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Growth and nitrogen accretion of dinitrogen-fixing *Alnus glutinosa* (L.) Gaertn. under elevated carbon dioxide

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Abstract

Short-term studies of tree growth at elevated CO_2 suggest that forest productivity may increase as atmospheric CO_2 concentrations rise, although low soil N availability may limit the magnitude of this response. There have been few studies of growth and N_2 fixation by symbiotic N_2-fixing woody species under elevated CO_2 and the N inputs these plants could provide to forest ecosystems in the future. We investigated the effect of twice ambient CO_2 on growth, tissue N accretion, and N_2 fixation of nodulated *Alnus glutinosa* (L.) Gaertn. grown under low soil N conditions for 160 d. Root, nodule, stem, and leaf dry weight (DW) and N accretion increased significantly in response to elevated CO_2. Whole-plant biomass and N accretion increased 54% and 40%, respectively. Delta-^{15}N analysis of leaf tissue indicated that plants from both treatments derived similar proportions of their total N from symbiotic fixation suggesting that elevated CO_2 grown plants fixed approximately 40% more N than did ambient CO_2 grown plants. Leaves from both CO_2 treatments showed similar relative declines in leaf N content prior to autumnal leaf abscission, but total N in leaf litter increased 24% in elevated compared to ambient CO_2 grown plants. These results suggest that with rising atmospheric CO_2 N_2-fixing woody species will accumulate greater amounts of biomass N through N_2 fixation and may enhance soil N levels by increased litter N inputs.

Introduction

As atmospheric CO_2 levels rise, growth of many woody plant species is expected to increase (Bazzaz et al. 1990; Ceulemans & Mouseau 1994). Low soil N availability, however, may limit the magnitude of both the photosynthetic (Tissue et al. 1993; Curtis et al. 1995) and the overall growth response (Pregitzer et al. 1995; Wong et al. 1992) of trees to CO_2 enrichment. Negative acclimation of photosynthesis is commonly observed in trees grown at elevated CO_2 (Gunderson & Wullschleger 1994) and may be due to the decline in leaf N content that often occurs at elevated CO_2, especially under conditions of low soil N availability (Curtis et al. 1995; Norby et al. 1986a). Linked soil-plant process models also indicate that the stimulation of growth by elevated CO_2 in N limited forest ecosystems will be considerably less than what has been observed in short term studies unless nutrient supply increases to match carbon supply (Comins 1994, Comins & McMurtrie 1993; Kirschbaum et al. 1994; Pastor and Post 1988).

The close coupling between carbon assimilation and symbiotic N_2 fixation suggests that N_2 fixation should increase as atmospheric CO_2 concentrations rise (Sinclaire 1992; Stulen and den Hertog 1993). Indeed, increased N_2 fixation with CO_2 enrichment has been well documented in annual legume species (Phillips et al. 1976; Ryle et al. 1992). This could have important consequences for forested ecosystems if increased N_2 fixation in woody species leads to greater soil N availability and an amelioration of nutrient limitations to CO_2 responses among associated non-fixing species. Results from a number of studies suggest that N_2-fixing woody plants have the capacity to

increase both C assimilation and total N_2 fixation as atmospheric CO_2 levels rise. Arnone & Gordon (1990) and Thomas et al. (1991) reported greater whole plant N_2 fixation and total plant N accretion in several N_2-fixing woody species grown at elevated compared to ambient CO_2. Norby (1987) observed increased whole plant N_2 fixation as well as an increase in the proportion of nodule mass to whole-plant mass in high CO_2 grown *Alnus glutinosa*. Also working with *A. glutinosa*, Vogel & Curtis (1995) found increased leaf N content and no downregulation of photosynthesis in plants grown under elevated CO_2 and low N soil conditions.

In this study we examined the relationship between growth at elevated CO_2 and N accretion (g N organ^{-1}) in plant fractions of N_2-fixing *A. glutinosa* growing on a low N soil. Actinorhizal (*Frankia* nodulated) symbiotic N_2-fixing species can contribute significant amounts of fixed N_2 to temperate forest ecosystems (Dawson 1983), leading to increased growth of co-occurring tree species (Cote & Camire 1984; Dawson 1986). For example, actinorhizal *A. glutinosa* and *Elaeagnus umbellata* Thunb. increased total and/or available soil N concentrations in mixed plantings of *Juglans nigra* L. compared to control plots containing *J. nigra* only (Friedrich & Dawson 1984; Paschke et al. 1989). Increased N_2 fixation under elevated CO_2, resulting in greater N levels in above- and belowground tissues, could therefore further increase soil availability of N for use by associated plants. We hypothesized that CO_2 enriched *A. glutinosa* would show increased growth, especially in nodule biomass and leaf area, leading to increased N_2 fixation and a concomitant increase in whole-plant N accretion.

Materials and methods

In mid-May, 1993, ten open bottom root boxes ($0.6 \times 0.6 \times 0.6$ m) were placed into the soil at the University of Michigan Biological Station, in northern Lower Michigan, USA (45°34′ N latitude, 84°40′ W longitude). The lower half of each box was filled with the Rubicon sand (164 μg N g^{-1}) that underlay the site and the upper half was filled with a mixture of 20% locally derived Kalkaska series topsoil and 80% Rubicon sand (202 μg N g^{-1}). The soil mixture had a PO_4^{-3} concentration of 110 μg PO_4^{-3} g^{-1} as determined by an acid extraction procedure.

Five, three-month old *Alnus glutinosa* (L.) Gaertn. (black alder) seedlings were transplanted into each of the root boxes on Julian Day (JD) 149 (May 29, 1993). The plants were grown from seed in sterilized peat:pearlite:vermiculite potting medium under greenhouse conditions of ambient CO_2. At the time of transplanting, seedlings had an average leaf area (LA) and stem height of 126 cm^2 and 16.3 cm, respectively, and nodulation was not apparent. Three days after transplanting, seedlings were inoculated with a homogenized suspension of locally collected *Alnus rugosa* (DuRoi) Sprengel nodules (0.075 *M* KPO_4, pH 7.0).

Immediately after transplanting, small open top chambers ($0.7 \times 0.7 \times 1.0$ m tall, Curtis & Teeri, 1992) were placed over the plants in each of the ten root boxes and CO_2 partial pressure elevated in five of the chambers. The experiment was arranged in a randomized block design with two CO_2 treatments (elevated and ambient) and five blocks (replicates). Carbon dioxide dispensing procedure and monitoring of temperature and CO_2 inside the chambers was as described previously (Vogel & Curtis, 1995). Daytime CO_2 partial pressure in the ambient and elevated CO_2 treatments were 34.9 $\pm$ 0.02 Pa and 69.5 $\pm$ 0.36 Pa (mean $\pm$ SE), respectively. Daytime temperature averaged 2.6 °C higher inside than outside chambers and there was no significant temperature difference between ambient and elevated CO_2 treatments.

Stem height and LA of each plant was measured non-destructively at the time of planting and on 5 other dates during the growing season. Individual leaf areas (ILA) of leaves were calculated using leaf length (l, cm) and width (w, cm) and the equation ILA = 0.762(lw) – 2.52, r^2 = 0.99, n=46, derived from the destructive harvest of a separate set of plants. Stem elongation and leaf growth had ceased before JD 293, the final date stem height and LA measurements were taken.

Leaf litter was collected daily as leaves abscised during late October and early November. Complete above- and below-ground harvest took place on JD 308 (November 4). At the time of harvest, 20% (%DW) of the leaves had not naturally abscised and were removed from the stems, dried, weighed, and added to previously collected leaf litter for bulk leaf mass and total N analysis. The rooting volume was excavated by hand and sieved to remove excess soil and the root fraction was stored frozen. Frozen roots and root nodules were washed free of soil and hand-separated. All organ fractions were dried to constant weight at 65 °C immediately after harvesting or washing to determine dry weight. Daily, whole plant net assimilation rate (NAR) was calculated as: NAR = (g whole-plant DW)/(m^2 *LA* $*$ *D*) where *LA* = maximal leaf area (JD 293) and *D* = duration of the experiment in days (160 d).

Seasonal changes in leaf N content (g N m^{-2}) were measured by sampling leaf tissue from the youngest mature leaf (most recent fully expanded leaf, generally the 7th leaf from the apex) six times from mid-summer through leaf abscission. The 7th leaf from the apex continued to be sampled for leaf N after leaf initiation had ceased in autumn. Leaf tissue was frozen on dry ice immediately after sampling and subsequently freeze dried. Freeze dried leaf and oven dried bulk leaf, stem, root and nodule samples were ground to a powder and analyzed for total N and C concentration with an elemental analyzer (Carlo Erba, Milan, Italy).

Delta ^{15}N of dried bulk leaf tissue from the harvest were compared to that of the soil in which the plants were grown in order to assess the source of nitrogen accumulating in plant tissue. Natural abundance of ^{15}N for each sample was measured using a SIRA Series II stable isotope ratio mass spectrometer (VG ISOGAS, Middlewich, UK). Following combustion of the samples in an elemental analyzer (Carlo Erba NA 1500, Milan, Italy) the gaseous emissions were passed to the mass spectrometer using helium as the carrier (Thomas et al. 1991). Isotopic composition ($\delta^{15}N$) was expressed as units of abundance of ^{15}N per mil ($^{o}/_{oo}$), where
$\delta^{15}N = (R_{sample}/R_{standard} - 1) \times 1000$ and $R = {}^{15}N/{}^{14}N$ with atmospheric N_2 as the standard.

Statistical analyses were performed using analysis of variance (ANOVA) for randomized block design where the unit of replication was a single chamber. Seasonal LA and stem height and final harvest growth, tissue N, and $\delta^{15}N$ data were expressed on a per plant basis from pooled chamber data (5 plants $chamber^{-1}$). Seasonal leaf N data were collected by sampling an individual plant from each chamber on each date.

Results

Growth at elevated CO_2 resulted in significantly greater leaf area $plant^{-1}$ (LA) and greater stem height compared to growth at ambient CO_2 (Table 1). These differences were evident 52 d after CO_2 treatment had begun and were sustained throughout the growing season (Figure 1). Treatment effects on final LA were due to an increase in both the total number of leaves $plant^{-1}$ (LN) and the size of individual leaves in elevated compared to ambient grown plants (Table 1). Specific leaf area (SLA) was significantly reduced with CO_2enrichment (Table 1).

Table 1. Leaf area $plant^{-1}$ (LA), stem height, mean area $leaf^{-1}$ (MAL), number of leaves $plant^{-1}$ (LN), and specific leaf area (SLA) of *A. glutinosa* seedlings grown at elevated (70 Pa) and ambient (35 Pa) CO_2 on JD 293 (post bud-set). Specific leaf area measurements were from the 7th leaf from the apex. Mean ± (SE), n = 5 (5 individuals pooled within chambers). * = P ≤ 0.05, ** = P ≤ 0.01, *** = P ≤ 0.001

	Elevated	Ambient	Δ %
LA (cm^2)	2521 (58.5)	2140 (28.8)	+18 ***
Stem Ht (cm)	111.6 (5.28)	92.9 (2.96)	+20 *
MAL (cm^2)	97.8 (1.33)	87.8 (0.58)	+11 ***
LN	25.8 (0.29)	24.4 (0.21)	+ 6 **
SLA (m^2/kg)	13.8 (0.35)	16.6 (0.57)	–17 **

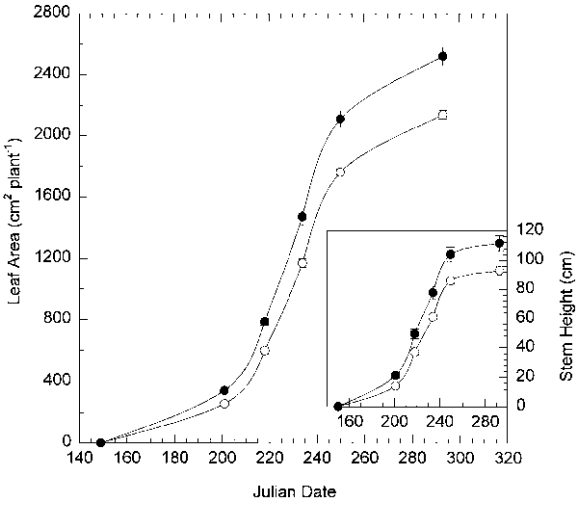

Figure 1. Leaf area $plant^{-1}$ and stem height $plant^{-1}$ of *A. glutinosa* seedlings grown at elevated CO_2 (70 Pa, ●) or ambient CO_2 (35 Pa, ○). Final measurements were taken after bud set when leaf area was maximal. Carbon dioxide treatments began on JD 149. Vertical bars represent one SE of mean (n = 5 with 5 individuals pooled within chambers).

Total above-ground biomass increased 50% under elevated compared to ambient CO_2, with stem and leaf biomass increases of 58% and 36%, respectively (Table 2). Below-ground growth was also stimulated, with significant CO_2 effects on both total root and nodule biomass. There was no difference between CO_2 treatments in fine root (<0.5 mm diameter) biomass, or in root:shoot ratio at the final harvest (Table 2). The relatively greater CO_2 effect on whole plant DW (+54%) compared to total LA (+18%) was reflected in

Table 2. Above and below ground biomass fractions, total biomass (g DW plant^{-1}), root: shoot ratio, and daily net assimilation rate (NAR) of *A. glutinosa* seedlings grown at elevated (70 Pa) and ambient (35 Pa) CO_2 for 160 d. Plants were harvested on November 4, 1993 after 80% of the leaves had naturally abscised. NAR based on maximal leaf area measured on JD 293. Mean $\pm$ (SE), $n = 5$ (5 individuals pooled within chambers), except for fine roots where $n = 3$. $^{**} = P \leq 0.01$, $^{***} = P \leq 0.001$, ns = not significant at $P \leq 0.05$.

	Elevated	Ambient	Δ %
Above ground DW (g)			
Leaf	15.9 (0.35)	11.7 (0.67)	+36 ***
Stem	35.7 (2.68)	22.6 (0.89)	+58 **
Total	51.6 (2.92)	34.3 (1.55)	+50 ***
Below ground DW (g)			
Fine Roots	6.1 (1.27)	4.8 (0.76)	+27 ns
All Roots	57.5 (1.82)	36.4 (1.11)	+58 ***
Nodule	1.6 (0.05)	0.9 (0.56)	+78 ***
Total	59.1 (1.82)	37.3 (1.16)	+58 ***
Whole plant DW (g)	110.7 (4.38)	71.7 (2.10)	+54 ***
Root: shoot	1.15 (0.05)	1.09 (0.04)	+ 5 ns
NAR (g m^{-2} d^{-1})	2.74 (0.07)	2.09 (0.04)	+31 ***

the significant increase in daily NAR with CO_2 enrichment (Table 2). The ratio of nodule DW to maximal LA (g nodule DW m^{-2} LA) also increased significantly (P = 0.01) in elevated (6.24 $\pm$ 0.26, mean $\pm$ SE) compared to ambient (4.32 $\pm$ 0.53) CO_2 grown plants.

Seasonal changes in leaf N content differed between plants in the two CO_2 treatments (Figure 2). Leaves from ambient grown plants had an essentially constant N content between JD 208 and 264 while those from elevated CO_2 plants showed a 50% increase during the same period. Apparent retranslocation of leaf N, calculated as the difference in N content of abscised leaves and leaves with maximal N content (Figure 2) was 30% higher at elevated compared to ambient CO_2, although this effect was only marginally significant (P = 0.15). The *relative* reduction in leaf N content, however, was not different between elevated (–42%) and ambient (–39%) CO_2 grown plants. While maximum leaf N content was significantly greater at high CO_2, there was no significant CO_2 effect on N content of abscised leaf number 7 or of the bulk leaf fraction (Figure 2).

Nitrogen accretion increased in both above- and belowground tissues with CO_2 enrichment, ranging from a 24% increase in leaves to a 74% increase in nodules (Table 3). Tissue N concentration (%DW) and C:N ratios were either not significantly changed (stem, roots and nodules) or changed only slightly (leaves). The relatively greater increase in whole-plant biomass (+58%) compared to N accretion (+40%) at elevated CO_2 resulted in a 9% reduction in whole-plant N concentration and a 10% increase in C:N ratio in elevated compared to ambient CO_2 grown plants (Table 3). Leaves from both CO_2 treatments were depleted in ^{15}N relative to the atmosphere resulting in negative $\delta^{15}N$ values (Table 3) while a pooled sample of the low N soil in which the plants were grown was enriched in ^{15}N ($\delta^{15}N_{soil}$ = 2.6 $^{o}/_{oo}$). While we can not determine the proportion of tissue N that was fixed by the plants to that attained from the soil, these data suggest that most of the N accumulated in the leaves of our plants could be attributed to N_2 fixation. In addition, the similar $\delta^{15}N$ values for ambient and elevated CO_2 grown black alder leaves suggest that similar proportions of N were fixed by plants from each CO_2 treatment.

Table 3. Leaf, stem, root, nodule, and whole plant N accretion, concentration, C:N ratio, and leaf δ^{15}N ($^{o}/_{oo}$) of *A. glutinosa* seedlings grown at elevated (70 Pa) and ambient (35 Pa) CO_2 for 160 d. Plants were harvested on November 4, 1993 after 80% of the leaves had naturally abscised. Mean $\pm$ (SE), $n = 5$ (5 individuals pooled within chambers). $^{*} = P \leq 0.05$, $^{**} = P \leq 0.01$, $^{***} = P \leq 0.001$, ns = not significant at $P \leq 0.05$.

	Elevated	Ambient	Δ %
N accretion (g organ^{-1})			
Leaf	0.301 (0.011)	0.243 (0.015)	+24 *
Stem	0.445 (0.017)	0.315 (0.017)	+41 ***
Root	0.721 (0.049)	0.491 (0.022)	+47 **
Nodule	0.033 (0.003)	0.019 (0.003)	+74 ***
Whole Plant	1.500 (0.069)	1.068 (0.047)	+40 ***
N concentration (% DW)			
Leaf	1.89 (0.042)	2.08 (0.051)	– 9 **
Stem	1.26 (0.067)	1.39 (0.029)	– 9 ns
Root	1.25 (0.051)	1.35 (0.030)	– 7 ns
Nodule	2.10 (0.145)	2.04 (0.100)	+ 3 ns
Whole Plant	1.35 (0.013)	1.49 (0.024)	– 9 ***
C:N ratio			
Leaf	25.9 (0.35)	24.0 (0.57)	+ 8 **
Stem	40.6 (1.93)	36.7 (0.75)	+11 ns
Root	38.2 (1.72)	35.0 (0.78)	+ 9 ns
Nodule	24.5 (1.54)	23.6 (0.71)	+ 4 ns
Whole Plant	36.0 (0.47)	32.8 (0.58)	+10 **
Leaf δ^{15}N ($^{o}/_{oo}$)	-1.6 (0.05)	-1.46 (0.07)	– 9 ns

Discussion

Increased growth and whole-plant N accretion in CO_2 enriched *A. glutinosa* grown in a low N soil was consistent with the positive CO_2 effects reported for other N_2-fixing woody species (Arnone & Gordon 1990; Norby 1987; Thomas et al. 1991). In our study, the significant increase in whole-plant LA, combined with a decline in SLA, lead to a large increase in leaf litter mass at elevated compared to ambient CO_2. This increase in litter mass, coupled with a small reduction in litter N concentration, resulted in a 24% increase in total N returned to the soil with CO_2 enrichment. The significant increase in leaf litter N accretion and only modest increase in litter C:N ratio (+8%) at high CO_2 suggests there will be little CO_2 effect on litter decomposition rates (Cotrufo and Ineson 1996; Couteaux et al. 1991), and supports the suggestion of Gifford (1992) that rising atmospheric CO_2 may lead to greater symbiotic N inputs to terrestrial ecosystems.

Ours is the first report of leaf N content in naturally abscised leaves of a high CO_2 grown, N_2-fixing tree and only limited data exist for litter N content in other woody species under elevated CO_2 conditions. Norby et al. (1986b) reported a 23% reduction in N concentration, and a 30% increase in C:N ratio, in elevated CO_2 grown *Quercus alba* L. leaf litter, while Couteaux et al. (1991) found a 48% decline in *Castanea sativa* Mill litter N concentration and a 85% increase in C:N ratio due to elevated CO_2. Diaz et al. (1993) found that on a productive soil foliar N concentrations of herbaceous species declined while soil microbial C and N increased in response to a doubling of ambient CO_2 concentration. The authors suggested that increased C assimilation at high CO_2 caused an increase in soil C, thus stimulating growth of microbial populations that

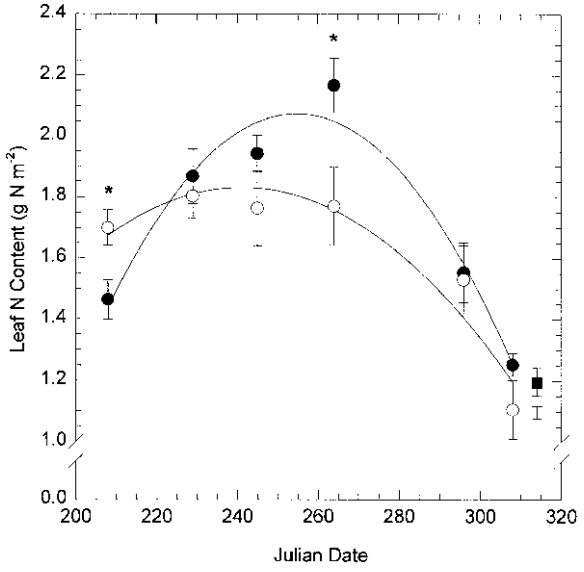

Figure 2. Seasonal leaf N content of the 7th leaf from the apex of *A. glutinosa* seedlings grown at elevated CO_2 (70 Pa, ●) or ambient CO_2 (35 Pa, ○) and whole canopy leaf N content after leaf abscission of plants grown at elevated (■) or ambient CO_2 (□). Final measurement of 7th leaf N content was of recently abscised leaves. All measurements were from leaves that had developed after CO_2 treatment had begun. Vertical bars represent one SE of mean (n = 4,5). * = $P \leq 0.05$.

consequently sequestered more soil N. Increased leaf litter C:N ratios at elevated CO_2 could also result in net immobilization of soil N. However, the contrast in the response of litter C:N ratio to elevated CO_2 observed for non-fixing species *vs*. that of *A. glutinosa* suggests that soil N availability in systems containing N_2 fixers would not decline, but rather would increase owing to the substantially greater litter N content of N_2 fixers at elevated CO_2.

To date, little work has been done on the retranslocation efficiency of N in leaves grown at elevated CO_2 and any effect that elevated CO_2 may have on this process could influence N return to forested ecosystems. Under ambient CO_2 conditions temperate woody deciduous plants typically resorb 40–80% of their leaf N prior to leaf abscission (Chapin & Kedrowski 1983). Dinitrogen-fixing species generally show somewhat lower net resorption, with 20–50% of leaf N being translocated to perennial tissues (Cote et al. 1989; Vogel & Dawson 1993). The apparent retranslocation of 39% to 42% of leaf N content we observed in *A. glutinosa* is within this range and suggests that elevated CO_2 will have little effect on the dynamics of autumnal leaf N retranslocation.

The negative leaf δ^{15}N in both ambient and elevated CO_2 grown plants from our study compared with the positive soil δ^{15}N indicated a significant proportion of leaf N was symbiotically fixed (Focht 1987; Shearer et al. 1983), as was expected given the low soil N status and the abundant root nodules we observed. There was, however, no CO_2 effect on the proportion of total N derived from N_2 fixation, as leaf δ^{15}N did not differ among treatments. Although we cannot determine the actual proportion of leaf N that was derived from symbiotic N_2 fixation, our data do rule out any greater or lesser reliance on nodule derived N at high CO_2.

An increase in whole-plant N content of 40% at high CO_2 combined with similar leaf δ^{15}N between CO_2 treatments suggests that high CO2 grown *A. glutinosa* had an approximately 40% greater whole-plant nitrogenase activity. Increased whole-plant nitrogenase activity has been found in other N_2-fixing woody plants grown under elevated CO_2 (Arnone & Gordon 1990; Norby 1987; Thomas et al. 1991). Greater whole-plant nitrogenase activity at elevated compared to ambient CO_2 was likely due both to increased nodule biomass and increased specific nitrogenase activity (Vogel and Curtis 1995). Furthermore, higher instantaneous (Vogel & Curtis 1995) and daily NAR under elevated CO_2 resulted in a significant increase in the ratio of nodule biomass to LA consistent with the close coupling of net CO_2 assimilation and nitrogenase activity in *A. glutinosa* (Gordon & Wheeler 1978; Dawson & Gordon 1979).

Ceulemans & Mousseau (1994), in their review of CO_2 responses in 64 woody species, concluded that root:shoot ratio typically increased at high CO_2, with more pronounced responses under conditions of low N availability. We found that root biomass in elevated CO_2 grown *A. glutinosa* increased to a similar extent as did shoot biomass, resulting in no CO_2 effect on root:shoot ratio. Norby (1987) also observed either no change or a decline in root weight ratio of N_2-fixing *Robinia pseudoacacia* L., *Elaeagnus angustifolia* L., and *A. glutinosa* in response to elevated CO_2. The difference in allocational responses to CO_2 among N_2-fixing and non-fixing plants may be due to the high metabolic cost of N_2 fixation. Root nodules are strong sinks for photosynthate (Tjepkema 1985) and the large increase in nodule biomass, producing comparatively more available N, due to high CO_2 observed in *A. glutinosa* and other N_2-fixing trees (Norby 1987; Thomas et al. 1991) could account for a significant consumption of photosynthate that might otherwise be available for root growth. Increased N availability due to greater

whole-plant nitrogenase activity at high CO_2 may also have influenced allocation of C to roots. Sellstedt et al. (1986) showed that root:shoot ratio increased, whole plant biomass decreased, and whole plant N content decreased in *Alnus incana* (L.) Moench inoculated with a *Frankia* strain inefficient in N_2 fixation compared to plants inoculated with a more efficient strain.

It is important to note, however, that a lack of a CO_2 effect on root biomass recovered at the final harvest does not remove the possibility of CO_2 effects on root turnover during the course of the experiment. For example, Pregitzer et al. (1995) found no difference between high and low soil N grown *Populus x euramericana* (Dode) Guinier in harvested fine root biomass, but using minirhizotron observations they found significant treatment effects on fine root production and mortality over the course of the growing season. The ability of nodulated *Alnus* species to increase soil N levels and the higher total N content of elevated compared to ambient CO_2 grown alder root tissues suggest that root turnover could play an important role in soil N accretion as atmospheric CO_2levels rise. Clearly, more intensive observations of root growth dynamics will be necessary before we can determine the magnitude of CO_2 effects on belowground C and N inputs in this system.

Our results suggest that future increases in atmospheric CO_2 will lead to greater whole plant nitrogenase activity in woody N_2-fixing species and that soil N inputs will increase due to greater leaf litter N content. Given the similarities in litter C:N ratio between elevated and ambient CO_2 treatments, there would be an increase in N mineralization rate that could sustain increased growth of associated non-fixing trees under the predicted higher CO_2 atmosphere.

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