Figure 2. Seedling nitrogen uptake (Panel A), Seedling growth rate (Panel B), Growth rate as a function of nitrogen content (Panel C), and Seedling nitrogen productivity (Growth rate per gram N; Panel D). Nitrogen treatments are indicated by symbol shape: circles, squares, and triangles for 0, 100, and 200 µg N per g soil (increment size), respectively. CO_2 treatments are indicated by the shading of the symbols: solid, half-tone, and open for 370, 525, and 700 µmol CO_2 per mole air. Points are plotted against an abscissa of the middle week of the interval between harvests. Panels A and B from Johnson *et al.*, (1994).

Figure 3. Fractional distribution of nitrogen and carbon between roots, stems and leaves, from the week 18,36, and 58 harvests. The three CO_2 treatments are grouped for each nitrogen treatment.

Figure 4. Root dry weight compared to shoot dry weight from the thinning harvest at four weeks to the final harvest at 58 weeks. Lines connect the data from plants within each treatment. Treatments may be identified from the legend which follows the same scheme as in Figure 2. As plants grow through time they tend to move from bottom left toward the top right side of the graph. The insert magnifies the bottom left corner of the plot revealing the responses of the lowest nitrogen treatment.

Figure 5. Root nitrogen content compared to shoot nitrogen content from the thinning harvest at four weeks to the final harvest at 58 weeks. Lines connect the data from plants within each treatment, in the same manner as Figure 4. Treatments may be identified from the legend which follows the same scheme as in Figure 2. As plants grow through time they tend to move from bottom left toward the top right side of the graph except as they shifted nitrogen into roots during the final growth interval.

Figure 6. Panel A. Root:Shoot biomass ratio as a function of whole seedling nitrogen concentration. The heavier, solid, line is the regression line. Lighter, dotted, lines connect different time points within a treatment as indicated by the harvest week next to the symbols. The symbol scheme (same as previous) is indicated in the legend. Panel B. Root nitrogen:Shoot nitrogen ratio as a function of whole seedling nitrogen concentration. As in Panel A, the heavier, solid, line is the regression line. Lighter, dotted, lines connect different time points within a treatment as indicated by the harvest week next to the symbols.



Biomass
Nitrogen Content
Seedling N Concentration
18 Weeks
36 Weeks
58 Weeks
g dw
mg
mg N/g dw
A 5 7
N treatment 0
1
2
CO$_2$ treatment A/ 370 uL L^{-1} 5/ 525 uL L^{-1} 7/ 700 uL L^{-1}
N treatment 0 = 0 ug g^{-1} 1 = 100 ug g^{-1} 2 = 200 ug g^{-1}
root
stem
leaf

Figure 1.

Figure 2

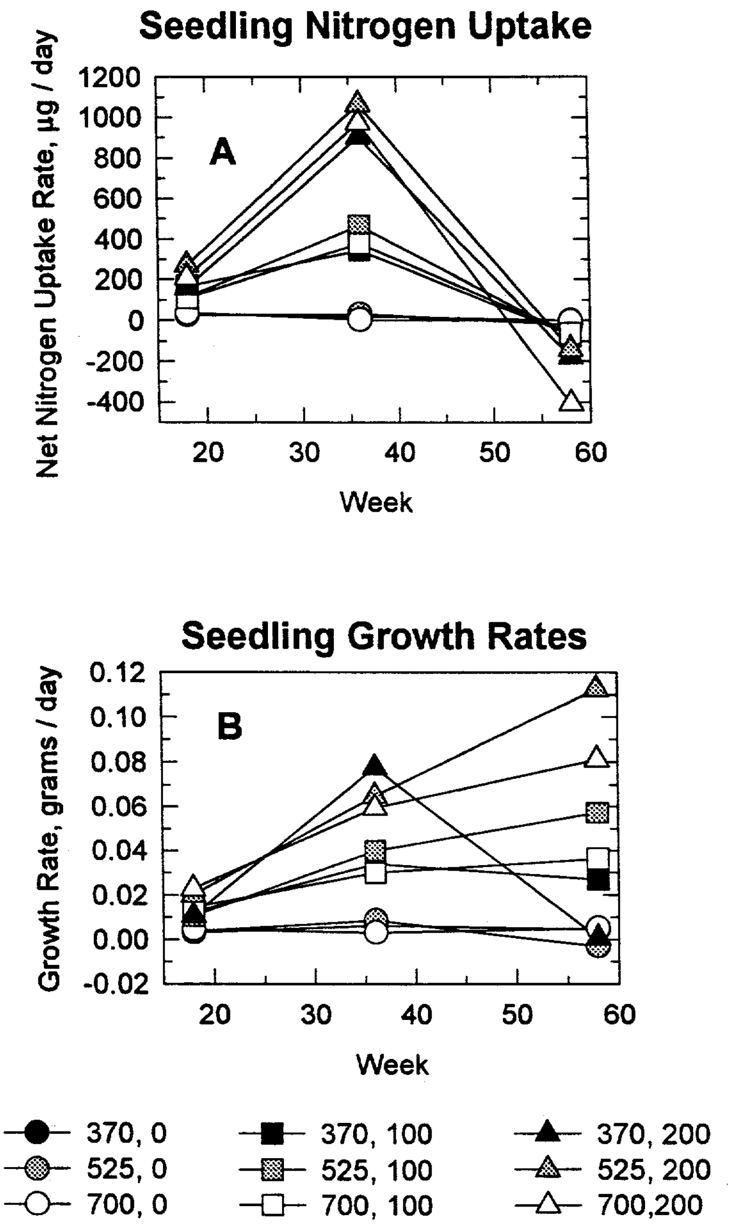


Figure 2 Panel C on next page

Figure 2 Panel C

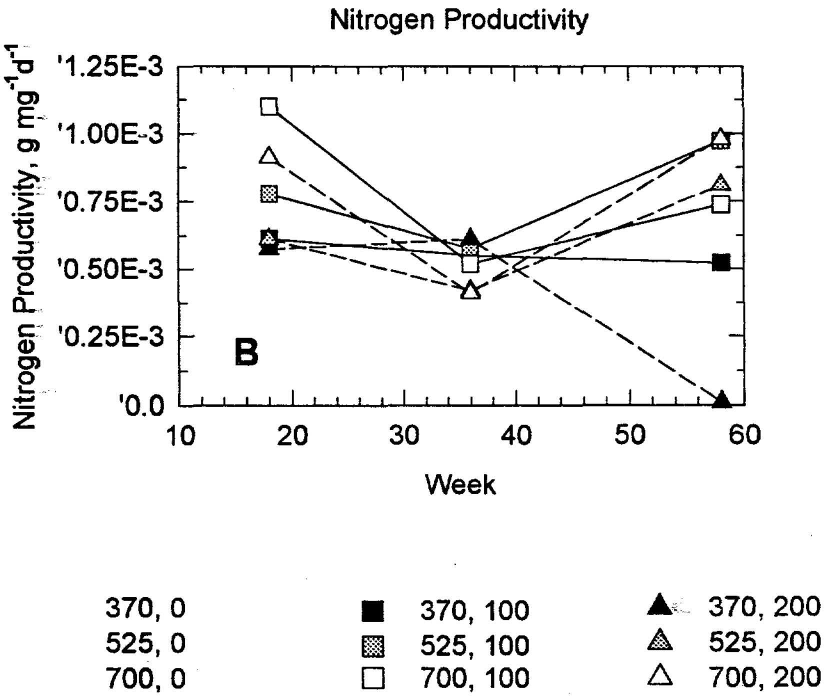
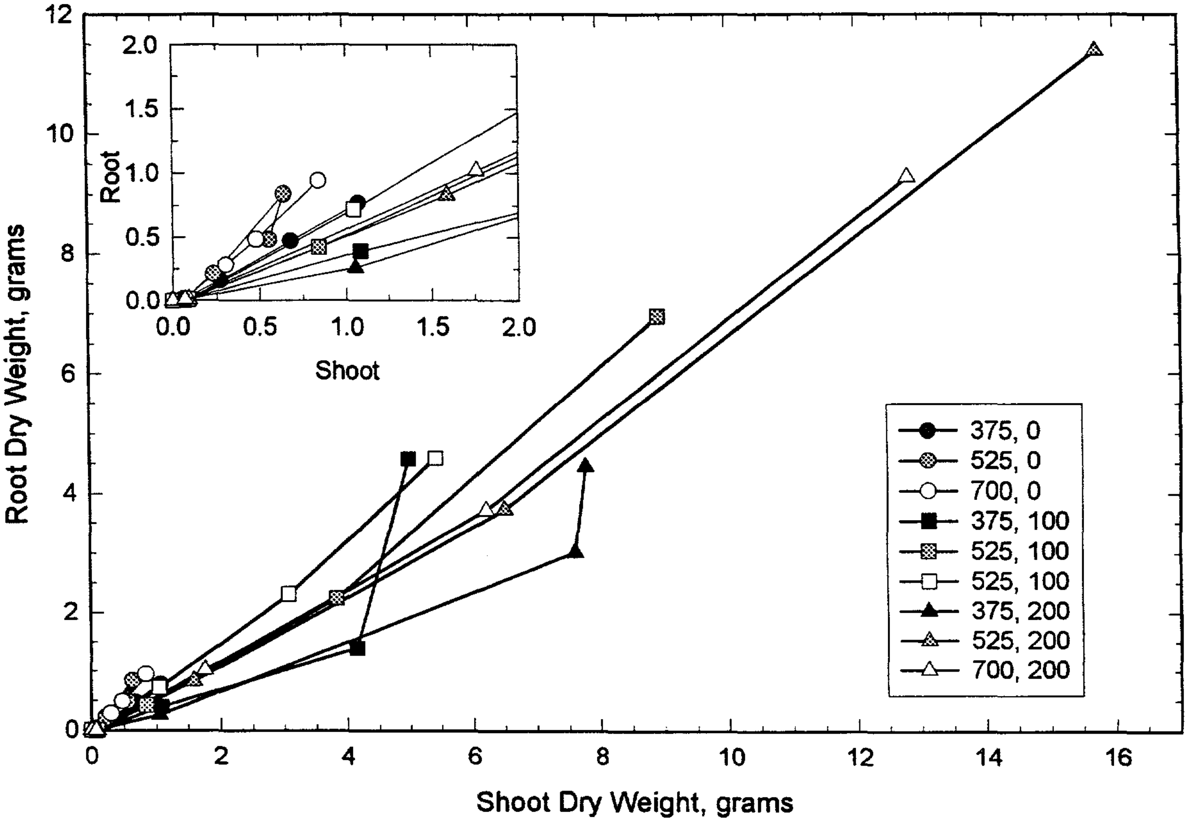


Figure 3

Root Weight Compared to Shoot Weight



Root and Shoot N Content Relationship

Figure 4

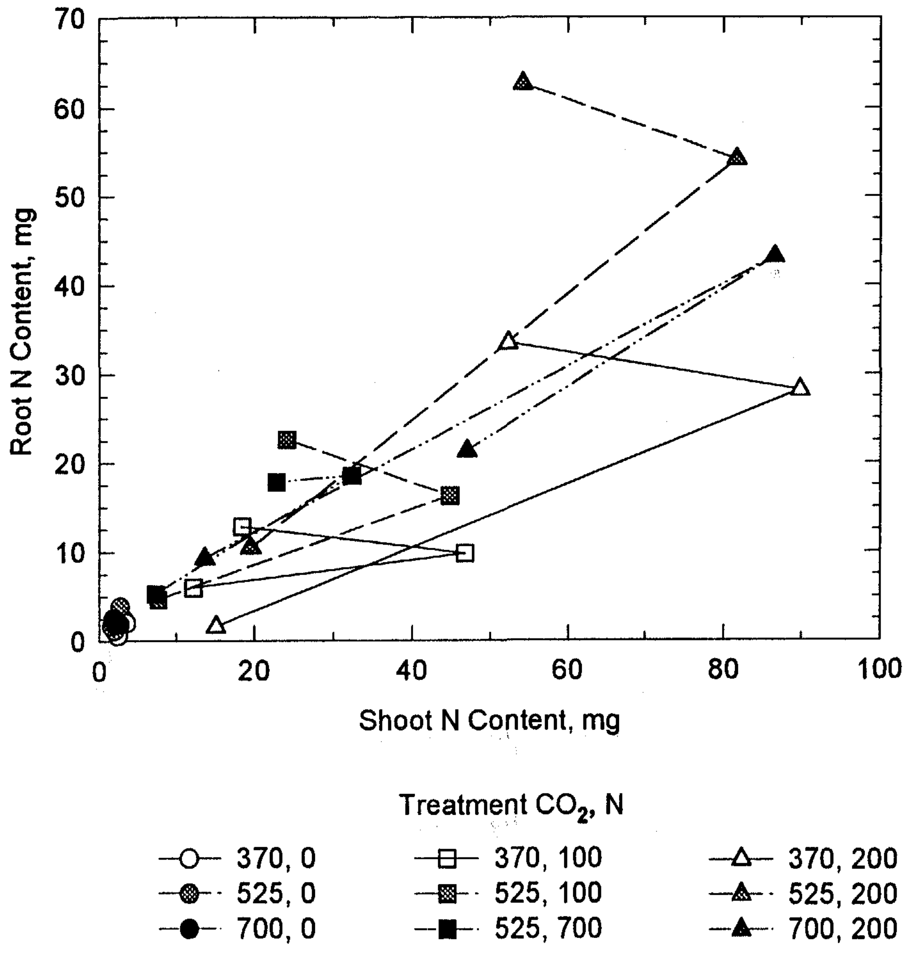
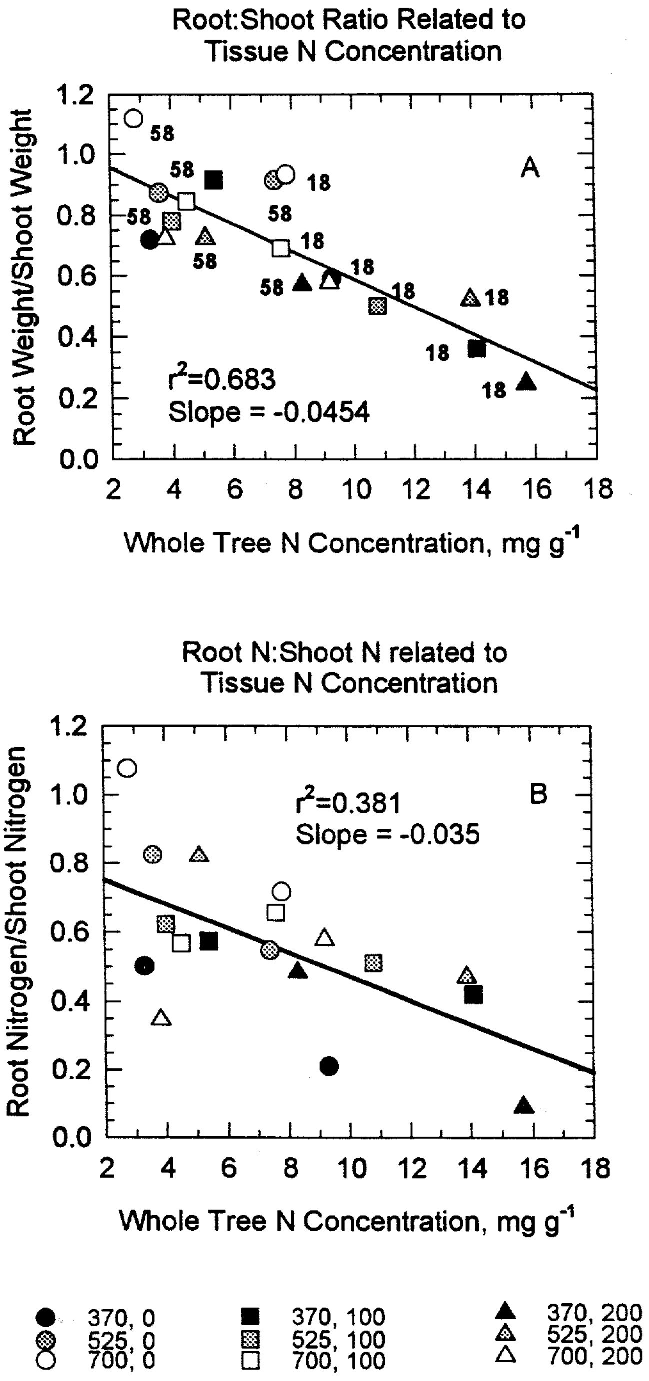


Figure 5



Root:Shoot Ratio Related to Tissue N Concentration

Root N:Shoot N related to Tissue N Concentration

R/SALL

Table 1

Nitrogen Concentration and Content by tissue

N Concentration mg/g											
		18 weeks			36 weeks			58 weeks			
		leaf	stem	root	leaf	stem	root	leaf	stem	root	
low n 1	amb co2	10.10	10.60	6.20	6.20	3.20	5.00	4.00	2.60	2.60	
2	1.5 co2	9.90	6.80	5.50	6.40	2.70	4.70	3.70	3.80	3.50	
3	2.0 co2	9.60	6.20	6.50	8.10	4.80	3.80	2.90	2.60	2.70	
med n 1	amb co2	14.20	11.60	14.90	13.90	7.20	7.00	7.70	3.90	4.20	
2	1.5 co2	11.50	8.10	10.90	14.40	12.00	7.20	4.50	3.30	3.90	
3	2.0 co2	8.50	4.50	7.40	14.90	9.40	8.10	6.50	4.40	3.30	
high N 1	amb co2	16.70	18.90	16.30	14.80	6.10	9.50	9.70	7.20	6.90	
2	1.5 co2	15.10	11.00	12.70	16.80	12.00	14.10	5.40	3.90	5.50	
3	2.0 co2	9.80	6.10	9.10	18.40	10.70	11.80	6.10	3.30	2.30	
N content											
		18 weeks			36 weeks			58 weeks			
		leaf	stem	root	leaf	stem	root	leaf	stem	root	
low N	amb co2	2.39	0.45	0.60	3.16	0.52	2.32	3.43	0.55	1.99	
	1.5 co2	1.92	0.24	1.18	2.63	0.61	3.88	1.52	0.52	1.68	
	2.0 co2	2.25	0.34	1.86	2.59	0.69	1.83	1.74	0.62	2.54	
med N	amb co2	12.15	2.03	5.96	46.75	5.21	9.79	18.41	3.90	12.82	
	1.5 co2	7.58	1.49	4.64	44.80	8.56	16.31	24.03	12.31	22.60	
	2.0 co2	7.20	0.86	5.29	32.28	6.99	18.56	22.70	8.71	17.84	
high N	amb co2	15.07	2.49	1.59	89.78	9.11	28.16	52.27	16.75	33.48	
	1.5 co2	19.51	2.73	10.45	81.63	19.12	54.19	54.19	22.14	62.65	
	2.0 co2	13.63	2.32	9.25	86.48	15.62	43.17	47.00	14.58	21.36	