AUTHOR: D. W. Johnson,* R. B. Thomas, K. L. Griffin, D. T. Tissue, J. T. Ball, B. R. Strain, and R. F. Walker
TITLE: Effects of Carbon Dioxide and Nitrogen on Growth and Nitrogen Uptake in Ponderosa and Loblolly Pine
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ABSTRACT
The purpose of this paper is to summarize the results of a series of greenhouse and open- top chamber studies on the effects of N and elevated atmospheric CO[sub2] on ponderosa and loblolly pine (Pinus ponderosa Laws. and P. taeda L.) to evaluate common patterns of response. Growth response to elevated CO[sub2] ranged from zero to more than 1000%, depending largely upon N status. In both species, growth response to CO[sub2] was greater under moderate N deficiency than under extreme N deficiency or N sufficiency/excess. Elevated CO[sub2] generally caused lowered tissue N concentrations in many (but not all) cases, which in turn resulted in smaller increases in N uptake than in biomass. Growth response to N ranged from -50 (in ponderosa pine) to more than 1000%, depending upon the N status of the control medium. Growth response to N was enhanced by elevated CO[sub2] when N was in the extreme deficiency range but not when N was in the moderate deficiency range. In two separate studies, ponderosa pine responded negatively to high N inputs, and in each case this response was mitigated by elevated CO[sub2]. Collectively, these results show that (i) N deficiency is a continuum rather than a step function, (ii) responses to elevated CO[sub2] vary across this continuum of N deficiency, and (iii) elevated CO[sub2] greatly enhances growth response to N additions when N is initially in the extremely deficient range.
Abbreviations: ANOVA, analysis of variance; DRI, Desert Research Institute; L-I-DRI, Laboratory Study I at DRI; L-II-DRI, Laboratory Study II at DRI; L-I-DUKE, Laboratory Study I at Duke; L-II-DUKE, Laboratory Study II at Duke; L-II-DUKE-L, Laboratory Study II at Duke, loblolly pine results; L-II-DUKE-P, Laboratory Study II at Duke, ponderosa pine results; F-DRI, Field Study at Placerville, CA; F-DUKE, Field Study at Duke; PAR, photosynthetically active respiration.

FOREST ECOSYSTEMS throughout the world are experiencing significant increases in both atmospheric CO[sub2] concentrations and N deposition (Strain and Thomas, 1992; Kauppi et al., 1992; Galloway et al., 1995). Either of these factors could cause increases in growth and C sequestration. Nitrogen is the most commonly limiting nutrient to forest growth in the northern hemisphere (Gessel et al., 1973; Aber et al., 1989; Johnson, 1992) and there is evidence that increases in N deposition have caused increased forest growth in Europe (e.g., Kauppi et al., 1992). On the other hand, excessive N deposition can result in Al toxicity and other nutrient deficiencies (Schulze, 1989).

Although some tree-ring studies have suggested a positive effect of increasing CO[sub2] (Graybill and Idso, 1993), there is as yet no direct evidence that elevated CO[sub2] has caused increases in forest growth under field conditions. Many studies have demonstrated that elevated CO[sub2] can cause increased growth of tree seedlings and saplings in greenhouse or open-top chamber studies, however. In many of these studies, elevated CO[sub2] caused reduced tissue N concentration (especially in foliage), allowing growth response under suboptimal N conditions (Brown, 1991; Campagna and Margolis, 1989; Norby et al., 1986a,b; Samuelson and Seiler, 1993). One explanation for the often-observed decreases in foliar N concentration with increased CO[sub2] is that plants may produce lower concentrations of the enzymes of the photosynthetic C reduction (PCR) cycle, particularly the carboxylating enzyme ribulose-1,5-bisphosphate (RuBP) carboxylase-oxygenase (Rubisco) (Tissue et al., 1993). If this response occurs under field conditions, the N deficiencies common to many forest ecosystems may not preclude a growth response to elevated CO[sub2].

As a part of the Forest Response to CO[sub2] Project, we have conducted a series of studies on the effects of CO [sub2] and N on ponderosa and loblolly pine seedlings (Griffin et al., 1993, 1995; Johnson et al., 1994a, 1995, 1996, 1997; Larigauderie et al., 1994; Thomas et al., 1994; Tissue et al., 1996, 1997; Walker et al., 1995). These studies have individually shown varying responses to both CO[sub2] and N, and the reader is referred to the original papers for details of growth, physiological, and nutrient responses. In this paper, we synthesize and normalize the growth and N uptake data from these various field and laboratory experiments to evaluate whether there are common patterns of response to CO[sub2] and N.

SITES AND METHODS
A brief summary of sites and methods is given below; the reader is referred to the original papers for details (Griffin et al., 1993, 1995; Johnson et al., 1994a, 1995, 1996, 1997; Larigauderie et al., 1994; Thomas et al., 1994; Tissue et al 1996, 1997; Walker et al., 1995).

FIELD STUDIES

PLACERVILLE FIELD SITE

An open-top chamber site for the ponderosa pine research is located at the Institute of Forest Genetics in Placerville, CA. The soil is an Aiken clay loam (Clayey, oxidic, mesic Xeric Haplohumult) derived from andesite. In the spring of 1991, hexagonal open-top chambers (3.6 m in diameter) were established. Ponderosa pine seeds were planted (21 per chamber), and treatments were initiated. The treatments consisted of three levels of N (0, 10, and 20 g m[sup-***2***] yr [sup-1] of N as NH[sub4]SO[sub4], applied in early spring) and three levels of CO[sub2] (ambient, 525, and 700 µL L[sup-1]) in triplicate. Due to funding restrictions, the 10 g m[sup-***2***] yr[sup-1] N, 525 µL L[sup-1] CO[sub2] combination was omitted. The seedlings were kept well watered with an automatic irrigation system throughout the experiment.

Harvests took place in October 1991, 1992, and 1993. Aboveground portions of three trees from each chamber were harvested each year. In order to prevent excessive plot disturbance, root systems from only one tree per chamber were harvested in 1992 and 1993. Biomass in the chambers before each harvest was estimated from allometric equations based upon diameter and height before harvest (Johnson et al., 1997). Soils were sampled by horizon (Ap, 0-18 cm and Bw, 18-30 cm) prior to treatment and in Year 3 (Johnson et al., 1997). Plant tissues and soils were analyzed for total C and N with a Perkin-Elmer 2400 CHN Analyzer at the University of Nevada, Reno.

DUKE FIELD SITE

Loblolly pine seeds were grown in the Duke University Phytotron glasshouses for 1 mo at three CO[sub2] levels (ambient, 500, and 650 µL L[sup-1]), inoculated with Pisolithus tinctorius (Pers.) Coker and Couch mycorrhizal fungus, and then transplanted into 3 m diam. by 3 m tall open top chambers in Duke Forest. There were three chambers for each CO[sub2] level and N treatment (0, 10, and 20 g N m[sup-***2***] yr[sup-1] as NH[sub4]SO[sub4]) plus two unchambered plots for each treatment combination. Because of high natural variability in soils at the site, the native soil in each chamber was excavated to a 1-m depth and replaced with a 1/1/1 mix of native clay soil, topsoil, and sand. The CO[sub2] treatments were applied 24 h per day for the entire experimental period. Seedlings were grown under ambient conditions of precipitation, light, and temperature.

LABORATORY STUDIES

DESERT RESEARCH INSTITUTE

In Laboratory Study no. I (Johnson et al., 1994a), Ponderosa pine was grown from half-sib seed (Eldorado County, CA source) in 1.1 L Rootrainer pots (Spencer-Lemaire Industries, Ltd.) in an artificially mixed soil consisting of 20% soil from a hydrothermally altered site near the Desert Research Institute (DRI) (Zephan very gravely loam, a clayey-skeletal, montmorillonitic, mesic Xerollic Haplargid), 20% peat moss, and 60% sand. The treatments consisted of three levels of atmospheric CO[sub2] (350, 525, and 700 µL L[sup-1]) and three levels of N (0, 100, and 200 mg N kg soil[sup-1]) applied by mixing the soil with appropriate amounts of (NH[sub4])[sub2]SO[sub4] at potting. The N treatments were reapplied at 26 wk as a top dressing. There were nine replicates per treatment, allowing three harvests of three replicates each at 18, 26, and 58 wk. In Laboratory Study no. II (Johnson et al., 1995), the same seed source was used to grow seedlings in ***2***.7 L pots filled with Ap horizon soil from the Placerville Field Site. The same CO[sub2] and N treatments were imposed. There were six replicates per treatment, allowing two harvests of three trees for each treatment combination at 16 and 46 wk. Plant tissues and soils were analyzed for total C and N with a Perkin-Elmer 2400 CHN Analyzer at the University of Nevada, Reno.

In both studies, the trees were grown in environment chambers (ecopods) at DRI where CO[sub2], temperature, and humidity were controlled. During these experiments, the ecopods were artificially lit with multivapor lamps to 600 µmol PAR m[sup-***2***] s[sup-1] for a 16-h photoperiod and subjected to atmospheric CO[sub2] levels of 370, 525, and 700 µL L[sup-1] and air temperature of 25°C.

DUKE UNIVERSITY

Loblolly pine seeds used in both experiments were from a single, wild-type tree growing in the North Carolina piedmont (North Carolina Forestry Commission, Lot no. LB-NC-P-84-***27***). Ponderosa pine seeds were from a half-sib family of trees collected at a plantation at 915 m elevation in Eldorado County California (California Department of Forestry, Lot no. CDF 526). All seeds were germinated and grown in the Duke University Phytotron. In Laboratory Study no. I (Larigauderie et al., 1994), the CO[sub2] levels were maintained at 375 and 710 µL L[sup-1] under natural light conditions in the Phytotron. Temperature in the greenhouse was 26°C during the day and 20°C at night. Seeds were germinated in styrofoam cups and transplanted into 24 L, 30 cm deep pots containing a mixture of

Turface, vermiculite, gravel, and soil (4:*2*:*2*:1) and one tablespoon of Pisolithus tinctorius inoculum, an ectomycorrhizal fungus. Plants were randomly placed in the greenhouse and watered three times weekly (Monday, Wednesday, and Friday) with a modified Hoagland's solution containing either 1.75 (low N) or 5.5 (high N) mM NH[sub4]NO[sub3]. Plants were harvested at 51, 86, 124, 150, and 172 d. Only the 172 d harvest was used in this comparison. Dried needles, stems, and roots were weighed and analyzed for N by Kjeldahl digestion.
In Laboratory Study no. II (Griffin et al., 1995), seeds of either species were planted in 3L PVC tubes filled with sterilized, acid-washed river sand and covered with 1 cm of similarly prepared gravel to reduce evaporative water loss. Seedlings were subjected to atmospheric CO[sub2] partial pressures of 375 and 710 µL L[sup-1]. Plants were randomly placed in the greenhouse and subjected to N treatments in a modified Hoagland's solution containing either 1.00 (low N) or 3.5 mM (high) NH[sub4]NO[sub3] applied daily (Griffin et al., 1995). Seven to 10 seedlings per treatment were harvested 166 d after planting. All seedlings were placed in a 10°C darkened chamber to minimize growth during the 3-d harvest. Plants were separated into roots, stems, primary needles, and fascicle needles and dried to a constant mass at 60°C. Leaf C and N concentrations were measured with an elemental analyzer (Model 2400 series II CHNS analyzer, Perkin Elmer Corp., Norwalk, CT).

STATISTICAL ANALYSES

Statistical analyses of biomass and N content data consisted of analysis of variance (for N and CO[sub2] effects) all laboratory studies and the Placerville field study (Johnson et al., 1994a, 1995, 1997; Griffin et al 1995). For the Duke field study statistical analyses consisted of student's t-test for CO[sub2] effects only. Means and standard errors are shown in all tables.

RESULTS

EFFECTS OF CARBON DIOXIDE AND NITROGEN ON BIOMASS AND NITROGEN UPTAKE

Biomass, N uptake, and biomass per unit N uptake for the various studies are in Tables 1 and ***2*** and the statistical analyses of these data are summarized in Tables 3 and 4. The reader is referred to the original papers for details of responses to treatments in each study; only a brief summary will be given here.
In Laboratory Study I at DRI (L-I-DRI), where the very low-N artificial soil was used, there were significant responses (ANOVA) in growth ($P < 0.01$), N uptake ($P < 0.05$), and biomass production per unit N (biomass/N) ($P < 0.01$) to N fertilization under both CO[sub2] treatments (Tables 1 and ***2***) (Johnson et al., 1994a). Biomass/N decreased by 60% with N fertilization in the ambient CO[sub2] treatment and by 24% in the elevated CO[sub2] treatment. There was no significant growth responses to CO[sub2] in the unfertilized treatment (student's t-test) but a 75% response to CO[sub2] in the fertilized treatment. The growth response to CO[sub2] in the fertilized treatment was accompanied by a doubling of biomass/N (due to the lower tissue N concentrations) (Johnson et al., 1994a).
In Laboratory Study II at DRI (L-II-DRI), where soil from the Placerville field studies was used, there were no overall significant effects of N fertilization by ANOVA, but there was was a significant ($P < 0.10$) negative (toxic) growth response (-38%) to N fertilization at ambient CO[sub2] (Tables 1 and ***2***). At elevated CO[sub2], however, this toxic response to N was mitigated: there was no significant response to N fertilization. There was no significant effect of elevated CO[sub2] on growth, N uptake, or biomass/N under either fertilizer treatment, but there was a nonsignificant trend toward greater biomass/N with elevated CO[sub2].
In the Placerville field study (F-DRI), there were significant, positive responses in growth and N uptake to both N fertilization and CO[sub2] in the Year 3 harvest (Tables 1 and ***2***). However, there were no treatment effects on biomass/N, and thus N uptake varied approximately in proportion to growth response (Johnson et al., 1997).
In Laboratory Study I at Duke (L-I-DUKE), there were significant overall growth responses to both CO[sub2] and N (Tables 3 and 4). The response to CO[sub2] was significant (student's t-test, $P < 0.05$) only in the High N treatment, however (Lauriguardie et al., 1994; Thomas et al., 1994). Biomass/N was significantly greater with elevated CO[sub2] in both Low and High N treatments and significantly lower with elevated N for both CO[sub2] treatments. As a result of lowered tissue N concentrations with elevated CO[sub2], there was significantly less (student's t-test, $P < 0.05$) N uptake with elevated CO[sub2] in the Low N treatment. In the High N treatment, there was no significant effect of CO[sub2] on N uptake despite the growth increase. Thus, the growth response to CO[sub2] in the High N treatment took place without any additional N uptake.
In Laboratory Study II at Duke (L-II-DUKE), there were substantial differences in how loblolly (L-II-DUKE-L) and ponderosa (L-II-DUKE-P) pine responded to N. For loblolly pine, there were significant positive responses of both growth and N uptake to N at both CO[sub2] levels (Tables 3 and 4) (Griffin et al., 1995). For ponderosa pine, there were significant, positive responses to CO[sub2] overall but no overall responses to N according to ANOVA analyses (Table 4). Within the ambient CO[sub2] treatments, however, there were significant effects of N treatment. At

ambient CO[sub2], there were a significant (student's t-test, P < 0.10) negative (toxic) responses of both growth and N uptake to N. As in the DRI study, however, this effect was mitigated at elevated CO[sub2]: there were significant (student's t-test, P < 0.10) positive responses of growth and N uptake to N in the elevated CO[sub2] treatment. Elevated CO[sub2] caused significantly greater overall (ANOVA analyses) biomass/N in both species, but this did not offset the growth increases and there was also a significant overall increase in N uptake with elevated CO[sub2] in both species (Tables 3 and 4).

In the Duke Field Study (F-DUKE), there was no response to N fertilization because of the high N availability in the topsoil used. Thus, data for both fertilized and unfertilized treatments were combined and placed in the High N category for this analysis. There was a nonsignificant (P = 0.15, student's t-test) increase in growth and a significant increase in biomass/N with elevated CO[sub2]. Because of lower tissue N concentrations at elevated CO[sub2], the growth increase with elevated CO[sub2] was accompanied by no increase in N uptake (Tables 3 and 4).

SYNTHESIS OF RESULTS

Figures 1 through 5 summarize normalized biomass and seedling N content responses as a percent of control. Figures 1, ***2***, and 5 (top) show the percent response to N within a given CO[sub2] treatment and Fig. 3, 4, and 5 (bottom) show percent response to CO[sub2] within a given N treatment. In Fig. 1 through 5, we have assigned a N status to each N treatment category based upon percent growth response to N additions. For example, the large growth response of ponderosa pine to N fertilization in L-I-DRI implies Extreme Deficiency in the absence of N fertilization. The more moderate but still positive growth response to N fertilization in F-DRI implies moderate deficiency in the absence of N fertilization, and the negative growth response to N in L-II-DRI implies that N was in the sufficiency/excess range in the absence of N fertilization (Fig. 1 and ***2***). With fertilization, N status in L-I-DRI and F-DRI is assumed to be in the sufficiency range, and N status in L-II-DRI is assumed to be in the toxicity range. Similarly, for loblolly pine, the large growth response to increased N in L-I-DUKE implies extreme deficiency; the more moderate response in L-II-DUKE-L implies moderate deficiency; and the lack of growth response in F-DUKE implies sufficiency or possibly excess (Fig. ***2***). With elevated N, N status is assumed to be in the sufficiency to excess (but not toxic) ranges in all cases.

For ponderosa pine, growth responses to N range from highly positive in L-I-DRI to negative in L-II-DRI and L-II-DUKE-P (Fig. 1). For loblolly pine, growth responses to N range from highly positive in L-I-DUKE to zero in F-DUKE (Fig. ***2***). In both species, elevated CO[sub2] greatly enhances growth responses to N within the Extreme N Deficiency range, but this response is accomplished with little or no additional N uptake (i.e., CO[sub2] has no effect on N uptake) (Fig. 1 and ***2***).

For both species, growth response to CO[sub2] is minimal to zero at both ends of the N status scale (extreme deficiency and sufficiency--Toxicity) and greatest with moderate deficiency (Fig. 3 and 4). This pattern is not an artifact of experimental conditions: Moderate deficiency for ponderosa pine occurred in F-DRI and for loblolly pine it occurred in L-II-DUKE-L. With N fertilization, N status is elevated to sufficiency--toxicity, and growth responses to CO[sub2] increase substantially over those in the unfertilized condition. In all cases, the increases in N content with elevated CO[sub2] are less than those in biomass, implying again that growth responses to elevated CO[sub2] are facilitated by lower N uptake per unit biomass. As noted above, this is especially true for fertilized treatments growing in the medium that produces extreme deficiency without fertilization. In the case of ponderosa pine, the mitigation of N toxicity by elevated CO[sub2] is readily apparent by comparing biomass in the fertilized and unfertilized treatments in the excess/toxicity range.

L-II-DUKE affords the opportunity to compare the two species under identical growing conditions. As in the individual studies summarized in Fig. 1 through 4, it appears that growth response to N is greater with elevated CO [sub2] in both species (Fig. 5, top). In the case of loblolly pine, growth response to N increases by nearly a factor of two (from [approximately equal to]50 to [approximately equal to]90%) at elevated compared to ambient CO[sub2]. In this study, however, CO[sub2] also causes approximately proportional increases in N uptake. For ponderosa pine, elevated CO[sub2] reverses the toxic effects of increased N, just as in L-II-DRI. In this case, elevated CO[sub2] has a disproportionately large effect upon N uptake (Fig. 5, top).

In contrast to the individual laboratory studies at DRI and Duke, there were substantial biomass responses to elevated CO[sub2] in the low N treatment for both species in L-II-DUKE, and these responses occurred with considerably less than proportional N uptake. Responses to elevated CO[sub2] were greater in the High N treatment, especially in the case of ponderosa pine where N toxicity was overcome by elevated CO[sub2]. Seedling N uptake increased with N fertilization such that the normal suppression of N uptake with elevated CO[sub2] was less in the high N treatments than in the low N treatments.

DISCUSSION

Both extreme deficiency and sufficiency/excess N status either greatly reduced or precluded growth response to CO [sub2] in both species. Two separate laboratory studies showed that the effects of N toxicity in ponderosa pine can be either eliminated (L-II-DRI) or reversed (L-II-DUKE-P) by elevated CO[sub2]. The mechanisms behind this mitigation of N toxicity deserve further investigation in light of increasing concern over N saturation of forest ecosystems (Aber et al., 1989; Johnson, 1992) including ponderosa pine ecosystems (Fenn et al., 1996).

Whereas extreme N deficiency precluded growth response to elevated CO[sub2], moderate N deficiency (such as occurred in F-DRI and in L-II-DUKE-L) did not. Thus, as noted previously (Johnson et al., 1994a, 1997), N deficiency should be thought of as a continuum rather than as a step function. Interestingly, it appears that the maximum potential for response to elevated CO[sub2] on a percentage basis occurs with moderate N deficiency and seems to lessen as N status approaches the excess range for both species. We did not conduct a full enough range of N treatments to assess whether any of the N treatments realized optimal N status; future studies should address the question of response to CO[sub2] under optimal as well as moderately deficient N conditions.

Elevated CO[sub2] increased biomass production per unit N uptake (reduced tissue N concentrations) in only one out of six cases for ponderosa pine (L-I-DRI, fertilized) but in four out of six cases for loblolly pine. The similarities in the numbers for biomass production per unit of N uptake in the L-II-DUKE study suggest that differences in growing conditions rather than species account for the differences in the other studies. It is also noteworthy that biomass production per unit N uptake was greaterwith elevated CO[sub2] during the first ***2*** yr of the Placerville field study (Johnson et al., 1997).

As noted by Coleman et al. (1993), some of these reductions may be size-dependent rather than a function of CO [sub2] treatment per se. As shown in Fig. 6, there was an increase in the proportion of woody tissues with elevated CO[sub2] in the field studies, a natural consequence of increased biomass. Thus, some of the increased biomass yield per unit of N uptake with elevated CO[sub2] is due to increasing proportions of woody tissues with low N concentrations.

Changes in woody tissue N concentration with elevated CO[sub2] can be of considerable significance in mature forests. Woody tissues contained only 30 to 60% of total tree C and 10 to 40% of total tree N in the studies described here, whereas wood contains more than 80% of tree C and more than 60% of total tree N in most mature forests. This is illustrated in the far right hand bar on Fig. 6 that shows the average organic matter distribution in trees from ponderosa pine and loblolly pine in Arizona and North Carolina (Klemmedson, 1975; Johnson and Lindberg, 1991). Foliage contains a disproportionate amount of tree N (ranging from ***27***-35%) compared to its biomass (6%) in these stands (Fig. 6). Wood accounts for the greatest proportion of tree biomass in both cases (74-76%); and, because of this, wood also accounts for the largest proportion of tree N (46-48%) despite its low N concentration. Foliage and fine root biomass reach an approximate steady-state after crown closure, and only woody plant biomass continues to accumulate. The N increment in this woody tissue must be balanced by inputs from either the soil or the atmosphere, whereas the annual uptake of N for building new foliage and fine roots can be balanced by cycling (decomposition of litterfall, root turnover, and internal translocation). Elevated CO[sub2] had no consistent effect upon woody tissue N concentration in the studies summarized here (Griffin et al., 1995; Johnson et al., 1994a, 1995, 1997; Larigauderie et al., 1994; Thomas et al., 1994); but given the importance of woody tissues in mature forests, the effects of CO[sub2] on woody N increment merit much more study.

In some cases (i.e., the fertilized seedlings growing in the medium that produced extreme deficiency without fertilization), growth response to elevated CO[sub2] was achieved without any additional N uptake. In other cases, especially for ponderosa pine, the decreases in tissue N concentration were insufficient to offset the increases in biomass and thus N uptake increased with elevated CO[sub2]. The latter results raise the question as to how this additional N can be obtained. Elevated CO[sub2] might facilitate greater N uptake by increasing soil N availability (e.g., Körner and Arnone, 1992; Zak et al., 1993), increasing root and mycorrhizal growth (Rogers and Runion, 1994), or perhaps by increasing free-living N fixation in the rhizosphere (see Bormann et al., 1993). On the other hand, some studies have shown that elevated CO[sub2] can cause reduced soil N availability (Diaz et al., 1993). The field studies at Placerville (F-DRI) revealed negative effects of elevated CO[sub2] on N availability in both the bulk soil and the rhizosphere (Johnson et al., 1996). Thus, it is clear that the additional N uptake caused by elevated CO [sub2] in that case was due either to increased root growth (which was noted to occur; Johnson et al., 1994b) and soil exploration or increased free-living N fixation. In mature forests where roots have been exploring soils for many decades, however, increased soil exploration may not be as efficient as in the seedling and sapling stages prior to canopy and root "closure" (e.g., Vogt et al., 1983).

There are many differences in the nature of nutrient cycling between seedlings and mature, closed canopy forests that leave serious questions as to how far studies of elevated CO[sub2] on seedlings or saplings can be carried (Johnson and Ball, 1996). Mature forests recycle >80% of the N they take up annually, primarily through the litterfall--root turnover--decomposition pathway (Cole and Rapp, 1981). Early suggestions of lowered litter quality because of lower

foliar N under elevated CO[sub2] (Strain, 1985) have been supported in some cases (Couteaux et al., 1991; Cotrufo et al., 1994) but not in others (O'Neill, 1994; Randlett et al., 1996).
Some perspective as to the relative importance of soil vs. vegetation C and N pools can be gained by comparing the Placerville data with that typical of mature stands. The loblolly pine and ponderosa pine seedlings contained < 15% of the total ecosystem C capital and < 5% of total ecosystem N capital (Fig. 7). In more mature stands, vegetation can comprise a substantially larger proportion of ecosystem C capital. Vegetation in the ponderosa and loblolly pine stands in Arizona and North Carolina contained approximately half of total ecosystem C capital but < 10% of ecosystem N capital (Fig. 7, right hand bars). The continuing importance of soil as a major N pool in mature forests is due to its lower C/N ratio (approximately 20) as compared to the vegetation (approximately 230) (Shaver et al., 1992; Johnson, 1994). Thus, while soils are certainly important components of ecosystem C capital in all cases, the relative importance of tree growth response (especially growth in wood volume) increases substantially as forests mature. In the case of N, soils are clearly the dominant pool in all successional steps.

SUMMARY AND CONCLUSIONS

Synthesis of a series of studies on the effects of N and CO[sub2] on ponderosa and loblolly pine showed the following:
1. Nitrogen constraints on growth responses to elevated CO[sub2] operated over a continuum. On a percentage basis, growth responses to elevated CO[sub2] were greatest under moderate N deficiency and least under extreme N deficiency and N excess/toxicity.
2. Elevated CO[sub2] enhanced growth responses to N fertilization when N status was initially in the extreme deficiency range but had little effect when N was in the moderate deficiency range.
3. Growth response to CO[sub2] was often accomplished with greater biomass production per unit N uptake, especially in loblolly pine. In most instances, however, this increase in N "use efficiency" was offset by growth such that elevated CO[sub2] caused increased N uptake as well as increased biomass.
4. In general, ponderosa pine appears to have less flexibility in dealing with varying N supply than loblolly pine. Ponderosa pine experienced a negative (toxic) response to high N in two separate studies; and in each case, elevated CO[sub2] mitigated this toxicity.
Collectively, these results show that the N constraints on growth responses to elevated CO[sub2] (i) are not always completely alleviated by greater growth per unit N uptake (lower tissue N concentrations) and (ii) vary continuously with N status. Nitrogen deficiency is a continuum rather than a step function, and responses to elevated CO[sub2] vary across this continuum. Whereas extreme N deficiency greatly reduced or precluded growth response to elevated CO[sub2], moderate N deficiency did not. In that most ecosystems would fall between extreme N deficiency and excess/toxicity, these results suggest that short-term, seedling responses to CO[sub2] are possible even under N limitations. However, the longer-term responses to CO[sub2] to ecosystem-level feedbacks via the N cycle are unknown.
Added material

D.W. Johnson, Desert Res. Inst., P.O. Box 60220, Reno, NV 89506 and Environ, and Resource Sciences, Univ. of Nevada-Reno, Reno, NV 89512; R.B. Thomas, West Virginia Univ., Morgantown, WV 26506-6057; K.L. Griffin, Lamont-Doherty Earth, Columbia Univ., Palisades, NY 10964; D.T. Tissue, Dep. of Biological Sciences, Texas Tech Univ., Lubbock, TX 79409-3131; J.T. Ball, Desert Res. Inst., P.O. Box 60220, Reno, NV 89506; B.R. Strain, Dep. of Botany, Duke Univ., Durham, NC 27707; and R.F. Walker, Environ, and Resource Sciences, Univ. of Nevada-Reno, Reno, NV 89512. Received 23 Apr. 1997. * Corresponding author (dwj@dri.edu)

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Table 1. Biomass and N content of ponderosa pine seedlings treated with elevated CO[sub2] and N in laboratory and field studies at the Desert Research Institute. (Means and standard errors are given.)

Nitrogen(FN+)	Low		High	
CO[sub2](FN++)	Ambient	Elevated	Ambient	Elevated
	Laboratory study I (L-I-DRI)(FN$$)			
Biomass, g/tree	1.8 +/- 0.***2***	1.8 +/- 0.1	12.6 +/- 0.6	22.1 +/- ***2***.1
N content, mg/tree	6 +/- 1	5 +/- 0.4	103 +/- 8	83 +/- 3

Biomass/ N, kg/kg	307 +/- 61	363 +/- 49	125 +/- 14	268 +/- 33
	Laboratory study II (L-II-DRI)(FN##)			
Biomass, g/tree	7.0 +/- 1.5	8.0 +/- 0.7	3.6 +/- 1.*2*	5.9 +/- *2*.5
N content, mg/tree	83 +/- 11	79 +/- 9	52 +/- 18	63 +/- 22
Biomass/ N,	82 +/- 21	101 +/- 14	72 +/- 34	89 +/- 9
	Field study (F-DRI)(FN#)			
Biomass, g/tree	689 +/- 76	1244 +/- 191	1407 +/- 174	2363 +/- 348
N content, g/tree	4.3 +/- 0.3	7.0 +/- 0.9	8.4 +/- 0.6	13.5 +/- 1.3
Biomass/ N, kg/kg	160 +/- 19	178 +/- 34	168 +/- 23	175 +/- 31

FOOTNOTES

+ Low = unfertilized, High = 10 g m[sup-*2*] yr[sup-1].
++ Low = 350 µl L[sup-1], High = 700 µl L[sup-1].
$$ Data from Johnson et al. (1994a).
Data from Johnson et al. (1995).
Data from Johnson et al. (1997).

Table *2*. Analysis of variance tests of biomass, N content, and biomass/N for the DRI studies.

Factor	CO[sub2]	N	Iteraction
	Laboratory study I (L-I-DRI)(FN+)		
Biomass	(FN***)	(FN***)	(FN***)
N content	(FN***)	(FN**)	(FN*)
Biomass/N	(FN***)	(FN***)	(FN***)
	Laboratory study II (L-II-DRI)(FN++)		
Biomass	NS	NS	NS
N content	NS	NS	NS
Biomass/N	NS	NS	NS
	Field study (F-DRI)(FN$$)		
Biomass	(FN**)	(FN**)	NS
N content	(FN**)	(FN**)	NS
Biomass/N	NS	NS	NS

FOOTNOTES

* P < 0.1

**** P < 0.05**

*** P < 0.01, NS = nonsignificant.
+ Data from Johnson et al. (1994a).
++ Data from Johnson et al. (1995) Student's t-tests showed a negative effect of N on biomass and N content at low CO[sub2].
$$ Data from Johnson et al. (1997).

Table 3. Biomass and N content of loblolly and ponderosa pine seedlings treated with elevated CO[sub2] and N in various laboratory and field studies at Duke University. (Means and standard errors are given.)

Nitrogen(FN+)	Low		High	
CO[sub2](FN++)	Ambient	Elevated	Ambient	Elevated
	Laboratory study I (L-I-DUKE)(FN$$)			
Biomass, g/tree	7.9 +/- 0.*2*	9.1 +/- 0.5	36.9 +/- 1.5	58.7 +/- 1.6
N content, mg/tree	54 +/- *2*	45 +/- 1	381 +/- 14	354 +/- 21
Biomass/ N, kg/kg	146 +/- 5	202 +/- 13	97 +/- 4	166 +/- 7
	Laboratory study II (loblolly pine) (L-II-DUKE-L)(FN##)			
Biomass,				

g/tree	5.6 +/- 0.5	8.8 +/- 1.8	8.6 +/- 1.2	16.4 +/- 2.6
N content, mg/tree	92 +/- 8	116 +/- 11	194 +/- 18	317 +/- 42
Biomass/ N, g/g	61 +/- 2	79 +/- 5	45 +/- 1	51 +/- 2
Greenhouse study II (ponderosa pine) (L-II-DUKE-P)				
Biomass, g/tree	6.6 +/- 0.5	9.9 +/- 0.9	3.3 +/- 1.3	14.2 +/- 2.4
N content, mg/tree	112 +/- 10	122 +/- 10	76 +/- 21	316 +/- 97
Biomass/ N, kg/kg	61 +/- 2	83 +/- 2	45 +/- 1	44 +/- 2
Field study (F-DUKE)(FN#)				
Biomass, g/tree	NA	NA	3151 +/- 447	4007 +/- 629
N content, g/tree	NA	NA	29 +/- 3	30 +/- 7
Biomass/ N, kg/kg	NA	NA	106 +/- 4	140 +/- 9

FOOTNOTES

+ For Laboratory Study I: Low = 1.75 and High = 5.5 mM as NH[sub4]NO[sub3]. For Laboratory study II: Low = 1.00 and High = 3.5 mM as NH[sub4]NO[sub3].

++ For Laboratory studies: Low = 375 µl L[sup-1], High = 715 µl L[sup-1]. For the field study: Low = ambient, High = +300 µl L[sup-1].

$$ Data from Lauriguardie et al. (1994) and Thomas et al. (1994).

Data from Griffin et al. (1995).

Data from Tissue et al. (1996, 1997).

Table 4. Analysis of variance tests of biomass, N content, and biomass/N for the Duke studies.

Factor	CO[sub2]	N	Interaction
Laboratory study I (L-I-DUKE)(FN+)			
Biomass	(FN***)	(FN***)	(FN***)
N content	NS	(FN***)	NS
Biomass/N	(FN***)	(FN***)	NS
Laboratory study II (loblolly pine) (L-II-DUKE-L)(FN++)			
Biomass	(FN***)	(FN***)	NS
N content	(FN**)	(FN***)	(FN**)
Biomass/N	(FN***)	(FN***)	(FN*)
Greenhouse study II (ponderosa pine) (L-II-DUKE-P)			
Biomass	(FN***)	NS	(FN*)
N content	(FN***)	(FN**)	(FN**)
Biomass/N	(FN***)	(FN***)	(FN***)
Filed study (F-DUKE)(FN$$)			
Biomass	NS	NS	NS
N content	NS	NS	NS
Biomass/N	(FN***)	NS	NS

FOOTNOTES

* P < 0.1

**** P < 0.05**

*** P < 0.01, NS = nonsignificant.

+ Data from Lauriguardie et al. (1994) and Thomas et al. (1994).

++ Data from Griffin et al. (1995). Student's t-tests showed a negative effect of N on biomass and N content at ambient CO[sub2] and a positive effect of N on biomass and N content at elevated CO[sub2] in ponderosa pine.

$$ Data from Tissue et al. (1996, 1997). Significance denotes results of student's t-test.

Fig. 1. Ponderosa pine response to N in the Desert Research Institute studies. Response is calculated as percent increase over unfertilized treatments for each CO[sub2] level. L-I-DRI = Laboratory Study I at DRI, L-II-DRI = Laboratory Study II at DRI, F-DRI = Field Study at Placerville, CA. (Data adapted from Johnson et al., 1994a, 1995, 1997.)

Fig. ***2***. Loblolly pine response to N in the Duke studies. Response is calculated as percent increase over unfertilized treatments for each CO[sub2] level. L-I-DUKE = Laboratory Study I at Duke, L-II-DUKE = Laboratory Study II at Duke, F-DUKE = Field Study at Duke (Data adapted from Laurguardie et al., 1994; Thomas et al., 1994; and Tissue et al., 1996, 1997).

Fig. 3. Ponderosa pine response to CO[sub2] in the Desert Research Institute studies. Response is calculated as percent increase over unfertilized treatments for each N treatment level. See Fig. 1 for legend. (Data adapted from Johnson et al., 1994a, 1995, 1997.)

Fig. 4. Loblolly pine response to CO[sub2] in the Duke studies. Response is calculated as percent increase over unfertilized treatments for each N treatment level. See Fig. ***2*** for legend. (Data adapted from Laurguardie et al., 1994; Thomas et al., 1994; and Tissue et al., 1996, 1997.)

Fig. 5. Loblolly and ponderosa pine response to CO[sub2] and N in the Duke common garden studies. Response is calculated as percent increase over controls for each CO[sub2] and N treatment level. (Data adapted from Griffin et al., 1995.)

Fig. 6. Distribution of C (top) and N (bottom) in loblolly pine after two growing seasons and ponderosa pine after three growing seasons in the field and values for the mature loblolly pine site at Duke (LOB) (Johnson and Lindberg, 1991) and the ponderosa pine site in Arizona (PON) (Klemmedson, 1975).

Fig. 7. Distribution of C (top) and N (bottom) in vegetation and soils at the Duke site after two growing seasons and the Placerville site after three growing seasons values for the mature loblolly pine site at Duke (LOB) (Johnson and Lindberg, 1991) and the ponderosa pine site in Arizona (PON) (Klemmedson, 1975).

REFERENCES

Aber, J.D., K.J. Nadelhoffer, P. Streudler, and J.M. Melillo. 1989. Nitrogen saturation in northern forest ecosystems. Bioscience 39:378-386.

Bormann, B.T., F.H. Bormann, R.S. Pierce, S.P. Hamberg, D. Wang, M.C. Snyder, and R.C. Ingersoll. 1993. Rapid N [sub2] fixation in pines, alder, and locust: Evidence from the sandbox ecosystem study. Ecology 74:583-598.

Brown, K.R. 1991. Carbon dioxide enrichment accelerates the decline in nutrient status and relative growth rate of Populus tremuloides Michx. seedlings. Tree Physiol. 8:161-173.

Campagna, M.A., and H.A. Margolis. 1989. Influence of short-term atmospheric CO[sub2] enrichment on growth, allocation patterns, and biochemistry of black spruce seedlings at different stages of development. Can. J. For. Res. 19:773-782.

Cole, D.W., and M. Rapp. 1981. Elemental cycling in forest ecosystems. p. 341-409. In D.E. Reichle (ed.) Dynamic properties of forest ecosystems. Cambridge University Press, London.

Coleman, J.S., K.D.M. McConnaughay, and F.A. Bazzaz. 1993. Elevated CO[sub2] and plant nitrogen use: Is reduced tissue nitrogen concentration size-dependent? Oecologia 93:195-200.

Couteaux, M.-M., M. Mousseau, M.-L. Celkerier, and P. Bottner. 1991. Increased atmospheric CO[sub2] and litter quality decomposition of sweet chestnut litter with animal food webs of different complexities. Oikos 61:54-64.

Cotrufo, M.F., P. Ineson, and A.P. Rowland. 1994. Decomposition of tree leaf litters grown under elevated CO[sub2]: Effect of litter quality. Plant Soil 163:121-130.

Diaz, S., J.P. Grime, J. Harris, and E. McPherson. 1993. Evidence of feedback mechanism limiting plant response to elevated carbon dioxide. Nature (London) 364:616-617.

Fenn, M.E., M.A. Poth, and D.W. Johnson. 1996. Evidence for nitrogen saturation in the San Bernardino Mountains in southern California. For. Ecol. Manage. 82:211-230.

Galloway, J.N., W.H. Schlesinger, H. Levy II, A. Michaels, and J.L. Schnoor. 1995. Nitrogen fixation: Anthropogenic enhancement-environmental response. Global Biogeochem. Cycles 9:235-252.

Gessel S.P., D.W. Cole, and E.C. Steinbrenner. 1973. Nitrogen balances in forest ecosystems of the Pacific Northwest. Soil Biol. Biochem. 5:19-34.

Graybill, D.A., and S.B. Idso. 1993. Detecting the aerial fertilization effect of atmospheric CO[sub2] enrichment from tree-ring chronologies. Global Biogeochem. Cycles 7:81-95.

Griffin, K.L., R.B. Thomas, and B.R. Strain. 1993. Effects of nitrogen supply and elevated carbon dioxide on construction cost in leaves of Pinus taeda (L.) seedlings. Oecologia 95:757-580.

Griffin, K.L., W.E. Winner, and B.R. Strain. 1995. Growth and dry matter partitioning in loblolly and ponderosa pine seedlings in response to carbon and nitrogen availability. New Phytol. 129:547-556.

Johnson, D.W. 1992. Nitrogen retention in forest soils. J. Environ. Qual. 21:1-12

Johnson, D.W. 1994. Role of carbon in the cycling of other nutrients in forest ecosystems. p. 299-328. In J.M. Kelly and W.M. McFee (ed.) Carbon: Forms and functions in forest soils. SSSA, Madison, WI.

Johnson, D.W., and J.T. Ball. 1996. Interactions between CO[sub2] and nitrogen in forests: Can we extrapolate from

the seedling to the stand level? p. 283-316. In G.W. Koch and H.A. Mooney (ed.) Carbon dioxide and terrestrial ecosystems. Academic Press, San Diego.
Johnson, D.W., J.T. Ball, and R.F. Walker. 1994a. Effects of CO[sub2] and nitrogen on nutrient uptake in ponderosa pine seedlings. Plant Soil 168:144-153.
Johnson, D.W., J.T. Ball, and R.F. Walker, 1997. Effects of CO[sub2] and nitrogen fertilization on vegetation and soil nutrient content in juvenile ponderosa pine. Plant Soil 190:20-40.
Johnson, D.W., D.R. Geisinger, R.F. Walker, J. Vose, K. Elliot, and J.T. Ball. 1994b. Soil pCO[sub2], soil respiration, and root activity in CO[sub2]-fumigated ponderosa pine. Plant Soil 165:111-121.
Johnson, D.W., P.L. Henderson, J.T. Ball, and R.F. Walker. 1996. Effects of CO[sub2] and N on growth and N dynamics in ponderosa pine: Results from the first two growing seasons. p. 23-40. In G.W. Koch and H.A. Mooney (ed.) Carbon dioxide and terrestrial ecosystems. Academic Press, San Diego.
Johnson, D.W., and S.E. Lindberg (ed.). 1991. Atmospheric deposition and forest nutient cycling: A synthesis of the integrated forest study. Ecol. Ser. 91. Springer-Verlag, New York.
Johnson, D.W., R.F. Walker, and J.T. Ball. 1995. Lessons from lysimeters: Soil N release from disturbance compromises controlled environment study. Ecol. Appl. 5:395-400.
Kauppi, P.E., K. Mielikäinen, and K. Kuusela. 1992. Biomass and carbon budget of European forests, 1971 to 1990. Science (Washington, DC) 256:70-74.
Klemmedson, J.O. 1975. Nitrogen and carbon regimes in an ecosystem of young dense ponderosa pine in Arizona. For. Sci. 21:163-168.
Körner, C., and J.A. Arnone. 1992. Responses to elevated carbon dioxide in artificial tropical ecosystems. Science (Washington, DC) 257:1672-1675.
Larigauderie, A, J.F. Reynolds, and B.R. Strain. 1994. Root response to CO[sub2] enrichment and nitrogen supply in pine. Plant Soil 165:21-32.
Norby, R.J., E.G O'Neill, and R.J. Luxmoore. 1986a. Effects of atmospheric CO[sub2] enrichment on the growth and mineral nutrition of Quercus alba seedlings in nutrient-poor soil. Plant Physiol. 82:83-89.
Norby, R.J., J. Pastor, and J.M. Melillo. 1986b. Carbon-nitrogen interactions in CO[sub2]-enriched white oak, physiological and long-term perspectives. Tree Physiol. ***2***:233-241.
O'Neill, E.G. 1994. Responses of soil biota to elevated atmospheric carbon dioxide. Plant Soil 165:55-65.
Randlett, D.L., D.R. Zak, K.S. Pregitzer, and P.S. Curtis. 1996. Elevated atmospheric carbon dioxide and leaf litter chemistry: Influences on microbial respiration and net nitrogen mineralization. Soil Sci. Soc. Am. J. 60:1571-1577.
Rogers, H.H., and G.B. Runion. 1994. Plant response to atmospheric CO[sub2] enrichment with emphasis on roots and the rhizosphere. Environ. Pollut. 83: 155-189.
Samuelson, L.J., and J.R. Seiler. 1993. Interactive role of elevated CO[sub2], nutrient limitations, and water stress in the growth responses of red spruce seedlings. Forest Sci. 39:348-358.
Schulze, E.-D. 1989. Air pollution and forest decline in a spruce (Picea abies) forest. Science (Washington, DC) 244:776-783.
Shaver, G.R., W.D. Billings, F.S. Chapin III, A.E. Giblin, K.J. Nadelhoffer, W.C. Oechel, and E.B. Rastetter. 1992. Global change and the caron balance of arctic ecosystems. Bioscience 42:433-441.
Strain, B.R. 1985. Physiological and ecological controls on carbon sequestering in terrestrial ecosystems. Biogeochemistry 1:219-232.
Strain, B.R., and R.B. Thomas. 1992. Field measurements of CO[sub2] enhancement and climate change in natural vegetation. Water Air Soil Pollut. 64:45-60.
Thomas, R.B., J.D. Lewis, and B.R. Strain. 1994. Effects of leaf nutrient status on photosynthetic capacity in loblolly pine (Pinus taeda L.) seedlings grown in elevated CO[sub2]. Tree Physiol. 14:947-960.
Tissue, D.T., R.B. Thomas, and B.R. Strain. 1993. Long-term effects of elevated CO[sub2] and nutrients on photosynthesis and rubisco in loblolly pine seedlings. Plant Cell Environ. 16:859-865.
Tissue, D.T., R.B. Thomas, and B.R. Strain. 1996. Growth and photosynthesis of loblolly pine (Pinus taeda L.) after exposure to elevated CO[sub2] for 19 months in the field. Tree Physiol. 16:49-59.
Tissue, D.T., R.B. Thomas, and B.R. Strain. 1997. Long-term atmospheric CO[sub2] enrichment increases growth and photosynthesis of loblolly pine (Pinus taeda L.): A four year experiment in the field. Plant Cell Environ. (in press).
Vogt, K.A., E.E. Moore, D.J. Vogt, M.J. Redlin, and R.L. Edmonds. 1983. Conifer fine root and mycorrhizal root biomass within the forest floors of Douglas-fir stands of different ages and site productivities. Can. J. For. Res. 13:429-437.
Walker R.F., D.R. Geisinger, D.W. Johnson, and J.T. Ball. 1995. Interactive effects of atmospheric CO[sub2] enrichment and soil N on growth and ectomycorrhizal colonization of ponderosa pine seedlings. For. Sci. 41:491-500.

Zak D.R., K.S. Pregitzer, P.S. Curtis, J.A. Teeri, R. Fogel, and D.L. Randlett. 1993. Elevated atmospheric CO[sub2] and feedback between carbon and nitrogen cycles. Plant Soil 151:105-117.