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Regenerating temperate forest mesocosms in elevated CO_2: belowground growth and nitrogen cycling

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Abstract The response of temperate forest ecosystems to elevated atmospheric CO_2 concentrations is important because these ecosystems represent a significant component of the global carbon cycle. Two important but not well understood processes which elevated CO_2 may substantially alter in these systems are regeneration and nitrogen cycling. If elevated CO_2 leads to changes in species composition in regenerating forest communities then the structure and function of these ecosystems may be affected. In most temperate forests, nitrogen appears to be a limiting nutrient. If elevated CO_2 leads to reductions in nitrogen cycling through increased sequestration of nitrogen in plant biomass or reductions in mineralization rates, long-term forest productivity may be constrained. To study these processes, we established mesocosms of regenerating forest communities in controlled environments maintained at either ambient (375 ppm) or elevated (700 ppm) CO_2 concentrations. Mesocosms were constructed from intact monoliths of organic forest soil. We maintained these mesocosms for 2 years without any external inputs of nitrogen and allowed the plants naturally present as seeds and rhizomes to regenerate. We used ^{15}N pool dilution techniques to quantify nitrogen fluxes within the mesocosms at the end of the 2 years. Elevated atmospheric CO_2 concentration significantly affected a number of plant and soil processes in the experimental regenerating forest mesocosms. These changes included increases in total plant biomass production, plant C/N ratios, ectomycorrhizal colonization of tree fine roots, changes in tree fine root architecture, and decreases in plant NH_4^+ uptake rates, gross NH_4^+ mineralization rates, and gross NH_4^+ consumption rates. In addition, there was a shift in the relative biomass contribution of the two dominant regenerating tree species; the proportion of total biomass contributed by white birch (*Betula papyrifera*) decreased and the proportion of total biomass contributed by yellow birch (*B. alleghaniensis*) increased. However, elevated CO_2 had no significant effect on the total amount of nitrogen in plant and soil microbial biomass. In this study we observed a suite of effects due to elevated CO_2, some of which could lead to increases in potential long term growth responses to elevated CO_2, other to decreases. The reduced plant NH_4^+ uptake rates we observed are consistent with reduced NH_4^+ availability due to reduced gross mineralization rates. Reduced NH_4^+ mineralization rates are consistent with the increases in C/N ratios we observed for leaf and fine root material. Together, these data suggest the positive increases in plant root architectural parameters and mycorrhizal colonization may not be as important as the potential negative effects of reduced nitrogen availability through decreased decomposition rates in a future atmosphere with elevated CO_2.

Key words *Betula* · CO_2 · Mycorrhizal fungi · Nitrogen · Pool dilution

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Introduction

The large and rapid rise in atmospheric CO_2 concentrations (Keeling and Whorf 1994) has the potential of directly altering earth's climate system (Schneider 1989) and net primary productivity (NPP) of terrestrial ecosystems (Melillo et al. 1996). The direct effects of elevated CO_2 on terrestrial ecosystems is important for two reasons. First, increased plant growth in elevated CO_2 may serve as an important negative feedback on the rate of rise of atmospheric CO_2 levels (Schimel 1995). Second, altered plant growth and physiology in elevated

CO_2 atmosphere may lead to significant changes in the structure and functioning of managed and natural ecosystems (Bazzaz 1990; Bazzaz and Sombroek 1996; Koch and Mooney 1996).

Temperate forest ecosystems are likely to play a key role in mediating future climate change. Recent theoretical and empirical analyses have suggested that the terrestrial biosphere, especially temperate forest ecosystems, are a globally important sink for atmospheric CO. (Ciais et al. 1995; Goulden et al. 1996; Tans et al. 1990, 1995; Wofsy et al. 1993). Current estimates suggest that, on average, forest species respond to a doubling of atmospheric CO_2 concentrations with about a 30% increase in NPP, (Wullschleger et al. 1995).

In addition to potential effects on NPP, elevated CO_2 may lead to changes in the composition of forest ecosystems through the differential effect of elevated CO_2 on the growth of different species (Ceulemans and Mousseau 1994; Poorter 1993; Wullschleger et al. 1995). Changes in species composition in an elevated CO_2 environment are likely to be especially important in regenerating forest ecosystems, which themselves are likely to be common within future landscapes (Bazzaz et al. 1996). There are a number of factors which may increase the frequency of incidents of forest regeneration in the future. These include changes in temperature and precipitation which may lead to shifts in tree species ranges (Davis 1989; Davis and Zabinski 1992); altered climatic regimes which could enhance disturbance and/or forest dieback through the increased frequency of pest outbreaks and/or forest fires (Kurz et al, 1995); and an increased frequency of extreme weather events which could lead to increased disturbance (Bassow et al. 1994; Katz and Brown 1992).

Much of our current understanding of how different species respond to elevated CO_2 are derived from studies of individually grown plants. Unfortunately, individually grown plants appear to respond to elevated CO_2 quite differently than plants grown in more natural, interacting assemblages (Ackerly and Bazzaz 1995; Bazzaz and McConnaughay 1992; Wayne and Bazzaz 1995). Natural assemblages of plants, and their associated natural soil environments, are increasingly being used to study plant community responses to elevated CO_2 (e.g., Arp et al. 1993; Díaz et al. 1993; Field et al. 1996; Körner et al. 1996; Navas et al. 1995; Owensby et al. 1994). This approach allows us to study an integrated set of interactions (plant-plant and plant-soil) which are critical components of natural ecosystem function.

Our current understanding of the direct effects of elevated CO_2 on aboveground growth and physiology is better developed than for belowground growth and physiology (Rogers et al. 1994). However, belowground plant growth responses and interactions with the soil environment may play a critical role in maintaining or constraining long-term plant growth responses to elevated CO_2 (Curtis et al. 1994). These belowground feedbacks consist of indirect effects of elevated CO_2 on nutrient cycling in the soil and direct effects on plant root architecture and mycorrhizal symbioses (Norby 1994; O'Neill 1994). Indirect effects of elevated CO_2 on nutrient cycling in the soil can be either positive or negative. Increases in plant C/N ratios can lead to reduced rates of mineralization in the soil (Berntson and Bazzaz 1997). Increases in carbon allocation belowground may either increase or decrease nutrient availability through changes in the soil microbial population (Díaz et al. 1993; Zak et al. 1993). And finally, changes in plant water use efficiency may increase soil water content which in turn enhances mineralization rates (Hungate et al. 1996). Changes in plant root architecture and mycorrhizal symbioses almost always lead to increased potential nutrient acquisition by plants and thus represent a mechanism whereby plants in elevated CO_2 atmospheres may be able to acquire more nutrients and thus maintain growth enhancement (Berntson 1994; Norby 1994; O'Neill 1994). To identify the relative importance of these multiple belowground feedbacks and mechanisms of sustained growth enhancement, it is important that they be simultaneously characterized within integrated experiments of terrestrial ecosystem responses to elevated CO_2 (Berntson and Bazzaz 1966a).

In this paper, we present a study of the impact of elevated CO_2 atmospheres on the first two years of regeneration in mesocosms consisting of intact monoliths of forest soil. Using this experimental system of a regenerating temperate forest, we test the following hypotheses regarding the effects of elevated CO_2:

1. Plant species composition is changed.
2. Belowground plant growth and mycorrhizal symbioses are enhanced.
3. Nitrogen cycling within the soil, including plant and microbial uptake of nitrogen, is changed.

Together, we assess the potential relative importance of these responses (especially 2 and 3) in relation to the availability and uptake of nitrogen by regenerating forest communities in elevated CO_2 atmospheres.

Materials and methods

Intact monoliths of forest floor soil (Oe + Oa) measuring 25×40 cm were collected at the Harvard Forest Long Term Ecological Research Site (Petersham, Mass.) in early May 1994. This site is located in the Transition Hardwood-White Pine-Hemlock forest region of New England (Westveld et al. 1956). Forest floor material was collected from several locations within an area of approximately 360 m^2 where the dominant canopy trees were (in order of decreasing percent basal area) *Quercus rubra*, *Acer rubrum*, *Pinus strobus*, *Betula alleghaniensis*, *B. papyrifera*, and *Prunus serotina* (Bassow 1995). We removed undecayed leaf litter from the monoliths.

Monoliths were carefully transported back to a controlled growth facility in Cambridge, Massachusetts, cut to size, and placed within plastic containers ($0.325 \times 0.25 \times 0.25$ m, 20 l) with drainage holes. The top of the monoliths was within a cm of the top of the tub. The bottom of the tubs contained a coarse silica sand which provided ample drainage and no additional nutrients. The total time between soil collection from the field and placement into the containers was less than 48 h. The average depth of the forest floor material was just over 7 cm and settled an insignificant amount (< 0.5 cm) through the duration of the experiment.

The plants present within the seed and rhizome bank within the soil were allowed to regenerate without interference. The mesocosms were never fertilized, but they were watered every 1–2 days so the regenerating plants were never water stressed. All senesced leaves were allowed to fall to the soil within each tub.

A total of 24 mesocosms were distributed among six controlled-environment glasshouses, and placed under 30% shade cloth to simulate a diffuse canopy, but otherwise received natural lighting. Half of the glasshouses (3 units) were maintained with ambient atmospheric CO_2 (375 ppm) and the other with elevated CO_2 (700 ppm). Day/night temperatures were maintained at 26°/19°C with a 13-h day until the beginning of September. At this time day/night temperatures were slowly ramped down to simulate the onset of autumn and harden off the plants for winter. At the end of the first growing season (November 1994), when >90% of leaves had senesced and the plants were dormant, the tubs were moved to an outside garden for the remaining winter months. Tubs were placed in trenches so that their soil surface was even with the external soil surface, and soil was filled in around the outside of each tub to equalize soil temperatures between the inside and outside of the tubs. The tubs were brought back into the glasshouses at the first sign of bud swelling (mid-March 1995).

^{15}N-NH_4^+ tracer application and measurement

In the second week of August 1995 (year 2), 120 ml of 2.5 mmol l^{-1} 98% ^{15}N-NH_4Cl was evenly distributed (vertically and horizontally) within each mesocosm by 120 1-ml injections using a specially built injection system (Berntson 1996). We selected this level of tracer addition because it represented approximately a 25% increase above ambient soil NH_4^+ levels measured in the field (Aber et al. 1993). Soil samples were collected immediately prior to injection, and 15 min, 24 h, and 48 h following injection (4 sampling times). Four cores of soil (1 cm diameter, full depth of forest floor) were collected at randomly selected locations within each mesocosm and pooled together. ^{15}N injections, soil processing, and plant harvesting were processed in three blocks (paired ambient + elevated CO_2 environments, four replicates in each) over a 6-day period.

Soil samples were extracted in 0.5 M K_2SO_4, (Davidson et al. 1989) with a soil/extract ratios (w/v) of 0.1. K_2SO_4 solutions were filtered on pre-combusted glass fiber filters. Samples were placed on a shaker for 24 h prior to filtration, and then frozen for storage. Concentrations of NH_4^+ in the soil extracts were determined using a Westco Scientific auto analyzer (Westco Scientific, Danbury, Conn.) using a modified Berthelot reaction with phenol (Maynard and Kalra 1993).

The level of ^{15}N enrichment (%^{15}N atoms) for the NH_4^+ recovered in the soil extracts taken prior to, after 15 min, 24 h, and 48 h after injection of the label was determined by diffusion to 0.625-cm-diameter pre-combusted glass fiber filters (Gelman AE). The filters contained 20 µl 2 M H_2SO_4 and were wrapped in polytetrafluoroethylene (Sørensen and Jensen 1991). To volatilize the NH_4^+, 0.25 g MgO was added to the extracts (80 ml) in air-tight urine specimen containers. To ensure that the MgO was sufficiently mixed within the K_2SO_4 extracts, acid washed boiling chips were added and the specimen containers were placed on a gently moving shaker table for 10 days. Glass fiber filters were dried in a desiccator which contained a concentrated H_2SO_4 trap to remove contamination by atmospheric NH_4^+. Blanks consisted of glass fiber filters with 20 µl 2 M H_2SO_4 which were placed in the desiccator with the samples. Determination of the total N and ^{15}N content of the glass fiber filters was performed using a Europa Scientific ANCA-sl coupled to a model 20–20 Stable Isotope Analyzer (Europa Instruments, England).

Soil microbial biomass was determined by chloroform fumigation (Jenkinson and Powlson 1976) of soil samples followed by direct extraction in 0.5 M K_2SO_4 (Brookes et al. 1985; Davidson et al 1989). This procedure was performed only on the soil samples collected 48 h after ^{15}N injection. For each these samples, half of the soil was directly extracted in K_2SO_4, and the other half was first fumigated with chloroform. Total-N and ^{15}N enrichment in the K_2SO_4 solutions were determined before and after fumigation. Measurement of C, N and ^{15}N content in the K_2SO_4 solutions was performed using the Europa ANCA-sl and 20–20 stable isotope analyzer. Dehydrated K_2SO_4 (to which no soil had been added) was used as a blank. To maximize the amount of solution which could be processed, the K_2SO_4 solutions were dehydrated by placing them in a drying oven for 24 h at 75°C. Samples of the dehydrated K_2SO_4 (75–150 mg) were then processed using the Europa ANCA-sl and stable isotope analyzer described above. To obtain maximum resolution when processing these samples an offset O_2 injection was used, providing resolution down to 0.5 µmol N (Owens and Rees 1989). The amount of ^{15}N recovered in microbial biomass was corrected by assuming that natural ^{15}N abundance of the microbial pool was equal to the natural ^{15}N abundance of the soil NH_4^+ pool.

Plant harvesting, mycorrhizal assessments and quantification of root architecture

After the 48-h incubation with the ^{15}N-NH_4 tracer, each mesocosm was harvested and all the plant material carefully collected. At the start of harvest, all plant stems were cut approximately 1 cm above the soil surface and tags were attached to the remaining stems so that each root system could be identified to species. The roots of all the plants for each different species of plant encountered were carefully washed from the soil by placing the entire soil block in a tub of water, and then carefully disentangling and extracting the roots by hand. It was inevitable that some of the fine roots were lost, but care was taken to keep each root system as intact as possible. Entire root systems for each species were wrapped in a moist paper towel, placed in an air tight plastic bag (with all the air removed) and frozen prior to the morphological and mycorrhizal measurements. Above-ground plant material was oven-dried for 96 h at 70°C and weighed to the nearest mg.

To characterize the root morphology and ectomycorrhizal status of each species, we made detailed measurements on subsamples of the fine roots of each species from each mesocosm. For all of the tree species, fine roots were defined as roots <0.5 mm in diameter which showed no clear signs of secondary growth or lignification. Woody roots consisted of those with clear secondary growth and lignification. For the herbaceous species, no attempt was made to separate different fractions of the root systems or characterize ectomycorrhizae. A total of approximately 200 individual root tips (215 ± 11, mean ± SE) were taken from three or four randomly selected locations within each root system. For each subsample, the total number of root tips, and for the tree species, the total number of root tips with a mycorrhizal sheath, were counted under a dissecting microscope. Root tips which were broken or dead were not included in these counts. Once the root tips had been counted, the subsample was laid out in a petri dish with ~2mm of water to minimize overlap of individual roots and scanned into a computer as 8-bit gray scale images at 177.2 pixels cm^{-1}. From these images total root length and average diameter of the fine root subsample were calculated using the procedures and algorithms proposed by Pan and Bolton (1991). From all of these measurements made on the (fine) root subsamples the following parameters were derived: percent mycorrhizal root tips, root diameter, specific root length (SRL, m g^{-1}) and specific root number (SRN, number of root tips g^{-1}). Total fine root length and number of root tips for each species within a given mesocosm were derived by multiplying SRL and SRN by (fine) root mass.

After taking the fine root subsamples, fine roots were separated from the woody roots after air drying. We defined fine roots as those that disintegrated when rubbed between the fingers; woody roots did not. Total root surface area (for the fine roots) was calculated by assuming roots were cylindrical and multiplying total root length by π times diameter. Total number of mycorrhizal root tips was calculated by multiplying total number of root tips by percent mycorrhizal root tips.

Total-N and percent ^{15}N enrichment for ground plant material were measured using the same procedure as for the glass fiber filters

used in the diffusions. Excess percent ^{15}N enrichment for the plant material was calculated as the difference between plant samples 48 h after the ^{15}N-NH_4^+ application and plant samples (leaves) collected prior to injection. For all of the tree species, leaves, stems, woody roots and fine roots were separated and determination of carbon and nitrogen content made using a Europa ANCA-sl Elemental Analyzer (Europa Instruments, England).

Nitrogen immobilization, uptake, and mineralization calculations

To estimate the gross rate of mineralization and NH_4^+ consumption from the soil pool, we used the pool dilution calculations which accounted for the base level of ^{15}N in the NH_4^+ produced during mineralization (Blackburn 1979; Wessel and Tietema 1992). The base level of ^{15}N in the $NH_4^+$was measured on the soil samples collected prior to the injection of ^{15}N. Average base levels of ^{15}N enrichment (%) in the soil NH_4^+ pool prior to injection was 0.391 ± 0.007 (mean ± SE) for ambient and 0.399 ± 0.013 for elevated CO_2 (not statistically different). Pool dilution calculations were performed separately for the first and second 24 h following the injection of the ^{15}N label, as well as the full 48-h period. Each of these three sets (0–24 h, 24–48 h, and 0–48 h) of pool dilution calculations were performed using actual data on NH_4^+ pool sizes and ^{15}N content of the soil solution at the beginning and end of the time interval.

We assumed that all NH_4^+ consumption was due to plant and soil microbial uptake and therefore used the relative amount of ^{15}N tracer acquired by plants and soil microbes to derive NH_4^+ uptake rates by partitioning the pool dilution estimate of gross immobilization. As an additional check, we also estimated plant and microbial uptake using the total amount of ^{15}N tracer recovered in plant and microbial biomass. However, because the ^{15}N is a small and changing proportion of the pool of available NH_4^+, recovery of ^{15}N in biomass will underestimate actual uptake rates (e.g., Barraclough 1991; Davidson et al. 1991). We did not include nitrification rates when partitioning gross NH_4^+ consumption rates because standing pools of nitrate were extremely low (< 1 ppm in soil extracts) making measurement of the ^{15}N tracer in this pool not possible. While there is some data to suggest that gross nitrification rates can be high even when standing pools of nitrate in the soil solution are low (Davidson et al. 1992; Stark and Hart 1997), recent experiments have found that elevated gross nitrification rates are linked to elevated N inputs to the soil (Tietema 1997). Because we maintained our mesocosms with no external nitrogen inputs for 2 years and nitrate levels were so low, we assumed that nitrification rates were negligible. We focused measurements on NH_4^+ rather than NO_3^- because we were interested in quantifying the fate of newly produced inorganic nitrogen from plant-derived soil detritus, and NH_4^+ is the dominant form of inorganic nitrogen in the soils we were examining (Aber et al. 1993; Magill et al. 1997).

Statistical analysis

For all one-mesocosm, one-measurement variables, data were analyzed using a two-way ANOVA, with the CO_2 treatment and block as independent variables. For measurements made separately for each species or separately for plants and soil microbes, a three-way ANOVA was used with the addition of species/organism as an additional independent factor. These ANOVAs treated block as a fixed factor because individual CO_2 glasshouses were not a priori paired by CO_2 levels. For a given species, the effect of CO_2 was tested using linear contrasts (SuperANOVA, v1.11, Abacus Concepts, Berkeley, Calif.). Assumptions of homoscedasticity and normality of residuals were tested with normal probability plots (DataDesk v4.0, Data Description, Inc., Ithaca, N.Y.) and data were transformed (log or arcsine square root) in cases where it improved compliance with the assumptions of parametric statistics.

Results

Elevated CO_2 led to significant increases in net plant biomass production in the mesocosms (Table 1). Pooling above- and belowground biomass, CO_2 enhancement (average elevated CO_2/average ambient CO_2) was 1.31. This is a level of net biomass enhancement close to the average level observed for most single plant studies (Ceulemans and Mousseau 1994; Poorter 1993; Wullschleger et al. 1995), and significantly greater than that observed in monocultures of yellow birch (Berntson and Bazzaz 1997; Wayne and Bazzaz 1995). The effect of elevated CO_2 on belowground biomass (62% increase) was greater than that for aboveground biomass (23%; Table 1). Total plant nitrogen content (above and belowground biomass for all species pooled together) was unaffected by elevated CO_2, and thus C/N ratios of plant biomass were significantly increased (Table 1).

In total, we cataloged 13 different species regenerating within the mesocosms (trees: *Betula papyrifera*, *B. alleghaniensis*, *B. populifolia*, *Quercus rubra*; other: *Gaultheria procumbens*, *Rubus alleghaniensis*, *Carex* sp., *Medeola virginiana*, *Trientalas americana*, *Dennstaedtia punctilobula*, *Hieracium* sp., *Lysimachea quadrifolia*, *Panicum* sp.). Aside from a single mesocosm which was completely dominated by *Dennstaedtia*, by the second year every mesocosm was dominated by *B. papyrifera* and *B. alleghaniensis* (>95% of total plant biomass). Except for the two dominant birch species and *G. procumbens*, no other species occurred in more than two

Table 1 Plant biomass, allocation patterns, molar C/N ratios for plants (all species pooled together) and soil microbes, and all measured pools of nitrogen. *P* = probability that null hypothesis is true (ambient CO_2 = elevated CO_2). (*ER* ratio of elevated CO_2 mean over ambient CO_2 mean)

	Units	Ambient CO_2		Elevated CO_2		*P*	ER
		Average	SE	Average	SE		
Plant biomass	g m^{-2}	893	42	1173	65	0.00	1.31
Root/shoot biomass	–	0.29	0.02	0.39	0.05	0.14	1.32
Aboveground biomass	g m^{-2}	695	38	853	49	0.08	1.23
Belowground biomass	g m^{-2}	198	12	320	34	0.01	1.62
Plant C/N	–	69.0	4.0	87.0	3.4	0.05	1.26
Soil microbial C/N	–	11.0	0.4	12.2	0.7	0.19	1.11
Plant nitrogen	g m^{-2}	10.6	0.6	10.9	0.8	0.77	1.03
Microbial N content	g m^{-2}	3.68	0.89	2.91	0.32	0.36	0.79
Extractable NH_4^+-N	g m^{-2}	0.218	0.025	0.222	0.038	0.94	1.02

mesocosms per CO_2 level. This high level of rarity made it impossible to infer anything about changes in species diversity or richness in response to elevated CO_2. However, there was a significant relative increase in *B. alleghaniensis* and a significant relative decrease in *B. papyrifera* biomass in elevated CO_2 (Fig. 1). This significant change in relative biomass contribution was driven by a significant absolute increase in *B. alleghaniensis* (Table 2A) biomass, and a non-significant change in absolute biomass in *B. papyrifera* (Table 2B) or any other species. The significant increase in *B. alleghaniensis* biomass was due to increases in the density as well as average size of

Table 2 A Summary of growth, allocation, nitrogen content, root architecture, mycorrhizal colonization and NH_4^+ uptake rates for *B. allegheniensis* and for *B. papyrifera*. *P* = probability that null hypothesis is true (ambient CO_2 = elevated CO_2) (*ER* ratio of elevated CO_2 mean over ambient CO_2 mean)

Betula alleghaniensis	Units	Ambient CO_2		Elevated CO_2		*P*	ER
		Average	SE	Average	SE		
A							
Growth and allocation							
Plant density	m^{-2}	39.0	7.4	80.6	16.7	0.00	2.07
Average plant biomass	g $plant^{-1}$	4.2	1.2	8.2	1.7	0.10	1.96
Total biomass	g m^{-2}	224	73	593	113	0.01	2.65
Root/shoot biomass	–	0.37	0.05	0.47	0.03	0.06	1.26
Fine root mass	g m^{-2}	13.0	4.7	46.3	8.9	0.00	3.57
Fine/woody root mass	–	0.37	0.04	0.37	0.04	0.95	0.99
Nitrogen content							
Whole plant nitrogen	g m^{-2}	2.98	0.99	4.82	0.64	0.18	1.62
C/N-leaves	–	21.4	1.0	28.8	1.7	0.00	1.35
C/N-stem	–	65.7	6.3	122.7	10.0	0.00	1.87
C/N-woody roots	–	67.9	6.4	111.8	9.8	0.00	1.65
C/N-fine roots	–	35.2	1.3	47.3	2.0	0.00	1.34
Root architecture & mycorrhizae							
Mycorrhizal colonization	% Tips	32.7	5.3	55.8	4.2	0.00	1.71
Root diameter	µm	162	3	154	4	0.14	0.95
Root length	km m^{-2}	2.95	1.08	12.44	2.24	0.00	4.22
Root surface area	m^{-2} m^{-2}	2.93	1.05	11.87	2.12	0.00	4.05
Specific root length	km g^{-1}	0.23	0.02	0.27	0.02	0.09	1.19
NH_4^+ uptake rate							
^{15}N recovery[a]	mg ^{15}N m^{-2} day^{-1}	6.9	3.6	4.3	0.8	0.22	0.61
Pool dilution 0–24 h[b]	mg N m^{-2} day^{-1}	41.6	20.9	16.6	6.8	0.13	0.40
Pool dilution 24–48 h[b]	mg N m^{-2} day^{-1}	17.6	12.2	1.9	4.7	0.30	0.11
Pool dilution 0–48 h[b]	mg N $m^{-2}day^{-1}$	43.2	25.2	9.2	2.5	0.75	0.21
B							
Betula papyrifera							
Growth and allocation							
Plant density	m^{-1}	64.6	8.0	43.6	6.9	0.02	0.68
Average plant biomass	g $plant^{-1}$	11.3	1.6	11.0	2.3	0.86	0.97
Total shoot biomass	g m^{-1}	658	70	593	116	0.69	0.90
Root/shoot biomass	–	0.25	0.01	0.35	0.05	0.05	1.42
Fine root mass	g m^{-2}	31.8	3.6	33.3	6.7	0.70	1.05
Fine/woody root mass	–	0.33	0.02	0.33	0.03	0.92	1.02
Nitrogen content							
Whole plant nitrogen	g m^{-2}	7.44	0.75	5.36	1.02	0.10	0.72
C/N-leaves	–	25.1	1.1	31.0	1.7	0.00	1.24
C/N-stem	–	115.6	3.7	118.3	1.2	0.73	1.02
C/N-woody roots	–	87.7	4.7	97.3	6.1	0.15	1.11
C/N-fine roots	–	38.3	1.8	48.0	2.0	0.00	1.25
Root architecture & mycorrhizae							
Mycorrhizal colonization	% Tips	36.3	5.2	52.7	2.3	0.00	1.45
Root diameter	µm	158	5	158	5	0.88	1.00
Root length	km m^{-2}	8.81	1.44	7.81	1.46	0.87	0.89
Root surface area	m^{-2} m^{-2}	8.5	1.3	7.57	1.4	0.87	0.89
Specific root length	km g^{-1}	0.27	0.02	0.25	0.03	0.50	0.92
NH_4^+ uptake rate							
^{15}N recovery[a]	mg ^{15}N m^{-2} day^{-1}	12.4	2.6	5.2	1.4	0.03	0.42
Pool dilution 0–24 h[b]	mg N m^{-2} day^{-1}	81.7	26.5	11.1	29.2	0.07	0.14
Pool dilution 24–48 h[b]	mg N m^{-2} day^{-1}	52.2	17.5	1.1	5.4	0.00	0.02
Pool dilution 0–48 h[b]	mg N m^{-2} day^{-1}	65.7	17.9	14.1	6.3	0.00	0.21

[a] Uncorrected uptake-total ^{15}N recovered in plant biomass
[b] Derived from partitioned pool dilution estimates of gross NH_4^+ consumption

plants (Table 2A). For *B. papyrifera*, there was no significant increase in total biomass or average plant biomass, but there was a significant decrease in plant density (Table 2B).

Because the two dominant birch species comprised more than 95% of the biomass in all the mesocosms in which they were present, we focused measurements of biomass allocation, root architecture, and mycorrhizal colonization rates on these species. In response to elevated CO_2 *B. alleghaniensis* showed significant increases ($P \leq 0.05$) in total biomass, fine root biomass, ectomycorrhizal colonization, fine root length, fine root surface area and specific root length; marginally significant increases ($P \leq 0.1$) in root/shoot ratios, and specific root length (SRL); and no significant change ($P > 0.1$) in total nitrogen content, the ratio of fine to woody root mass, or fine root diameter (Table 2A). In contrast, *B. papyrifera* showed significant increases only in root/shoot ratios and mycorrhizal colonization rates; and a marginally significant decrease in nitrogen content (Table 2B). Both species of *Betula* showed significant increases in C/N ratios for the leaves and fine roots in elevated CO_2 (Table 2A, B). For *B. papyrifera*, no significant changes in the C/N ratios of woody stems or roots were seen. In contrast, for *B. alleghaniensis* the C/N ratios of woody stems and roots increased significantly with elevated CO_2. We observed no significant change in NH_4^+ uptake rates for *B. alleghaniensis*, regardless of the method used to estimate uptake rates (Table 2A). In contrast, we observed significant reductions in uptake rates of NH_4^+ for *B. papyrifera* for every method we used to estimate uptake rates (Table 2B).

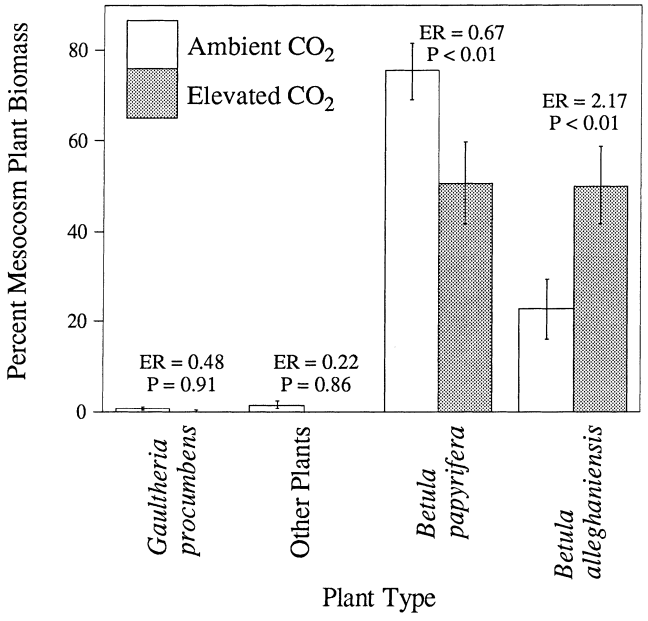

Fig. 1 Summary of the present of total biomass for each of the four groups of plants which were present in more than one mesocosm per CO_2 level. Percent biomass was calculated as the total biomass of a given plant group divided by the total plant biomass within the microcosm. The single soil monolith which was completed dominated by the fern was not included in this analysis (*P* probability that elevated CO_2 mean = ambient CO_2 mean, *ER* ratio of elevated CO_2 mean over ambient CO_2 mean). *Error bars* are 1 SEM

Observed rates of gross mineralization and NH_4^+ consumption were strongly dependent on the time interval over which the flux rate was determined. On average, mineralization rates decreased by 71.5% and immobilization rates by 63.6% from the first to the second 24 h of the 48-h labeling period (Table 3). Estimates over the full 48 h incubation period were intermediate. Elevated CO_2 led to significant reductions in gross mineralization and consumption rates of NH_4^+ for

Table 3 Results of pool dillution calculations, including gross mineralization and consumption, along with partitioned estimates of gross consumption (see Materials and methods for details) and ^{15}N recovery in plant and soil microbial biomass. *P* = probability that null hypothesis is true (ambient CO_2 = elevated CO_2) (*ER* ratio of elevated CO_2 mean over ambinet CO_2 mean)

	Units	Time interval	Ambient CO_2		Elevated CO_2		*P*	ER
			Average	SE	Average	SE		
Gross mineralization rate	mg N $m^{-2}day^{-1}$	0–24 h	242.0	64.0	189.0	90.0	0.56	0.78
		24–48 h	106.0	26.0	17.0	32.0	0.04	0.16
		0–48 h	201.2	41.1	103.7	25.0	0.06	0.52
Gross consumption rate	mg N $m^{-2}day^{-1}$	0–24 h	262.0	66.0	173.0	89.0	0.40	0.66
		24–48 h	139.0	36.0	20.0	32.0	0.01	0.14
		0–48 h	215.3	45.7	101.1	28.1	0.05	0.47
Plant NH_4^+ uptake rate	mg N $m^{-2}day^{-1}$	0–24 h	122.8	33.9	53.3	39.3	0.19	0.43
		24–48 h	69.6	19.8	7.2	9.1	0.00	0.10
		0–48 h	107.5	28.0	39.3	18.3	0.04	0.37
^{15}N recovery-plant[a]	%	–	35.5	7.1	20.6	4.8	0.10	0.58
Microbial NH_4^+ uptake rate	mg N $m^{-2}day^{-1}$	0–24 h	138.9	49.8	119.9	58.4	0.78	0.86
		24–48 h	68.7	23.7	12.7	23.7	0.10	0.18
		0–48 h	107.8	35.1	61.7	15.6	0.23	0.57
^{15}N recovery-microbial[a]	%	–	30.5	7.8	39.7	5.0	0.35	1.30
Fraction plant ^{15}N[b]	%	–	55.7	8.2	32.9	4.7	0.02	0.59

[a] Percent of total amount of ^{15}N injected recovered in given pool
[b] ^{15}N acquisition by plants relative to the total amount recovered in plant and soil microbial biomass

the full 48 h incubation period, and for second 24 h, but not for the first 24 h.

Partitioned pool dilution estimates of plant NH_4^+ uptake rates were significantly reduced for the full 48 h incubation period and the second 24 h. However, we observed non-significant reductions in soil microbial uptake rates of NH_4^+, though for the second 24 h of the incubation reduction in uptake was nearly significant ($P = 0.10$). The amount of ^{15}N recovered in plant and soil microbial biomass generally supported the pool dilution estimates. The decrease observed for plants was nearly significant ($P = 0.10$) but a non-significant change was observed for soil microbes. Expressed as a percentage of the total amount of ^{15}N recovered in plant and soil microbes, we observed a significant reduction in the amount of ^{15}N recovered by plants relative to soil microbes.

Discussion

Species composition

Species composition plays an important role in determining terrestrial ecosystem structure and function (Bassow 1995; Clark 1993; Pastor and Post 1986). Changes in composition of regenerating forests in an elevated CO_2 environment may itself represent a mechanism of long-term shifts in ecosystem productivity and nutrient cycling. The effects of elevated CO_2 on early tree growth and establishment may be a critical determinant of future forest composition. For example, assuming that the effect of elevated CO_2 on growth enhancement for several tree species is transitory, Bolker et al. (1995) used a forest simulator (Pacala et al. 1993) to show that elevated CO_2 may potentially lead to large changes in the composition and productivity of temperate forests.

In this study, we observed a significant shift in relative dominance of the two most abundant tree species regenerating within the mesocosms. In elevated-CO_2 environments, yellow birch (*B. alleghaniensis*) increased in frequency and white birch (*B. papyrifera*) decreased in frequency. Trees in the genus *Betula* produce large numbers of small seeds, typically have fast seedling growth rates, and often dominate early successional forest communities (Burns and Honkala 1990). These characteristics explain why the two dominant regenerating species were *Betula*. A review of the average growth enhancement of *B. alleghaniensis* and *B. papyrifera* in elevated CO_2 from individually grown plants does not *a priori* suggest that either species would benefit more than the other in response to elevated CO_2. Net growth enhancement in a doubled-CO_2 atmosphere for individually grown *B. alleghaniensis* range between 23 and 86% (Rochefort and Bazzaz 1992; Wayne and Bazzaz 1995) and for *B. papyrifera* between 24 and 100% (Bazzaz et al. 1990; Berntson and Bazzaz 1996b; Rochefort and Bazzaz 1992). Thus, the increased representation of *B. alleghaniensis* which we observed could not have been predicted on the basis of average individual-plant CO_2 growth enhancements. This discrepancy between actual changes in community composition and predicted changes is a critical challenge in predicting community and ecosystem effects of elevated CO_2 (Bazzaz and McConnaughay 1992; Körner 1996).

Shifts in the dominance from *B. papyrifera* to *B. alleghaniensis* in regenerating forest ecosystems in the Northeastern U.S. in response to elevated CO_2 is likely to be the result of a an increase in water use efficiency in elevated CO_2 (Field et al. 1995) *B. alleghaniensis* typically regenerates in much moister microsites than does *B. papyrifera* (Burns and Honkala 1990). We observed a 13% increase in gravimetric moisture content with elevated CO_2, and this may be an important factor leading to the differential performance of these species. It is not clear what the long term implications of such a shift would be. *B. alleghaniensis* is a longer lived, much larger tree than is *B. papyrifera*. However, communities dominated by *B. alleghaniensis* may be more sensitive to periods of drought.

Root architecture and mycorrhizal colonization

Along with effects on biomass production and allocation, elevated CO_2 levels can lead to changes in root architecture, physiology, and mycorrhizal symbiosis. In general, elevated CO_2 appears to lead to changes in root form and function which lead to increases in potential nutrient acquisition (Berntson and Bazzaz 1996a; Norby 1994; Rogers et al. 1994). Changes in plant root system architecture in elevated-CO_2 environments include greater total root length, an increase in the amount of soil explored, and more root tips (Berntson and Woodward 1992; Kaushal et al. 1989; Larigauderie et al. 1994; Rogers et al. 1992). In the majority of studies to date, elevated CO_2 has led to significant increases in the colonization of trees by ectomycorrhizal fungi (Berntson et al. 1997; Godbold and Berntson 1997; Ineichen et al. 1995; O'Neill 1994). Together, these changes in root architecture and mycorrhizal symbiosis have the potential of increasing the total amount of nutrients a plant can acquire from the soil (Berntson 1994; Yanai et al. 1995).

In this study we found that elevated CO_2 led to a significant increase in the overall size of the root systems of *B. alleghaniensis* but not *B. papyrifera*, as measured by fine-root length, number of root tips, and root surface area. This differential belowground response between the two dominant tree species was not due to enhanced allocation of biomass belowground, as *B. papyrifera* showed a greater enhancement in root/shoot biomass than did *B. alleghaniensis* in elevated CO_2 (Table 2A, B). Differences in root system size between these species is largely related to plant size (e.g., biomass). In contrast to root architecture, elevated CO_2 led to increases in the degree of ectomycorrhizal colo-

nization for every tree species in the experimental mesocosms. Taken together, the changes in root architecture and mycorrhizal symbioses imply that elevated CO_2 leads to an increase in the potential of regenerating trees to acquire nutrients from the soil.

Plant productivity and plant C/N

In this study, elevated atmospheric CO_2 resulted in a 31% increase in plant biomass (pooling all plant species, above and belowground biomass), without a significant change in total plant nitrogen content (Table 1). This imbalance in biomass production relative to nitrogen acquisition led to a 26% increase in whole-plant C/N ratios. From the perspective of plant growth, increased C/N ratios represent an increase in nutrient use efficiency (Sheriff et al. 1995; Vitousek 1982), allowing plants to maintain or increase biomass production with limited N.

However, from the perspective of decomposing plant detritus, increased C/N ratios of plant biomass in elevated CO_2 has important implications for the long term storage of biomass in terrestrial ecosystems as well as potential feedbacks on nitrogen cycling and nitrogen availability (Rastetter et al. 1992; Shaver et al. 1992). Studies of decomposition rates in the field have shown that higher C/N ratios can lead to slower decomposition rates and increased nitrogen immobilization (Aber et al. 1990; Downs et al. 1996; Melillo et al. 1982). However, it has not been conclusively demonstrated that atmospheric CO_2-induced changes in plant C/N ratios will lead to alterations in the rate of plant detritus decomposition. Some studies have observed reduced decomposition (mass-loss) rates of plant detritus from elevated CO_2 environments which have elevated C/N ratios (Boerner and Rebbeck 1995; Cotrufo and Ineson 1995). Other studies have found that reductions in decomposition rates can be either insignificant or transitory (Coûteaux et al. 1991; O'Neill and Norby 1996).

The study we present here was carried out for just under 2 years. At the end of this time, we found no effect of elevated CO_2 on total plant-N content. Thus, we would not expect any N limitations due to the sequestration of N in plant biomass or detritus for early stages of forest regeneration. Instead, we suggest the relationship between plant C/N ratios and mineralization of plant-derived detritus may be a more important feedback on nitrogen availability, and thus possibly plant growth responses to elevated CO_2. To assess the potential for changes in plant C/N ratios to influence decomposition rates in the context of this study, it is important the we examine those plant organs which turnover rapidly and therefore represent the sources of plant-derived detritus in the soil (e.g., leaves and fine roots in this study). The C/N ratios of leaves and fine roots were significantly increased in elevated CO_2 (by 24–35%) for both of the dominant tree species.

The interplay between nitrogen cycling and plant capacity for nitrogen uptake

We used pool dilution techniques to quantify *in situ* gross mineralization and immobilization rates of nitrogen within the soil. Pool dilution techniques are more useful for characterizing potential changes in nutrient availability than the dynamics of mass loss because they directly quantify the fluxes, and thus potential availability, of nitrogen. The dynamics of mass loss do not provide a valid estimate of nitrogen mineralization because plant detritus can immobilize a significant amount of nitrogen during early decomposition phases, especially when C/N ratios are high (Aber and Melillo 1991; Aber et al. 1990; Downs et al. 1996). This disparity between mass and nitrogen dynamics in decaying litter suggests that to determine potential effects of altered plant detritus quantity and/or quality on nitrogen availability within the soil we need to directly quantify fluxes of nitrogen.

Using pool dilution calculations, we observed reductions in NH_4^+ mineralization and consumption rates under elevated CO_2 (Table 3). These results are consistent with and possibly the result of the increases in C/N ratios for the fine roots and leaves. These results do not support either of the feedback hypotheses presented by Zak et al. (1993) or Díaz et al. (1993). We found no evidence to support the suggestion of Zak et al. (1993) that elevated CO_2 would lead to increased nitrogen availability. Rather, we observed significant reductions in gross mineralization and consumption rates of NH_4^+ in elevated CO_2. The results of this study only partially support the suggestion of Díaz et al. (1993) that increased nitrogen immobilization by soil microbes for inorganic nitrogen will reduce plant available N. We found that NH_4^+ uptake was reduced for plants, but not clearly so for soil microbes. In terms of the relative amount of ^{15}N tracer recovered in plant and soil microbial biomass, there was a shift from what appeared to be a balance in ambient CO_2 (a 50/50 split in acquired tracer) to a significant reduction in the relative amount of tracer acquired by plants in elevated CO_2 (Table 3). However, neither total microbial-N content nor total plant-N content were significantly affected by elevated CO_2 (Table 1). The results we present here are more consistent with a previous study we carried out examining the growth and nitrogen dynamics in monocultures of *B. alleghaniensis* (Berntson and Bazzaz 1997) than with either Zak et al. (1993) or Diaz et al. (1993).

A critically important clarification to make regarding the data we present is that all the data on estimated fluxes (mineralization, consumption, and uptake of NH_4^+) correspond to the last 48 h of the experiment, while all of the data on carbon and nitrogen pool sizes integrate processes over a much longer period of time. This clarification is important because our conclusion regarding the presence or absence of a negative feedback on potential plant productivity through nitrogen avail-

ability will vary depending on which set of data we examine.

Judging by total nitrogen content measured in plant and soil microbial biomass at the end of the experiment, elevated CO_2 had no effect on net nitrogen acquisition for either group of organisms, nor on their relative ability to acquire nitrogen (Table 1). The effect of elevated CO_2 on nitrogen mineralization, consumption, and uptake rates which we quantified at the end of the experiment lead to a very different conclusion regarding the potential impact of elevated CO_2 on nitrogen cycling and nitrogen availability to plants. The large, significant reductions in nitrogen cycling (production and consumption of NH_4^+) and plant uptake we observed, if extrapolated into the future, suggest that reduced plant availability of nitrogen is likely to constrain positive growth enhancements in elevated CO_2 due to nitrogen limitation.

The key question which follows from these apparently contrasting data is "How is it possible for these data to be so different from each other?" One possible answer to this question is simply that rates of nitrogen production and consumption were not constant throughout the duration of the experiment. This hypothesis, while not directly testable with our data, could explain the apparent discrepancy in pool sizes of nitrogen and measured flux rates. If the early effects of elevated CO_2 on plant growth were increases in plant size and plant ability to acquire available nitrogen (though changes in root architecture, mycorrhizal symbioses, and possibly root physiology), there may have been an early enhancement in plant growth and nitrogen uptake. This a reasonable assumption, as elevated CO_2 typically leads to larger growth enhancements early in plant ontogeny (Bazzaz et al. 1993). Further, when the mesocosms were first established, all of the nitrogen supplied within the soil via mineralization was derived from labile organic matter within the soil. Presumably, the different mesocosms had about the same amount of nitrogen available because they were all watered the same amount and maintained in the same temperature regimes. If, through time, the production of high C/N detritus in elevated CO_2 led to a reduction in NH_4^+ mineralization rates and thus consumption rates, the early increases in growth and nitrogen acquisition would diminish. This scenario is consistent with all of the data presented in this study. However, to critically test this hypothesis, future studies must examine the ontogeny of nitrogen cycling and nitrogen uptake in plant communities.

One additional important factor which this study has demonstrated is that even though we observed changes in root architecture (root length, root surface area; particularly in *B. alleghaniensis*) and ectomycorrhizal colonization which would, all other factors being equal, increase the ability of plants to acquire nitrogen, we observed no increase in plant uptake of NH_4^+. There are two possible hypotheses for this pattern. First, that changes in root architecture and mycorrhizal colonization are not important in determining plant ability to acquire soil nutrients (NH_4^+ in this case). Second, that the reduction in the rate of supply of NH_4^+ led to a constraint in nitrogen availability which was more important than the observed changes in root growth. We suggest that the first hypothesis is not consistent with our data. In *B. alleghaniensis* we observed a significant increases in root architectural parameters and mycorrhizal colonization (Table 2A), and no statistically significant drop in total ^{15}N acquisition or estimated NH_4^+ uptake. In contrast, in *B. papyrifera* we observed no change in root architectural parameters (though we did see an increase in mycorrhizal colonization; Table 2B) and we observed a decrease in both total ^{15}N acquisition or estimated NH_4^+ uptake. These data suggest that changes in root architecture do play an important role in mediating plant ability to acquire nitrogen, even when total plant uptake is reduced to reductions in availability due to environmental limitations.

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References

Aber JD, Melillo JM (1991) Terrestrial ecosystems. Saunders College, Philadelphia

Aber JD, Melillo JM, McClaugherty CA (1990) Predicting long-term patterns of mass loss, nigrogen dynamics and soil organic matter formation from initial fine litter chemistry in temperate forest ecosystems. Can J Bot 68:2201–2208

Aber JD, Magill A, Boone R, Melillo JM, Steudler P, and Bowden R (1993) Plant and soil responses to chronic nitrogen additions at the harvard forest, Massachusetts. Ecol App 3:156–166

Ackerly DD, Bazzaz FA (1995) Plant growth and reproduction along CO_2 gradients: non-linear responses and implications for community change. Global Change Biol 1:199–207

Arp WJ, Drake BG, Pockman WT, Curtis PS, Whigham DF (1993) Interactions between C_3 and C_4 salt marsh species during four years of exposure to elevated atmospheric CO_2. Vegetatio 104/105:133–143

Barraclough D (1991) The use of mean pool abundances to interpret nigrogen-15 tracer experiments. I. Theory. Plant Soil 131:89–96

Bassow SL (1995) Canopy photosynthesis and carbon cycling in a deciduous forest: implications of species composition and rising concentrations of CO_2, Harvard University Cambridge

Bassow SL, McConnaughay KDM, Bazzaz FA (1994) The response of temperate tree seedlings grown in elevated CO_2 to extreme temperature events. Ecol Appl 4:593–603

Bazzaz FA (1990) The response of natural ecosystems to the rising global CO_2 levels. Annu Rev Ecol Syst 21:167–196

Bazzaz, FA, and McConnaughay, KDM (1992) Plant-plant interactions in elevated CO_2 environments. Aust J Bot 40:547–563

Bazzaz, FA, Sombroek W (1996) Global climate change and agricultural production. Wiley, Chichester

Bazzaz FA, Coleman JS, Morse SR (1990) Growth responses of seven major co-occurring tree species of the northeastern United States to elevated CO_2. Can J For Res 20:1479–1484

Bazzaz FA, Miao SL, Wayne PW (1993) CO_2-induced growth enhancements of co-occuring tree species decline at different rates. Oecologia 96:478–482

Bazzaz FA, Basssow SL, Berntson GM, Thomas SC (1996) Elevated CO_2 and terrestrial vegetation: implications for and beyond the global carbon budget. In: Walker B, Steffen W (eds) Global change and terrestrial ecosystems. Cambridge University Press, Cambridge, pp. 43–76

Berntson GM (1994) Modelling root architecture: are there tradeoffs between efficiency and potential of resource acquisition? New Phytol 127:483–493

Berntson GM (1996) Root growth and nitrogen cycling in temperate deciduous forests in an elevated CO_2 world. Harvard University, Cambridge

Berntson GM, Bazzaz FA (1996a) Belowground positive and negative feedbacks on CO_2 growth enhancement. Plant Soil 187:119–134

Berntson GM, Bazzaz FA (1996b) The influence of elevated CO_2 on the allometry of root production and root loss in *Acer rubrum* and *Betula papyerifera*. Am J Bot 83:608–616

Berntson GM, Bazzaz FA (1997) Nitrogen cycling in microcosms of yellow birch exposed to elevated CO_2: simultaneous positive and negative feedbacks. Global Change Biol 3:247–258

Berntson GM, Woodward FI (1992) The root system architecture and development of *Senecio vulgaris* in elevated carbon dioxide and drought. Funct ecol 6:324–333

Berntson GM, Wayne PM, Bazzaz, FA (1997) Belowground architectural and mycorrhizal population-level responses to elevated CO_2 in *Betula alleghaniensis*. Funct ecol (in press).

Blackburn TH (1979) Method for measuring rates of NH_4^+ turnover in anoxic marine sediments, using a ^{15}N-NH_4^+ dilution technique. Appl Environ Microbiol 37:760–765

Boerner REJ, Rebbeck J (1995) Decomposition and nitrogen release from leaves of three hardwood species grown under elevated O_3 and/or CO_2. Plant Soil 170:149–157

Bolker BM, Pacala SW, Bazzaz FA, Canham CD, Levin SA (1995) Species diversity and ecosystem response to carbon dioxide fertilization: conclusions from a temperate forest model. Global Change Biol 1:373–381

Brookes PC, Landman A, Pruden G, Jenkinson DS (1985) Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol Biochem 17:837–842

Burns RM, Honkala BH (1990) Silvics of North American trees, vol 2 hardwoods. US Department of Agriculture, Washington

Ceulemans R, Mousseau M (1994) Effects of elevated atmospheric CO_2 on woody plants. New Phytol 127:425–446

Ciais P, Tans PP, Trolier M, White JWC, Francey RJ (1995) A large norther hemisphere terrestrial CO_2 sink indicated by the $^{13}C/^{12}C$ ratio of atmospheric CO_2. Science 269:1098–1102

Clark JS (1993) Scaling the population level: effects of species composition and population structure. In: Ehleringer J, Field CB (eds) Scaling physiological processes: leaf to globe. Academic Press, San Diego, pp 255–285

Cotrufo MF, Ineson P (1995) Effects of enhanced atmospheric CO_2 and nutrient supply on the quality and subsequent decomposition of fine roots of *Betula pendula* Roth. and *Picea sitchensis* (Bong.) Carr. Plant and Soil 170:267–277

Coûteaux MM, Mousseau M, Célérier ML, Bottner P (1991) Increased atmospheric CO_2 and litter quality: decomposition of sweet chestnut leaf litter with animal food webs of different complexities. Oikos 61:54–64

Curtis PS, O'Neill EG, Teeri JA, Zak DR, Pregitzer KS (1994) Belowground responses to rising atmospheric CO_2: implications for plants, soil biota, and ecosystem processes. Kluwer, Dordrecht

Davidson EA, Eckert RW, Hart SC, Firestone MK (1989) Direct extraction of microbial biomass nitrogen from forest and grassland soils of California. Soil Biol Biochem 21:773–778

Davidson EA, Hart SC, Firestone MK (1991) Measuring gross nitrogen mineralization, immobilization, and nitrification by ^{15}N isotope dilution in intact soil cores. J Soil Sci 42:335–349

Davidson EA, Hart SC, Firestone MK (1992) Internal cycling of nitrate in soils of a mature coniferous forest. Ecology 73:1148–1156

Davis MB (1989) Insights from paleoecology on global change. Ecol Soc Am Bull 70:222–228

Davis MB, Zabinski C (1992) Changes in geographical range resulting from greenhouse warming: effects on biodiversity in forests. In: Pefers RL, Lovejoy TE (eds) Global warming and biological diversity. Yale University Press, New Haven

Díaz S, Grime JP, Harris J, McPherson E (1993) Evidence of a feedback mechanism limiting plant response to elevated carbon dioxide Nature 364:616–617

Downs MR, Nadelhoffer KJ, Melillo JM, Aber JD (1996) Immobilization of a ^{15}N-labeled nitrate addition by decomposing forest litter. Oecologia 105:141–150

Field CB, Jackson, RB, Mooney HA (1995) Stomatal responses to increased CO_2: implications from plant to global scale. Plant Cell Environ 18:1214–1225

Field CB, Chapin FS, Chiariello NR, Holland EA, and Mooney HA (1996) The Jasper Ridge CO_2 experiment: design and motivation. In Koch GW, Mooney HA (eds) Carbon dioxide and terrestrial ecosystems. Academic Press, San Diego, pp 121–145

Godbold DL, Berntson GM (1997) Elevated atmospheric CO_2 concentrations lead to changes in ectomycorrhizal morphotype assemblages in *Betula papyrifera*. Tree Physiol 17:347–350

Goulden ML Munger JW, Fan SM Daube, BC, Wofsy SC (1996) Exchange of carbon dioxide by a deciduous forest: response to interannual climate variability. Science 271:1576–1578

Hungate BA, Chapin FSI, Zhong H, Holland EA, Field CB (1997) Stimulation of grassland nitrogen cycling under carbon dioxide enrichment. Oecologia 109:149–153

Ineichen K, Wiemken V, Wiemken A (1995) Shoots, roots and ectomycorrhiza formation of pine seedlings at elevated atmospheric carbon dioxide. Plant Cell Environ 18:703–707

Jenkinson DS, Powlson DS (1976) The effects of biocidal treatments on metabolism in soil. V. A method for measuring soil biomass. Soil Biol Biochem 8:209–213

Katz RW, Brown BG (1992) Extreme events in a changing climate: variability is more important than averages. Climatic Change 21:289–302

Kaushal P, Guehl JM, Aussenac G (1989) Differential growth response to atmospheric carbon dioxide enrichment in seedlings of *Cedrus atlantica* and *Pinus nigra* ssp. Laricio var. Corsicana. Can J For Res 19:1351–1358

Keeling CD, Whorf TP (1994) Atmospheric CO_2 records from sites in the SIO air sampling network. In: Boden TA, Kaiser DP, Sepanski JP, Stoss FW (eds) Trends '93: a compendium of data on global change. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, Oak Ridge, pp 16–26

Koch GW, Mooney HA, (eds) (1996) Carbon dioxide and terrestrial ecosystems. Academic Press, San Diego

Körner C (1996) The response of complex multispecies systems to elevated CO_2. In: Walker B, Steffen W (eds) Global change and terrestrial ecosystems. Cambridge University Press, Cambridge, pp 20–42

Körner C, Diemer M, Schäppi B, Zimmermann L (1996) Response of alpine vegetation to elevated CO_2. In: Koch G W, Mooney HA (eds) Carbon dioxide and terrestrial ecosystems. CA, Academic Press, San Diego, pp 177–196

Kurz WA, Apps MJ Stocks BJ, Volney WJA (1995) Global climate change: Disturbance regimes and biospheric feedbacks of temperate and boreal forests. In: Woodwell GM, Mackenzie FT (eds) Biotic feedbacks in the global climatic system: will the warming feed the warming. Oxford University Press, Oxford, pp 119–133

Larigauderie A, Reynolds JF, Strain BR (1994) Root response to CO_2 enrichment and nitrogen supply in loblolly pine. Plant Soil 165:21–32

Magill AH, Aber JD, Hendrichs JJ, Bowden RD, Melillo JM Steudler P (1997) Biogeochemical response of forest ecosystems to simulated chronic nitrogen deposition. Ecol Appl 7:402–415

Maynard DG, Kalra, YP (1993) Nitrate and exchangeable ammonium nitrogen. In: Carter MR (ed) soil sampling and methods of analysis. Lewis, Boca Raton, pp 25–38

Melillo JM, Aber JD, Muratore JF (1982) Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. Ecology 63:621–626

Melillo JM, Prentice IC, Farquhar GD, Schulze ED, Sala OE (1996) Terrestrial biotic responses to enrionmental change and feedbacks to climate. In: Houghton JT, Meira Filho LG, Callanderet BA (eds) climate change 1995:the science of climate change. Cambridge University Press, Cambridge, pp 445–481

Navas ML, Guillerm JL, Fabreguettes J, Roy J (1995) The influence of elevated CO_2 on community structure, biomass and carbon balance of mediterranean old-field microcosms. Global Change Biol 1:325–336

Norby RJ (1994) Issues and perspectives for investigating root responses to elevated atmospheric carbon dioxide. Plant Soil 165:9–20

O'Neill EG (1994) Responses of soil biota to elevated atmospheric carbon dioxide. Plant Soil 165:55–65

O'Neill EG, Norby RJ (1996) Litter quality and decomposition rates of foliar litter produced under elevated CO_2 In: Koch GW, Mooney HA (eds) Carbon dioxide and terrestrial ecosystems. Academic Press, San Diego, pp 87–103

Owens NJP, Rees AP (1989) Determination of nitrogen-15 at submicrogram levels of nitrogen using automated continuous-flow isotope ratio mass spectrometry. Analyst 114:1655–1657

Owensby CE, Auen LM, Coyne PI (1994) Biomass production in a nitrogen-fertilized, tallgrass prairie ecosystem exposed to ambient and elevated levels of CO_2. Plant Soil 165:105–113

Pacala SW, Canham CD, Silander, JA (1993) Forest models defined by field measurement I. The design of a northeastern forest simulator. Can J For Res 23:1980–1988

Pan WL, Bolton RP (1991) Root quantification by edge discrimination using a desktop scanner. Agronomy Journal. 83:1047–1052

Pastor J, Post WH (1986) Influence of climate, soil moisture, and succession on forest carbon and nitrogen cycles. Biogeochemistry 2:3–27

Poorter H (1993) Interspecific variation in the growth response of plants to an elevated ambient CO_2 concentration. Vegetatio 104/105:77–97

Rastetter EB, McKane RB, Shaver GR, Melillo JM (1992) Changes in C storage by terrestrial ecosystems: how carbon-to-nitrogen interactions restrict responses to CO_2 and temperature. Water Air Soil Pollut 64:327–344

Rochefort L, Bazzaz FA, (1992) Growth response to elevated CO_2 in seedlings of four co-occurring birch species. Can J For Res 22:1583–1587

Rogers HH, Peterson CM, McCrimmon JN, and Cure JD (1992) Response of plant roots to elevated atmospheric carbon dioxide. Plant Cell Environ 15:749–752

Rogers HH, Runion GB, Krupa SV (1994) Plant responses to atmospheric CO_2 enrichment with emphasis on roots and the rhizosphere. Environ Pollut 83:155–189

Schimel DS (1995) Terrestrial ecosystems and the carbon cycle. Global Change Biol 1:77–91

Schneider SH (1989) The greenhouse effect: science and policy. Science 243:771–781

Shaver GR, Billings WD, Chapin FSI, Giblin AE, Nadelhoffer KJ, Oechel WC, and Rastetter EB (1992) Global change and the carbon balance of Arctic ecosystems. BioScience 42:433–441

Sheriff DW, Margolis HA, Kaufmann MR, Reich PB (1995) Resource use efficiency. In: Smith WK, Hinckley TMC Physiological ecology of coniferous forests: acquisition, allocation and utilization. Academic Press, New York, pp 143–178

Sørensen P, Jensen ES (1991) Sequential diffusion of ammonium and nitrate from soil extracts to a polytetrafluoroethylene trap for ^{15}N determination. Anal Chim 252:201–203

Stark JM, Hart SC (1997) High rates of nitrification and nitrate turnover in undisturbed coniferous forests. Nature 385:61–64

Tans PP, Fung IY, Takahashi T (1990) Observational constraints on the global atmospheric CO_2 budget. Science 247:1431–1438

Tans PP, Fung IY, Enting IG (1995) Storage versus flux budgets: the terrestrial uptake of CO_2 during the 1980s. In: Woodwell GM, Mackenzie FT (eds) Biotic feedbacks in the global climatic system: will the warming feed the warming? Oxford University Press, Oxford, pp 351–366

Tietema A (1997) Microbial carbon and nitrogen dynamics in coniferous forest floor material collected along a European nitrogen deposition gradient For Ecol Manage (in press)

Vitousek PM (1982) Nutrient cycling and nutrient use efficiency. Am Nat 119:553–572

Wayne PM, Bazzaz FA (1995) Seedling density modifies growth responses of yellow birch maternal familes to elevated carbon dioxide. Global Change Biol 1:315–324

Wessel WW, Tietema A (1992) Calculating gross N transformation rates of ^{15}N pool dilution experiments with acid forest litter: analytical and numerical approaches. Soil Biol Biochem 24: 931–942

Westveld M, Ashman RI, Baldwin HI, Woldsworth RP, Johnson RS, Lambert JH, Lutz HJ, Swain L, Standish M (1956) Natural forest vegetation zones of New England. J For 54:332–338

Wofsy SC, Goulden ML, Munger JW, Fan SM, Bakwin PS, Daube BC, Bassow SL, Bazzaz FA (1993) Net exchange of carbon dioxide in a mid-latitude forest. Science 260:1314–1317

Wullschleger SD, Post WM, Emanuel AW (1995) On the potential for a CO_2 fertilization effect in forests: estimates of the biotic growth factor based on 58 controlled-exposure studies. In: Woodwell GM and Mackenzie FT (eds) Biotic Feedbacks in the global climatic system: will the warming feed the warming? Oxford University Press, Oxford, pp. 85–107

Yanai RD, Fahey TJ, Miller SL (1995) The efficiency of nutrient acquisition by fine roots and mycorrhizae. In: Smith WK, Hinckley TM (eds) Physiological ecology of coniferous forests: acquisition, allocation and utilization. Academic Press, New York, pp 75–103

Zak DR, Pregitzer KS, Curtis PS, Teeri JA, Fogel R, Randlett DL (1993) Elevated atmospheric CO_2 and feedback between carbon and nitrogen cycles. Plant Soil 151:105–117