Global Change Biology (1997) **3,** 247–258

Nitrogen cycling in microcosms of yellow birch exposed to elevated CO_2: simultaneous positive and negative below-ground feedbacks

G. M. BERNTSON* and F. A. BAZZAZ
Harvard University, Department of Organismic and Evolutionary Biology, Biological Laboratories, 16 Divinity Avenue, Cambridge, MA 02138 USA

Abstract

This study investigated simultaneous plant and soil feedbacks on growth enhancement with elevated [CO_2] within microcosms of yellow birch (*Betula alleghaniensis* Britt.) in the second year of growth. Understanding the integrated responses of model ecosystems may provide key insight into the potential *net* nutrient feedbacks on [CO_2] growth enhancements in temperate forests. We measured the net biomass production, C:N ratios, root architecture, and mycorrhizal responses of yellow birch, *in situ* rates gross nitrogen mineralization and the partitioning of available NH_4^+ between yellow birch and soil microbes. Elevated atmospheric [CO_2] resulted in significant alterations in the cycling of N within the microcosms. Plant C/N ratios were significantly increased, gross mineralization and NH_4^+ consumption rates were decreased, and relative microbial uptake of NH_4^+ was increased, representing a suite of N cycling negative feedbacks on N availability. However, increased C/N ratios may also be a mechanism which allows plants to maintain higher growth with a constant or reduced N supply. Total plant N content was increased with elevated [CO_2], suggesting that yellow birch had successfully increased their ability to acquire nutrients during the first year of growth. However, plant uptake rates of NH_4^+ had decreased in the second year. This discrepancy implies that, in this study, nitrogen uptake showed a trend through ontogeny of decreasing enhancement under elevated [CO_2]. The reduced N mineralization and relatively increased N immobilization are a potential feedback which may drive this ontogenetic trend. This study has demonstrated the importance of using an integrated approach to exploring potential nutrient-cycling feedbacks in elevated [CO_2].

Keywords: Betula alleghaniensis, CO_2, feedback, immobilization, mineralization, nitrogen, uptake

Received 29 May 1996; revision accepted 7 September 1996

Introduction

Recent theoretical and empirical analyses have demonstrated that the terrestrial biosphere, especially temperate and boreal forests, is a large sink for atmospheric [CO_2] (Tans *et al*. 1990; Wofsy *et al*. 1993; Ciais *et al*. 1995; Tans *et al*. 1995; Goulden *et al*. 1996). This carbon sequestration may act to slow the rate of future rises in atmospheric [CO_2] levels and thus partially ameliorate the greenhouse effect (Woodwell & Mackenzie 1995). The mechanisms whereby temperate forest ecosystems are able to sequester as much carbon as they do are not well understood, but the primary factors seem to be forest regrowth (Kauppi *et al*. 1992) and fertilization by anthropogenic N inputs (Peterson & Melillo 1985; Schindler & Bayley 1993; Townsend *et al*. 1996). Predictions of the future capacity of forests to sequester carbon with increasing concentrations of [CO_2] in the atmosphere remain tentative. Currently the evidence that increases in atmospheric [CO_2] levels since pre-industrial times have resulted in increased forest productivity is inconclusive (LaMarche *et al*. 1984; Kienast & Luxmoore 1988). Several recent reviews have suggested that the intrinsic capacity for forest ecosystems to increase biomass production in elevated [CO_2] is high (Poorter 1993; Ceulemans & Mousseau 1994; Wullschleger *et al*. 1995). However, several models and experiments have raised serious concerns about whether high growth enhancements in elevated [CO_2] will be realized due to ecosystem-level feedbacks which may reduce nutrient

*Correspondence: tel +1/617-496-4062, fax +1/617-496-5223, e-mail berntson@oeb.harvard.edu

supply and thus constrain actual growth enhancement (reviewed in Schimel 1995).

The hypothesized feedbacks which may constrain long-term growth enhancement all rest on the common assumption that increased growth under elevated [CO_2] is driven by increases in carbon assimilation (Berntson & Bazzaz, in press). Greater productivity itself can place a limitation on [CO_2] growth enhancement due to the stoichiometry of carbon and N within plant biomass, soil organic matter (SOM), and detritus (Comins & McMurtrie 1993; Comins 1994; McMurtrie & Comins 1996). Experimental evidence in support of this hypothesis is rare and inconsistent. Díaz *et al*. (1993) found that elevated [CO_2] resulted in increased microbial population sizes, thereby resulting in a reduction in the amount of available N to plants. In contrast, Zak *et al*. (1993) found that elevated atmospheric [CO_2] increased microbial biomass in the rhizosphere of *Populus tremuloides* and this increase was accompanied with an increase in net N mineralization rates.

Nutrient feedbacks on enhanced growth in an elevated [CO_2] world are not restricted to SOM and soil microbes. Higher plant carbon to nitrogen ratios (C/N) typically lead to slower decomposition rates (Melillo *et al*. 1982; Aber *et al*. 1990). A few studies have experimentally verified this pattern in elevated [CO_2] environments (Boerner & Rebbeck 1995; Cotrufo & Ineson 1995; but see Coûteaux *et al*. 1991; O'Neill & Norby 1996). Increases in plant C/N may result in a negative feedback on N availability through reduced mineralization rates. However, increases in C/N ratios can also be viewed as a mechanism whereby plants are able to maintain a given NPP with a lower or equivalent supply of nutrients (higher nutrient use efficiency, NUE; Vitousek 1982; Sheriff *et al*. 1995). Several studies have also shown that elevated [CO_2] can lead to alterations in the architecture of plant root systems (Berntson & Woodward 1992; Rogers *et al*. 1992) and increases in mycorrhizal symbiosis, especially ectomycorrhizae (reviewed in O'Neill 1994). These changes in root growth and mycorrhizal symbioses may represent important mechanisms of increased soil exploration potential (Berntson 1994; Yanai *et al*. 1995). Additionally, ectomycorrhizae may allow plants to directly utilize SOM nitrogen through ectomycorrhizal production of extracellular enzymes (Maijala *et al*. 1991; Bending & Read 1995). Overall, the direct effects of elevated [CO_2] on plant growth patterns may themselves represent important mechanisms which maintain sustained growth enhancement in elevated [CO_2].

Current experimental data on integrated, whole-system level feedbacks on and mechanisms of sustained growth enhancement under elevated [CO_2] are inconclusive. Studies which have attempted to identify plant- and microbially mediated feedbacks have focused on a relatively narrow subset of possible feedbacks. For example, previous studies have not simultaneously examined *in situ* mineralization rates and the partitioning of available N between plants and soil microbes (Díaz *et al*. 1993; Zak *et al*. 1993). To directly demonstrate that altered microbial activity – mineralization and immobilization – with elevated [CO_2] leads to a negative feedback, it is critical that we simultaneously examine these processes.

The goal of this study is to explore these processes within microcosms of yellow birch (*Betula alleghaniensis* Britt.) during the second year of growth in both ambient and elevated [CO_2]. We chose this experimental system for several reasons. First, due to climatic change and subsequent potential impacts on current forests, regeneration may be a critically important component of forest ecosystem responses to global change (Bazzaz 1990; Bazzaz *et al*. 1996). Studying model systems of regenerating tree saplings can provide valuable insight into this important regeneration stage. Secondly, temperate forest ecosystems represent an important sink for atmospheric [CO_2] (see references above). Knowledge of potential feedbacks on future growth responses to elevated [CO_2] is critical for predicting future carbon sink potential in these systems. Third, yellow birch is a significant component of northern temperate hardwood ecosystems (Harlow *et al*. 1979; Erdmann 1990). For these reasons, our microcosms of regenerating yellow birch may provide valuable insight into the potential nutrient feedbacks on [CO_2] growth enhancement in temperate forests. In this study we measured the net biomass production and plant tissue composition of yellow birch microcosms, *in situ* gross rates of N mineralization and the partitioning of available NH_4^+ between yellow birch and soil microbes.

Materials and methods

Plant preparation, planting design and growth conditions

Seeds of yellow birch were collected from three separate trees in the Prospect Hill tract of the NSF LTER site, Harvard Forest, Petersham, MA. Seeds were germinated in a vermiculite: peat: sand mixture and transplanted to the microcosms when they were 2–3 cm tall and had 3–4 leaves (mid March 1993). Only seedlings of equal size were transplanted. Within each tub a total of 54 seedlings were planted resulting in a density of 150 plants m^{-2}.

Microcosms consisted of large tubs ($0.75 \times 0.5 \times 0.25$ m, 94 L) which contained O (Oe + Oa) and mineral forest soil. The top 10–15 cm of each tub contained a forest floor material (excluding litter) including fine roots. Forest floor material was collected from a 100 m^2 area within the Prospect Hill Tract of the Harvard Forest NSF LTER Site and thoroughly homogenized (removing rocks

> 0.5 cm diameter and roots > 1 mm diameter). The bottom 15 cm of each tub was lined with a mineral soil (A + B horizons) and coarse sand (1:1) mixture to provide adequate drainage below the O layer. Soil at this site is a brown podzolic of the Charlton series (well drained, sandy loam; Lyford *et al*. 1963; Allen 1995). In total, there were 12 tubs. Each tub was used as a replicate microcosm. During the growing season these tubs were distributed within six controlled environment glasshouses. Half of the glasshouse modules were maintained at ambient [CO_2] (≈375 ppm) and the other half at elevated [CO_2] (700 ppm). Adjacent ambient/elevated [CO_2] glasshouse modules were treated as blocks (four replicate microcosms per block). The tubs received natural light. Day/night temperatures were maintained at 26°/19 °C with a 13-h day until the beginning of September (1993). 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 (1993), when > 90% of leaves had senesced (November), the tubs were moved to an outside garden and placed in trenches for the remaining winter months. Tubs were placed in the 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 in 1994 (early March). Fibreglass dividers were inserted through the forest floor material to divide each tub into two halves with equal soil surface area and equal numbers of plants.

^{15}N application

After canopy closure (mid May), we injected highly enriched (98 atom percentage enrichment, Cambridge Isotope Laboratories, Andover, MA) ^{15}N-NH_4Cl into the tubs. 204 one-mL injections of 2.5 mmol ^{15}N labelled solutions were injected into each tub. We selected this level of ^{15}N-NH_4^+ because it represented only 25% of ambient NH_4^+ levels measured in the field (Aber *et al*. 1993). We explored the use of a ^{15}N-NO_3^- label for quantifying fluxes of NO_3^-, but extremely low levels of NO_3^-, which are typical of measurements in the field (Aber *et al*. 1993), precluded reliable measurement of NO_3^- pool sizes and ^{15}N tracer recovery. The injection system we used consisted of 12 one mL syringes which had been mounted on a sliding rack. The syringe cylinders were attached to one half of the rack and the plungers to the other. This design allowed all 12 syringes to be filled and emptied simultaneously. To each syringe we attached 7.62 cm long 22 gauge stainless steel hypodermic needles (Popper and Sons, New Hyde Park, NY, USA) which allowed us to inject to a depth of 7 cm into the O layer. While injecting into the soil, the syringe plungers remained in a fixed location while the needles were pulled through the soil and the solution was evenly distributed vertically through the soil.

Measurements of soil NH_4^+

Soil samples were collected from the O horizon prior to (Pre), immediately after (T_i, within 15 min) and 2 days after injection (T_f). Between 6 and 12 soil cores measuring 0.5 × 8.5 cm were taken from each tub half at each sampling. For each sample type, taken from a given tub (pre, T_i, and T_f), all the cores were pooled into a single sample. Soil pH was measured in suspensions of 2 g air-dried soil in 20 mL of deionized water (Hendershot *et al*. 1993). Soil samples were extracted in 200 mL 0.5 M K_2SO_4 (Brookes *et al*. 1985; Davidson *et al*. 1989). Samples were placed on a shaker for 24 h prior to filtration to remove soil debris and frozen for storage. Concentrations of NH_4^+ in the soil extracts were determined using a Lachat QuickChem AE auto analyser. NH_4^+ was measured using a modified Berthelot reaction with phenol (Maynard & Kalra 1993).

The level of ^{15}N enrichment (atom percentage enrichment) for the NH_4^+ recovered in the soil extracts was determined by sequential diffusion to 0.625 cm diameter pre-combusted glass fibre filters (Gelman AE™) with 20 µL 2 M H_2SO_4 (Sørensen & Jensen 1991). To volatilize the NH_4^+, 0.25 g MgO was added to the extracts (70–90 mL) at the beginning of the diffusion period which lasted for 10 days. Solutions were kept in air-tight urine specimen containers during the diffusion period. 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 swirled by hand two times a day. Glass fibre filters were dried in a desiccator which contained a concentrated H_2SO_4 trap to remove contamination by atmospheric ammonia. Blanks consisted of glass fibre filters with 20 µL 2 M H_2SO_4 which were placed in the desiccator with the samples. Determination of the total N content and ^{15}N content of the glass fibre filters was done using a Europa elemental analyser (model ANCA-sl) attached to a Europa model 20–20 Stable Isotope Analyser via a capillary interface (Europa Instruments, England). The diffusions recovered 103 ± 3% of the N-NH_4^+ within the solutions.

Microbial N content

An estimate of soil microbial biomass was determined by chloroform fumigation (Jenkinson & Powlson 1976) of soil samples followed by direct extraction in 0.5 M K_2SO_4 (Brookes *et al*. 1985; Davidson *et al*. 1989). We selected this method of estimated microbial biomass

because it allowed us to determine the amount of ^{15}N tracer incorporated into the microbial biomass pool. Chloroform-extractable N was determined only on the T_f soil samples. For each T_f sample, half of the sample was directly extracted in K_2SO_4, and the other half was fumigated with chloroform. Total-N and atom percentage enrichment in the K_2SO_4 solutions were determined pre- and post-chloroform fumigation extractions. No Kn or Kc factors were used to estimated microbial N or carbon values. Measurement of carbon, N and ^{15}N content in the K_2SO_4 solutions was performed using the Europa ANCA-sl and 20–20 stable isotope analyser. Dehydrated K_2SO_4 solutions to which no soil had been added were used as blanks. 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. 100–125 mg samples of the dehydrated K_2SO_4 were then processed using the Europa ANCA-sl and Stable Isotope Analyser 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 & Rees 1989). Excess atom percentage enrichment ^{15}N for microbial biomass (^{15}N immobilized by microbes) was calculated as atom percentage enrichment ^{15}N in excess of the natural abundance of ^{15}N (0.3665%).

Plant harvesting, mycorrhizal assessments and root architecture

After the 2 day incubation with the ^{15}N tracer, each microcosm was harvested and all the plant material carefully collected. At the start of harvest, all plant stems were cut approximately one cm above the soil surface. The roots of every plant were carefully washed from the soil by placing the entire soil block in a tub of water, and then carefully disentangling and removing the roots by hand. It was inevitable that some of the fine roots were lost during this procedure, but care was taken to keep each root system as intact as possible. Entire root systems were wrapped in a moist paper towel, placed in an air tight plastic bag (with all the air removed) and frozen prior to root morphological and mycorrhizal measurements. Above-ground plant material was separated into leaves and stems and oven dried for 96 h at 70 °C and weighed to the nearest mg.

To characterize root morphology and mycorrhizal status, we made detailed measurements on subsamples of the fine roots of 20–24 individuals from each microcosm. 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. When taking the root subsamples, ≈ 300 root tips from each sampled individual plant were examined for a total of 6000–7200 root tips per microcosm. For each subsample, the total number of root tips 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, each subsample was laid out in a petri dish with ≈ 2 mm of water to minimize overlap of individual roots and scanned into a computer as 8-bit greyscale images at 118.1 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 & Bolton (1991). From all of these measurements made on the fine root subsamples the following parameters were derived for each microcosm: percentage 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 microcosm 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. Similar criteria were used to separate fine roots from woody roots for air dried samples of tree root systems. One of the more convenient characteristics which allowed us to rapidly separate the fine roots from the woody roots is that the fine roots would disintegrate (fall apart) when rubbed between the fingers, the lignified 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 percentage mycorrhizal root tips.

Total-N and ^{15}N atom percentage enrichment for ground plant material were measured using the same procedure as for the glass fibre filters used in the diffusions and dehydrated K_2SO_4 solutions. Excess ^{15}N atom percentage enrichment for the plant material ($At\%_{p\text{-}excess}$) was calculated as the difference between plant samples following the ^{15}N application and plant samples (leaves) collected prior to injection. Leaves, stems, woody roots and fine roots were separated and determination of carbon and N content made using a Europa ANCA-sl Elemental Analyser. All C/N ratios reported herein are molar C/N ratios (moles carbon per mole N).

N immobilization, uptake, and mineralization calculations

To estimate the gross rate of mineralization (M) and NH_4^+ consumption from the soil pool (C), we used pool dilution calculations. Application of pool dilution equations requires that several assumptions be made. A complete review of these assumptions is beyond the scope of this

paper, but is discussed in detail elsewhere (Davidson *et al*. 1991; Wessel & Tietema 1992; Knowles & Blackburn 1993; Schimel 1993). We used Wessel & Tietema's (1992) formulation of Blackburn's (1979) isotope dilution equations for calculating M and C. We assumed that natural abundance of ^{15}N-NH_4^+ in the soil solution was equal to the natural abundance of ^{15}N (0.3665%). We calculated plant (C_P) and soil microbial (C_M) NH_4^+ consumption rates by partitioning C on the basis of the relative amount of tracer recovered in the plant and soil microbial biomass in excess of natural abundance (Berntson 1996). We used this method for calculating plant and microbial NH_4^+ consumption rates in order to provide a balanced set of N flux estimates. Alternative methods, such as mean tracer pool abundance estimates (Barraclough 1991; Barraclough 1991; Davidson *et al*. 1991), can lead to results which are inconsistent with pool dilution estimates (Berntson 1996). To characterize plant uptake rates in relation to biomass production and root architecture, we derived specific NH_4^+ uptake rates (SNU). Six different SNU indices were derived by dividing C_P by total plant biomass, fine root biomass, root length, root surface area, number of root tips, and number of mycorrhizal root tips.

Statistical analysis

Data were analysed with analysis of variance (ANOVA). Blocks (adjacent pairs of ambient and elevated [CO_2] glasshouse modules) were treated as fixed factors, and the [CO_2], block, and [CO_2] by block interaction terms were tested with the residual mean square as the denominator (Data Desk v4.2, Velleman 1994). For each ANOVA, assumptions of normality and homoscedasticity were tested using a combination of normal probability plots of residuals. When necessary, variables were log transformed to improve compliance with assumptions of normality and homoscedasticity.

Results

Plant growth responses to elevated [CO_2]

Total plant biomass within each tub increased with elevated [CO_2] by an average of 14% (Table 1), though this increase was not statistically significant at $P < 0.05$. Despite significant increases in C/N ratios, increases in plant biomass were accompanied by a significant increase in the total amount of N in plant tissue (Table 1). Allocation of biomass to roots was marginally increased in elevated [CO_2] ($P < 0.10$), but the amount of fine root biomass relative to woody root biomass was not affected by elevated [CO_2] (Table 1). Fine root morphology (root diameter, specific root length, and specific root number) were not significantly changed by elevated [CO_2]. Total fine root mass, root length and root surface area were all significantly increased with elevated [CO_2], though the number of root tips was not. The single largest below-ground response was with ectomycorrhizae. The percentage of root tips which were colonized and the total number of colonized root tips showed large increases which were highly significant (Table 1).

The C/N ratios of plant organs were significantly increased by elevated [CO_2] only for leaves and fine roots (Table 2). These increases in leaves and fine roots were by 12% and 6%, respectively. Stems and woody roots, the organs which showed the overall highest C/N ratios, showed no significant changes with elevated [CO_2]. Whole plant C/N ratios showed a marginally significant ($P < 0.10$) increase of 6.6% (Table 2).

Nitrogen cycling

Levels of extractable NH_4^+ within the soil prior to the injection of the ^{15}N solutions were not different for the two [CO_2] levels (Figs 1 and 2). Changes in pool sizes from time i to f were not significantly different from zero ($P = 0.54$). Soil NH_4^+ levels were 25–50% of those typically observed in the field (Aber *et al*. 1993). The total recovery (in the soil NH_4^+, plant, and chloroform-extractable microbe pools) of ^{15}N was about 45% of the total amount of tracer applied for both ambient and elevated [CO_2]. It is likely that large size and 'open system' design of the soil monoliths (O layer lying directly on the sand/mineral soil which was not measured) contributed a large fraction of this loss. Given that the loss was equal between the [CO_2] treatments, this suggests that a mechanism(s) common to both experimental treatments and therefore is not likely to have biased the ^{15}N treatment comparisons.

Elevated [CO_2] resulted in no significant change in chloroform-extractable microbial biomass-N, but a marginally significant increase in total plant N (Figs 1 and 2). From the pool dilution calculations, elevated [CO_2] resulted in a decrease in M which was only nearly significant ($P = 0.12$) and a significant decrease in C (Figs 1 and 2). Soil pH averaged 3.86 with a standard error of 0.05. C_M decreased with elevated [CO_2], corresponding to the decrease in C, but this reduction was not statistically significant ($P = 0.33$) due to fairly large variation in ^{15}N M. In contrast, C_P showed a significant decrease with elevated [CO_2]. Thus, the reduction in C in elevated [CO_2] was associated with a greater reduction in C_P than in C_M.

Plant specific N uptake rates

The increases in plant biomass and root architectural measures with elevated [CO_2] (Table 1) in conjunction with decreases in C_P (Figs 1 and 2) led to large decreases

Table 1 Yellow birch NPP, allocation patterns, root size, architecture, and mycorrhizal infection. *P*, probability that null hypothesis (ambient CO_2 = elevated CO_2). ER, enhancement ratio (elevated CO_2/ambient CO_2 average).

		Ambient CO_2		Elevated CO_2			
Variable	Units	Average	SE	Average	SE	P	ER
Plant biomass	g m^{-2}	338	21	386	21	0.145	1.14
Total plant nitrogen	g m^{-2}	2.72	0.11	2.96	0.11	0.075	1.09
Root/shoot mass	–	0.39	0.01	0.41	0.01	0.073	1.05
Fine/woody root mass	–	0.80	0.04	0.79	0.05	0.823	0.99
Fine root mass	g m^{-2}	57.8	4.7	69.1	4.7	0.043	1.19
Root length	km m^{-2}	11.00	1.05	12.78	1.05	0.051	1.16
Root surface area	m^2 m^{-2}	6.22	0.57	7.16	0.57	0.052	1.15
#Root tips	M m^{-2}	3.44	0.34	4.05	0.34	0.123	1.18
#Mycorrhizal root tips	M m^{-2}	1.36	0.22	2.46	0.22	0.000	1.81
Mycorrhizal root tips	% Colonization	39%	3%	61%	3%	0.000	1.56
Root diameter	μm	0.18	0.00	0.18	0.00	0.500	0.99
Specific root length	cm mg^{-2}	18.9	0.6	18.4	0.6	0.457	0.97
Specific root number	K g^{-1}	59.4	2.4	58.5	2.4	0.853	0.98

Table 2 Molar C/N ratios for entire NPP (whole plant) and separate plant organs. *P*, probability that null hypothesis (ambient CO_2 = elevated CO_2). ER, enhancement ratio (elevated CO_2/ambient CO_2 average).

	Ambient CO_2		Elevated CO_2			
	Average	SE	Average	SE	*P*	ER
Total	65.2	1.3	69.5	2.1	0.096	1.07
Leaves	44.3	1.1	49.7	1.2	0.000	1.12
Stem	125.9	3.7	122.7	4.2	0.555	0.97
Fine roots	35.1	0.8	37.3	1.2	0.015	1.06
Woody roots	124.9	5.6	113.1	6.5	0.259	0.91

in SNU (Table 3). These decreases were least apparent in relation to biomass, as the magnitude (as estimated by the ratio of elevated to ambient [CO_2] means and the level of significance) of [CO_2] enhancement on biomass was less than for fine root mass and root architectural measures. The single greatest decrease in SNU was for the total number of mycorrhizal root tips due to the large increase in mycorrhizal infection rates in elevated [CO_2].

Discussion

Negative feedbacks

Within this study, we found several plant and microbial responses which may represent potentially important negative feedbacks on [CO_2]-induced growth enhancement. These responses to elevated [CO_2] include increases in the C/N ratios of leaf and fine root material, reductions *in situ* mineralization (M) and consumption rates (C), and increased NH_4^+ microbial uptake rates (C_M) relative to plant uptake rates (C_P).

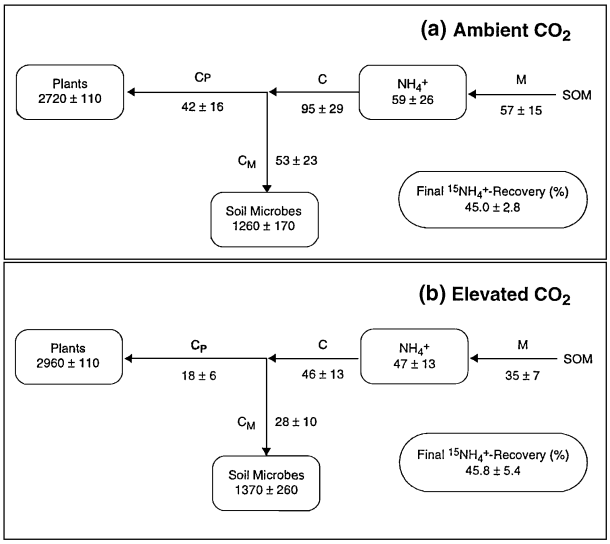

Fig. 1 Diagramatic representation of the measured nitrogen pools and fluxes within the experimental microcosms. Rectangles represents pools of nitrogen. Each arrow represents a flux of nitrogen between pools. All pool sizes are given in mg N m^{-2}. All fluxes are given in mg N m^{-2} d^{-1}. The values given for every pool and flux term are averages ± a single standard error of the mean. The oval in the bottom right of each sub-figure gives the total recovery of the applied ^{15}N label in the pools included in the figure at the end of the experiment relative to the amount of label added. (a) shows the results for the replicate soil microcosms/monoliths in ambient (375 ppm) [CO_2]. (b) shows the results for the replicate soil microcosms/monoliths in elevated (700 ppm) [CO_2].

The significant increase in C/N ratios for leaves and fine

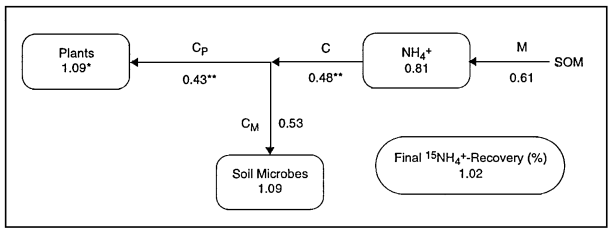

Fig. 2 Diagrammatic representation of the effects of elevated CO_2 on nitrogen pools and fluxes within experimental micrcosms. See Fig. 1 for a description of the layout of the figure. The values associated with each pool and flux term are ratios of elevated over ambient $[CO_2]$ means from Fig. 1. An asterisk is added to a $[CO_2]$ enhancement ratio to indicate the level of significance between CO_2 levels (**$P < 0.05$, *$P < 0.10$).

roots (Table 2) represents a potential negative feedback. In this relatively short study, the input of organic carbon to the soil from plant biomass is most likely to have come from the leaf and fine root pools which can show high turnover within period less than a year (Waring & Schlesinger 1986; Berntson *et al*. 1995). The increases in C/N ratios we observed with elevated $[CO_2]$ may have been the mechanism behind our decrease decomposition rates (Boerner & Rebbeck 1995; Cotrufo & Ineson 1995). However, several researchers have suggested that elevated $[CO_2]$ may lead to transitory or non-significant reductions in decomposition rates (Coûteaux *et al*. 1991; O'Neill & Norby 1996). Uncertainties about predicting decomposition rates in elevated $[CO_2]$ stem primarily from three sources. First, plant-derived detritus which decomposes in natural ecosystems has usually senesced naturally. The studies which have observed decreases in decomposition rates of plant material associated with elevated C/N ratios have typically studied fresh, not senesced plant material. If the net effects of retranslocation of nutrients prior to senescence equalizes C/N ratios, then we might not expect alterations in decomposition rates (O'Neill & Norby 1996). Second, short-term reductions in decomposition rates are likely the result of increases in C/N ratios due to increased non-structural carbohydrates rather than increases in structural components (lignin, holocellulose) which are more resistant to decay (Melillo *et al*. 1989; Aber *et al*. 1990). Third, biotic and abiotic factors can play a critically important role in determining decomposition rates. Changes in soil biota, soil moisture, and temperature which may be indirectly altered through global change may represent controls over decomposition rates which are more important that small but statistically significant changes in tissue quality or decomposition rates in 'ambient' environments (O'Neill 1994; O'Neill & Norby 1996). These caveats aside, our measurements of *in situ* gross NH_4^+ transformation rates within the soil provide us with additional evidence in support of the role increased C/N ratios of plant material. While not highly statistically significant, there was a clear trend toward decreasing gross M in elevated $[CO_2]$. In addition to altered chemistry of above- and below ground derived litter, it is also possible that changes in the chemistry and quantity of root exudates could have also played a role in altering observed mineralization rates (Whipps 1985; Norby *et al*. 1987; Rouhier *et al*. 1994).

In addition, C was significantly reduced while soil NH_4^+ levels (standing and net changes) were not significantly affected by elevated $[CO_2]$. Thus, the *in situ* rate of N cycling within our microcosms was significantly reduced in the second year of exposure to elevated $[CO_2]$. Our observations of reduced N cycling within the soil do not allow us to infer whether changes in carbon loss through decomposition were significantly affected. It has been clearly established that changes in N and carbon fluxes associated with decomposition are not identical. Within the first few months to years of decomposition N is typically immobilized by decaying litter and the extent (duration and magnitude) of immobilization tends to be greater when carbon, lignin and/or holocellulose to N ratios are high (Aber & Melillo 1982; Melillo *et al*. 1982; Melillo *et al*. 1983; Melillo *et al*. 1989; Taylor *et al*. 1989; Aber *et al*. 1990). Thus, it is possible that the reduced N cycling we observed was temporally disconnected from changes in carbon (or mass) loss from decomposing material. This observation is important because reductions in N cycling rates, independent of changes in rates of carbon loss through decomposition, may place a constraint on maximum biomass production through nutrient limitation.

Along with the overall reduction in C, C_M and C_P showed reductions with elevated $[CO_2]$. However, the relative magnitude of reduction in C_M was not as great as C or C_P, nor was it a statistically significant decrease, due to the greater amount of ^{15}N recovered in chloroform-extractable microbial biomass than in plant biomass. This observation is counter to the predictions of Díaz *et al*. (1993) and Zak *et al*. (1993) who predicted greater microbial biomass and activity with the increased plant-derived carbon inputs into the soil in elevated $[CO_2]$. It is possible that the increased C/N ratios led to a N limitation which constrained maximum microbial growth and N immobilization. In contrast, C_P was significantly reduced in elevated $[CO_2]$. Thus, while elevated $[CO_2]$ reduced overall gross consumption rates from the NH_4^+ pool, these reductions were greater for plants than soil microbes. The observed reduction in C_P was accompanied by a marginally significant *increase* in the total amount of N in plant biomass, even with increases in C/N ratios. Several studies have documented transitory enhancements in plant growth in elevated $[CO_2]$ (Bazzaz *et al*. 1993), which may be driven by factors including artificial limitations imposed by small growth containers (Thomas

Table 3 Plant nitrogen uptake rates (C_P) and specific nitrogen (SNU) in yellow birch in relation to root size, architecture, and mycorrhizal infection for both NH_4^+ and NO_3^-. *P*, probability that null hypothesis (ambient CO_2 = elevated CO_2). er, enhancement ratio (elevated CO_2/ambient CO_2 average).

			Ambient CO_2		Elevated CO_2			
NH_4^+		Units	Average	SE	Average	SE	*P*	ER
	C_P	mg N m^{-2} day^{-1}	42.3	16.4	18.1	6.1	0.020	0.43
SNU	Total biomass	mg N g^{-1} day^{-1}	0.124	0.043	0.050	0.019	0.148	0.41
SNU	Fine root mass	mg N g^{-1} day^{-1}	0.680	0.211	0.260	0.080	0.072	0.38
SNU	Root length	mg N km^{-1} day^{-1}	3.41	0.93	1.36	0.38	0.056	0.40
SNU	Root surface area	mg N m^{-2} day^{-1}	6.10	1.71	2.44	0.70	0.059	0.40
SNU	Root tips	mg N M day^{-1}	10.9	3.1	4.3	1.2	0.070	0.39
SNU	Mycorrhizal root tips	mg N M day^{-1}	24.5	5.4	7.8	1.8	0.040	0.32

& Strain 1991; but see McConnaughay *et al*. 1993), or plant ontogeny (Coleman *et al*. 1993). The greater total plant N content we observed implies that average net N uptake was greater over the full duration of the study. However, our measurements of C_P during a short period during the second year of growth show decreased uptake rates. This discrepancy implies that, in this study, N uptake shows a trend through ontogeny of decreasing enhancement under elevated [CO_2]. If this pattern continues or is maintained, future reductions in NH_4^+ consumption and therefore negative feedbacks on future plant growth enhancement in elevated [CO_2] are possible.

Positive feedbacks and mechanisms of sustained growth enhancement

We observed two distinct plant-mediated potential positive feedbacks on and mechanisms of sustained growth enhancement under elevated [CO_2] that may be particularly important when the supply of N is limited. First is the increased C/N ratio of plant material. From the perspective of inputs into SOM, this increase represents a negative feedback via nutrient supply. However, from the perspective of plant growth, this alteration in tissue composition represents an increase in resource or nutrient use efficiency (NUE or RUE, Vitousek 1982; Sheriff *et al*. 1995) which means that plants can maintain or increase total biomass production even when N uptake is unchanged or possibly even decreased.

Second, the increase in plant fine root biomass, root architectural measures, and especially mycorrhizal symbiosis in elevated [CO_2] represents a potential positive feedback on sustained growth enhancement (Table 1). O'Neill and colleagues (O'Neill *et al*. 1987; O'Neill & Norby 1988; O'Neill *et al*. 1991; O'Neill 1994) have suggested that increases in mycorrhizal infection (especially ectomycorrhizae) under elevated [CO_2] represent a potentially critical mechanism whereby plants may increase their ability to acquire soil resources. Increases in mycorrhizal infection can lead to significant increases in the capacity of resource acquisition even in the absence of changes in root architecture or physiology (Yanai *et al*. 1995). The increases in fine root mass and length, and surface area may have further increased the capacity of roots to forage within the soil. For the microcosms of yellow birch which we studied, increases in mycorrhizal symbiosis were the most dramatic alteration which may have increased plant capacity for nutrient acquisition under elevated [CO_2].

The potential increase in nutrient capture which we might have expected with the changes in root architecture and increases in ectomycorrhizal colonization did not take place. Instead, we observed decreases in whole plant NH_4^+ uptake rates (C_P). Thus, when we look at specific nutrient uptake rates, they show quite large decreases under elevated [CO_2] (Table 3). This is especially the case for the rate of NH_4^+ for a given number of mycorrhizal root tips, where we saw the ratio of elevated [CO_2] to ambient [CO_2] means of only 0.32. Without having directly estimated uptake rates, we would have expected that the yellow birch would have shown increases in NH_4^+ acquisition. The significant reductions in C_P and SNU suggest that controls of N supply and availability within the soil which may be more important than potential changes in plant growth form and mycorrhizal symbioses.

Plant–microbe interactions

The ability of microbes to compete for soil inorganic N is strongly linked to the supply of labile organic compounds from plants (Riha *et al*. 1986). This dependency makes it difficult to make *a priori* predictions on the expected behaviour of heterotrophic competition for N with plants under elevated [CO_2]. We know that net biomass production under elevated [CO_2] is enhanced (for a reviews see

Bazzaz 1990 and Poorter 1993). Also, there is growing evidence of enhanced continuous input of plant-derived SOM through exudation and root turnover (Norby *et al*. 1987; van Veen *et al*. 1991; Billès *et al*. 1993; Pregitzer *et al*. 1995; Berntson 1996). This increase in supply of organic carbon into the soil could result in an increase in the size and or activity of the microbial population thereby increasing the relative competitive ability of soil microbes, as was observed by Díaz *et al*. (1993). However, increases in soil microbial populations may result in increases in decomposition rates, thereby increasing the supply of nutrients to both plants and soil microbes (Zak *et al*. 1993). However, it is also possible that plant derived SOM in elevated [CO_2] atmospheres will be of poorer quality, thereby resulting in reduced rates of utilization by microbes as well as decomposition rates (see above, Hobbie & Melillo 1984), so that the increased input of plant detritus to the soil could have no net effect on microbial populations. Thus, if we look at the increased supply of carbon to the soil on an absolute basis we may overestimate the potential carbon reserves which can be utilized by microbes and thus their altered role in immobilizing/supplying nutrients. Furthermore, changes in the size and architecture of plant root systems (Berntson & Woodward 1992; Berntson 1994) and increases in the level of mycorrhizal infection (reviewed in O'Neill 1994; Ineichen *et al*. 1995) which have been documented with increasing [CO_2] levels may result in plants which are better able to compete for available N. In this study, we found preliminary evidence that plant uptake was more strongly negatively affected by elevated [CO_2] than was microbial uptake. This represents a shift in rates of NH_4^+ uptake in favour of the soil microbes.

Caveats on extrapolation

The results derived from this study should be viewed as indicators of the potential for feedbacks on future forest productivity in elevated [CO_2] environments. This study was carried out with microcosms of forest soil, planted with a high density of yellow birch, and maintained in controlled environments in ambient or approximately double ambient atmospheric [CO_2] concentrations for just over a year. The results of this study suggest that rising levels of atmospheric [CO_2] concentrations can lead to simultaneous changes in plant growth, mycorrhizal associations, and N cycling. Together, the contrasting potential positive and negative feedbacks appear to be outweighed by reduction in N cycling rates. This suggests that reductions in N availability may over-ride the increased capacity for nutrient acquisition by plants through changes in root architecture and mycorrhizal associations. To assess this onset of N limitation, future studies should place an emphasis on documenting how instantaneous gross fluxes of N within the soil lead to changes in various plant and soil pool sizes through time. The possibility of whether the results we have documented here will be observed in future forest ecosystems in an elevated [CO_2] environment depends on several factors which this study has not directly examined. These factors include the potential for other components of environmental change (N deposition, climatic change), changes in forest cover and in species composition, and ontogeny of plant (seedlings and saplings vs. mature trees) and soil responses.

Acknowledgements

Special thanks for this project belong to R. Boone for the introduction to ^{15}N techniques, and J. Nevins for invaluable help with the mass spectrometer. A. Townsend, S. Catovsky, C. Field, B. Hungate and two anonymous reviewers provided insightful comments on a previous draft of this manuscript. For GMB, this research was performed under appointment to the Graduate Fellowships for Global Change Program administered by Oak Ridge Institute for Science and Education for the U.S. Department of Energy, Office of Health and Environmental Research, Atmospheric and Climate Research Division.

References

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

Aber JD, Melillo JM (1982) Nitrogen immobilization in decaying hardwood leaf litter as a function of initial nitrogen and lignin content. *Canadian Journal of Botany*, **60**, 2263–2269.

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. *Canadian Journal of Botany*, **68**, 2201–2208.

Allen A (1995) *Soil Survey and Land-Use Map Update Activities at Harvard Forest*. 6th Annual Harvard Forest Ecology Symposium, Petersham, MA.

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

Barraclough D (1991) The use of mean pool abundances to interpret nitrogen-15 tracer experiments. II. Application. *Plant and Soil*, **131**, 97–105.

Bazzaz FA (1990) The response of natural ecosystems to the rising global CO_2 levels. *Annual Review of Ecology and Systematics*, **21**, 167–196.

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

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.

Bending GD, Read DJ (1995) The structure and function of the vegetative mycelium of ectomycorrhizal plants: VI. Activities of nutrient mobilizing enzymes in birch litter colonized by *Paxillus involutus* (Fr.) Fr. *New Phytologist*, **130**, 411–417.

Berntson GM (1994) Modelling root architecture. Are there tradeoffs between efficiency and potential of resource acquisition? *New Phytologist*, **127**, 483–493.

Berntson GM (1996) *Root Growth and Nitrogen Cycling in Temperate Deciduous Forests in an Elevated CO_2 World*. PhD thesis, Harvard University, Cambridge, MA, USA.

Berntson GM, Bazzaz FA (in press) Belowground positive and negative feedbacks on CO_2 growth enhancement. *Plant and Soil*.

Berntson GM, Farnsworth EJ, Bazzaz FA (1995) Allocation, within and between organs, and the dynamics of root length changes in two birch species. *Oecologia*, **101**, 439–447.

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

Billès G, Rouhier H, Bottner P (1993) Modifications of the carbon and nitrogen allocations in the plant (Triticum aestivum L.) soil system in response to increased atmospheric CO2 concentration. *Plant and Soil*, **157**, 215–225.

Blackburn TH (1979) Method for measuring rates of NH_4^+ turnover in anoxic marine sediments, using a ^{15}N-NH_4^+ dilution technique. *Applied and Environmental Microbiology*, **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 and Soil*, **170**, 149–157.

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 Biology and Biochemistry*, **17**, 837–842.

Ceulemans R, Mousseau M (1994) Effects of elevated atmospheric CO_2 on woody plants. *New Phytologist*, **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.

Coleman JS, McConnaughay KDM, Bazzaz FA (1993) Elevated CO_2 and plant nitrogen use: is reduced tissue nitrogen concentration size-dependent? *Oecologia*, **93**, 195–200.

Comins HN (1994) Equilibrium analysis of integrated plant-soil models for prediction of the nutirent limited growth response to CO_2 Enrichment. *Journal of Theoretical Biology*, **71**, 369–385.

Comins HN, McMurtrie RE (1993) Long-term response of nutrient-limited forests to CO_2 enrichment: Equilibrium behavior of plant-soil models. *Ecological Applications*, **3**, 666–681.

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. & *Picea sitchensis* (Bong.) Carr. *Plant and Soil*, **170**, 267–277.

Coûteaux MM, Mousseau M, Célérier ML, Bottner P (1991) Increased atmospheric CO2 and litter quality: decomposition of sweet chestnut leaf litter with animal food webs of different complexities. *Oikos*, **61**, 54–64.

Davidson EA, Eckert RW, Hart SC, Firestone MK (1989) Direct extraction of microbial biomass nitrogen from forest and grassland soils of California. *Soil Biology and Biochemistry*, **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. *Journal of Soil Science*, **42**, 335–349.

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.

Erdmann GG (1990) Betula alleghaniensis Britton. Yellow birch. In: *Silvics of North America*, Vol. 2, *Hardwoods* (eds Burns, Honkala), pp. 158–171. Agriculture Handbook 654. U.S. Department of Agriculture, Washington, D.C.

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.

Harlow WM, Harrar ES, White FM (1979) *Textbook of Dendrology*. McGraw-Hill, New York.

Hendershot WH, Lalande H, Duquette M (1993) Soil reaction and exchangeable acidity. In: *Soil Sampling and Methods of Analysis* (ed. Carter MR), pp. 141–145. Lewis Publishers, Boca Raton.

Hobbie JE, Melillo JM (1984) Comparative carbon and energy flow in ecosystems. Role of microbes in global carbon cycling. *Current Perspectives in Microbial Ecology*, **6**, 389–393.

Ineichen K, Wiemken V, Wiemken A (1995) Shoots, roots and ectomycorrhiza formation of pine seedlings at elevated atmospheric carbon dioxide. *Plant, Cell, and Environment*, **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 Biology and Biochemistry*, **8**, 209–213.

Johnson DW (1992) Nitrogen retention in forest soils. *Journal of Environmental Quality*, **21**, 1–12.

Kauppi PE, Mielikäinen K, Kuusela K (1992) Biomass and carbon budget of European forests, 1971–1990. *Science*, **256**, 70–74.

Kienast F, Luxmoore RJ (1988) Tree-ring analysis and conifer growth responses to increased atmospheric CO_2 levels. *Oecologia*, **76**, 487–495.

Knowles R, Blackburn TH (1993) *Nitrogen Isotope Techniques*. Academic Press, Inc., San Diego.

LaMarche VC, Graybill DA, Fritts HC, Rose MR (1984) Increasing atmospheric carbon dioxide: Tree ring evidence for growth enhancement in natural vegetation. *Science*, **225**, 1019–1021.

Lyford WH, Goodlett JC, Coates WH (1963) *Landforms, Soils with Fragipans, and Forests on a Slope in the Harvard Forest. Harvard University*, Harvard Forest. Report No. 8.

Maijala P, Ragerstadt KV, Raudaskoski M (1991) Detection of extracellular cellulolytic and proteolytic activity in ectomycorrhizal fungi and *Heterobasidion annosum* (Fr.) Bref. *New Phytologist*, **117**, 643–648.

Maynard DG, Kalra YP (1993) Nitrate and exchangeable ammonium nitrogen. In: *Soil Sampling and Methods of Analysis* (ed. Carter MR), pp. 25–38. Lewis Publishers, Boca Raton.

McConnaughay KDM, Berntson GM, Bazzaz FA (1993) Limitations to CO_2-induced growth enhancement in pot studies. *Oecologia*, **94**, 550–557.

McMurtrie RE, Comins HN (1996) The temporal response of forest ecosystems to doubled atmospheric CO_2 concentration. *Global Change Biology*, **2**, 49–58.

Melillo JM, Aber JD, Linkins AE, Ricca A, Fry B, Nadelhoffer KJ (1989) Carbon and nitrogen dynamics along the decay continuum: Plant litter to soil organic matter. *Plant and Soil*, **115**, 189–198.

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

Melillo JM, Naiman RJ, Aber JD, Eshleman KN (1983) The influence of substrate quality and stream size on wood decomposition dynamics. *Oecologia*, **58**, 281–285.

Norby RJ, O'Neill EG, Hood WG, Luxmoore RJ (1987) Carbon allocation, root exudation and mycorrhizal colonization of Pinus echinata seedlings grown under elevated CO2 enrichment. *Tree Physiology*, **3**, 203–210.

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

O'Neill EG, Luxmoore RJ, Norby RJ (1987) Increases in mycorrhizal colonization and seedling growth in *Pinus echinata* and *Quercus alba* in an enriched CO_2 atmosphere. *Canadian Journal of Forest Research*, **17**, 878–883.

O'Neill EG, Norby RJ (1988) Differential responses of ecto- and endomycorrhizae to elevated atmospheric CO2. *Bulletin of the Ecological Society of America*, **69**, 248–249.

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

O'Neill EG, O'Neill RV, Norby RJ (1991) Hierarchy theory as a guide to mycorrhizal research on large-scale problems. *Environmental Pollution*, **73**, 271–284.

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

Peterson BJ, Melillo JM (1985) The potential storage of carbon caused by eutrophication of the biosphere. *Tellus*, **37B**, 117–127.

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

Pregitzer KS, Zak DR, Curtis PS, Kubiske ME, Teeri JA, Vogel CS (1995) Atmospheric CO_2, soil nitrogen and turnover of fine roots. *New Phytologist*, **129**, 579–585.

Riha SJ, Campbell GS, Wolfe J (1986) A model of competition for ammonium among heterotrphs, nitrifiers and roots. *Soil Science Society of America Journal*, **50**, 1463–1466.

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

Rouhier H, Billés G, El Kohen A, Mousseau M, Bottner P (1994) Effect of elevated CO_2 on carbon and nitrogen distribution within a tree (*Castanea sativa* Mill.) – soil system. *Plant and Soil*, **162**, 281–292.

Schimel DS (1993) *Theory and Application of Tracers*. Academic Press, Inc., San Diego.

Schimel DS (1995) Terrestrial ecosystems and the carbon cycle. *Global Change Biology*, **1**, 77–91.

Schindler DW, Bayley SE (1993) The biosphere as an increasing sink for atmospheric carbon: Estimates from increased nitrogen deposition. *Global Biogeochemical Cycles*, **7**, 717–733.

Sheriff DW, Margolis HA, Kaufmann MR, Reich PB (1995) Resource use efficiency. In: *Physiological Ecology of Coniferous Forests: Acquisition, Allocation and Utilization* (eds Smith WK, Hinckley TM), pp. 143–178. Academic Press, New York.

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

Tans PP, Fung IY, Enting IG (1995) Storage vs. flux budgets: The terrestrial uptake of CO_2 during the 1980s. In: *Biotic Feedbacks in the Global Climatic System: Will the Warming Feed the Warming?* (eds Woodwell GM, Mackenzie FT), pp. 351–366. Oxford University Press, Oxford.

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

Taylor BR, Parkinson D, Parsons WFJ (1989) Nitrogen and lignin content as predictors of litter decay rates: A microcosm test. *Ecology*, **70**, 97–104.

Thomas RB, Strain BR (1991) Root restriction as a factor in photosynthetic acclimation of cotton seedlings grown in elevated carbon dioxide. *Plant Physiology*, **96**, 627–634.

Townsend AR, Braswell BH, Holland EA, Penner JE (1996) Spatial and temporal patterns in potential terrestrial carbon storage resulting from deposition of fossil fuel derived nitrogen. *Ecological Applications*, **6**, 806–814.

van Veen JA, Liljeroth E, Lekkerkerk LJA (1991) Carbon fluxes in plant-soil systems at elevated atmospheric CO_2 levels. *Ecological Applications*, **1**, 175–181.

Velleman PF (1994) *Data Desk®* Statistics Guide. Volume II. Data Description Inc., Ithaca, NY, USA,

Vitousek PM (1982) Nutrient cycling and nutrient use efficiency. *American Naturalist*, **119**, 553–572.

Waring RH, Schlesinger (1986) *Forest Ecosystems: Conepts and Management*. Academic Press, New York.

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 Biology and Biochemistry*, **24**, 931–942.

Whipps JM (1985) Effect of CO2-concentration on growth, carbon distribution and loss of carbon from the roots of maize. *Journal of Experimental Botany*, **36**, 644–651.

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.

Woodwell GM, Mackenzie FT (1995) *Biotic Feedbacks in the Global Climatic System: Will the Warming Feed the Warming?* Oxford, Oxford University Press.

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: *Biotic Feedbacks in the Global Climatic System: Will the Warming Feed the Warming?* (eds Woodwell GM, Mackenzie FT), pp. 85–107. Oxford University Press, Oxford.

Yanai RD, Fahey TJ, Miller SL (1995) The efficiency of nutrient acquisition by fine roots and mycorrhizae. In: *Physiological Ecology of Coniferous Forests: Acquisition, Allocation and Utilization* (eds Smith NK, Hinckley TM), pp. 75–103. Academic Press, New York.

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 and Soil*, **151**, 105–117.