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Combined effects of atmospheric CO_2 and N availability on the belowground carbon and nitrogen dynamics of aspen mesocosms

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Abstract It is uncertain whether elevated atmospheric CO_2 will increase C storage in terrestrial ecosystems without concomitant increases in plant access to N. Elevated CO_2 may alter microbial activities that regulate soil N availability by changing the amount or composition of organic substrates produced by roots. Our objective was to determine the potential for elevated CO_2 to change N availability in an experimental plant-soil system by affecting the acquisition of root-derived C by soil microbes. We grew *Populus tremuloides* (trembling aspen) cuttings for 2 years under two levels of atmospheric CO_2 (36.7 and 71.5 Pa) and at two levels of soil N (210 and 970 μg N g^{-1}). Ambient and twice-ambient CO_2 concentrations were applied using open-top chambers, and soil N availability was manipulated by mixing soils differing in organic N content. From June to October of the second growing season, we measured midday rates of soil respiration. In August, we pulse-labeled plants with $^{14}CO_2$ and measured soil $^{14}CO_2$ respiration and the ^{14}C contents of plants, soils, and microorganisms after a 6-day chase period. In conjunction with the August radio-labeling and again in October, we used ^{15}N pool dilution techniques to measure in situ rates of gross N mineralization, N immobilization by microbes, and plant N uptake. At both levels of soil N availability, elevated CO_2 significantly increased whole-plant and root biomass, and marginally increased whole-plant N capital. Significant increases in soil respiration were closely linked to increases in root biomass under elevated CO_2. CO_2 enrichment had no significant effect on the allometric distribution of biomass or ^{14}C among plant components, total ^{14}C allocation belowground, or cumulative (6-day) $^{14}CO_2$ soil respiration. Elevated CO_2 significantly increased microbial ^{14}C contents, indicating greater availability of microbial substrates derived from roots. The near doubling of microbial ^{14}C contents at elevated CO_2 was a relatively small quantitative change in the belowground C cycle of our experimental system, but represents an ecologically significant effect on the dynamics of microbial growth. Rates of plant N uptake during both 6-day periods in August and October were significantly greater at elevated CO_2, and were closely related to fine-root biomass. Gross N mineralization was not affected by elevated CO_2. Despite significantly greater rates of N immobilization under elevated CO_2, standing pools of microbial N were not affected by elevated CO_2, suggesting that N was cycling through microbes more rapidly. Our results contained elements of both positive and negative feedback hypotheses, and may be most relevant to young, aggrading ecosystems, where soil resources are not yet fully exploited by plant roots. If the turnover of microbial N increases, higher rates of N immobilization may not decrease N availability to plants under elevated CO_2.

Key words Atmospheric CO_2 · C cycle · N cycle · *Populus tremuloides* Michx. · Rhizodeposition

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Introduction

The stimulation of photosynthesis by rising atmospheric CO_2 has the potential to increase carbon (C) storage in terrestrial ecosystems and slow rates of global climate change (Broecker et al. 1979). There is some evidence that temperate and boreal forests may already be significant sinks for anthropogenic C (Tans et al. 1990; Jarvis 1995). However, given the widespread nature of nitrogen

(N) limitation to plant growth, ecologists have questioned the likelihood of sustained increases in the sequestration of terrestrial C (Field et al. 1992; Rastetter et al. 1992; McGuire et al. 1995). Understanding processes that govern the cycling of N in soil will thus be important in predicting the broader responses of terrestrial ecosystems and the biosphere to increasing atmospheric CO_2.

Although research to date has focused primarily on the aboveground physiology of CO_2-enriched plants, there is evidence that root responses to CO_2 may influence the activities of soil microorganisms and the cycling of N (Körner and Arnone 1992; Diaz et al. 1993; Zak et al. 1993; Berntson and Bazzaz 1997; Hungate et al. 1996). Roots are often the single largest source of carbon (C) for microbial metabolism and the formation of soil organic matter (SOM) (Coleman 1976). The available energy content of fine roots and of root-derived C compounds is high, and is expected to increase under elevated CO_2 (van de Geijn and van Veen 1993). Greater fine-root biomass and rates of turnover observed in CO_2-enriched plants suggest that roots may be an important pathway by which microbial substrate availability could increase under elevated CO_2 (Norby 1994; Rogers and Runion 1994; Pregitzer et al. 1995; Fitter et al. 1997).

Labile C compounds can decrease plant access to N by promoting rapid microbial growth and the incorporation of available N into microbial proteins and nucleic acids. Plants often respond to elevated CO_2 with lower tissue N concentrations (Coleman et al. 1993). If CO_2-enriched roots are relatively rich in energy but poor in N, external N requirements for the synthesis of new microbial biomass may increase, leading to direct competition with plants for available N. There is some experimental evidence to support a negative feedback effect of CO_2 on soil N availability (Diaz et al. 1993; Berntson and Bazzaz 1997).

Equally compelling reasons exist to predict that plant N availability may increase in response to elevated CO_2 (Luxmoore 1981; Zak et al. 1993). By stimulating rates of C input to soils, elevated CO_2 will likely increase the flow of energy through microbial food webs. For example, microbial growth in the presence of roots can ultimately increase net N mineralization by stimulating microfaunal predation (Clarholm 1985; Griffiths 1994; Kuikman et al. 1990). Microbial responses to CO_2 vary widely (O'Neill 1994; Sadowsky and Schortemeyer 1997), but a number of studies have reported significant increases in microbial biomass or rates of N cycling under elevated CO_2 (Körner and Arnone 1992; Billes et al. 1993; Zak et al. 1993; Schenk et al. 1995; Diaz et al. 1993; Rice et al. 1994; Newton et al. 1995; Klironomos et al.1996; Ross et al. 1996; Hungate et al. 1997a).

Here we present results of a 2-year study on the combined effects of atmospheric CO_2 and soil N availability on C and N dynamics beneath trembling aspen (*Populus tremuloides* Michx.). We included N availability as an experimental treatment because of its recognized potential to modify plant responses to CO_2 (Curtis et al. 1994; McGuire et al. 1995); any interactive effects of N and CO_2 on plant biomass, allometry, or tissue chemical composition will in turn influence the amount or quality of C compounds entering the soil C cycle. Our specific objective was to examine the utilization of root-derived C by soil microbes and resulting effects on the competitive balance for N between CO_2-enriched plants and microorganisms. To accomplish this, we used a dual-labeling method in which plants were photosynthetically labeled with $^{14}CO_2$ and soils were simultaneously labeled with $^{15}NH_4^+$. This approach allowed us to quantify the partitioning of discrete pulses of C and N between plants, soils, and microbes.

In soil, as opposed to solution culture, it is difficult to distinguish between soluble root exudates and insoluble products that are secreted, sloughed, or abraded from growing roots (Curl and Truelove 1986; Darrah 1996). Pulse-labeling techniques of short duration examine assimilation of the most labile fractions of root-derived C by microbes (cf. Norton et al. 1990; Meharg 1994). By using a short (6-day) chase period between ^{14}C pulse and harvest, our experiment was designed to limit the influence of compounds deposited over longer periods, such as root turnover, on microbial ^{14}C dynamics. Photosynthetic and fine-root dynamics for the previous year of this experiment were reported by Kubiske et al. (1997, 1998).

Methods

Experimental design

The experiment was conducted over two growing seasons (1994–1995) at the University of Michigan Biological Station in northern Lower Michigan (45°34′N, 84°40′W). Rooted softwood cuttings of *P. tremuloides* were grown in a fully factorial, randomized block design that included two levels of soil N availability (low and high), two partial pressures of atmospheric CO_2 (p[CO_2]; ambient and elevated) and two harvest dates (August and October 1995). Each experimental unit consisted of an open-top chamber (0.64 m^2×1.5 m high) over an open-bottom root box (0.49 m^2×1.3 m deep; cf. Zak et al. 1993). Each of the eight CO_2×N×date treatment combinations was replicated four times, for a total of 32 experimental units. The chambers were arranged in four linear blocks of eight, oriented perpendicular to the midsummer solar angle.

One month prior to planting, the tops of the root boxes were buried level with the soil surface and filled with well-homogenized low- and high-N soils. High-N soil was the A horizon of a local Kalkaska sandy loam (Typic Haplorthod). Low-N soil was a mixture (1:4 by volume) of Kalkaska A horizon and the C horizon of a local Rubicon sand, resulting in a sandy texture. Organic C and total N were determined for each root box using a Carlo Erba NA 2500 CHN analyzer (CE Instruments, Milan, Italy). Low-N soil contained significantly less organic C and total N (3,104 µg C g^{-1}, 210 µg N g^{-1}) than high-N soil (12,936 µg C g^{-1}, 970 µg N g^{-1}). The C:N ratios of low- (14.8) and high-N (13.3) soils were not significantly different. Initial net N mineralization rates, measured over 5 days in the laboratory at 20°C, were 89±7 ng g^{-1} day^{-1} in low-N soil, and 333±16 ng g^{-1} day^{-1} in high-N soil. A 1-cm layer of sand was applied to all soil surfaces to minimize temperature differences between darkly colored high-N and lightly colored low-N soils. Mean daytime air temperatures in chambers were elevated by 3–6°C relative to unchambered ambi-

ent air; mean day- and night-time temperatures were not affected by soil or CO_2 treatments during either growing season.

Softwood cuttings of four aspen genotypes were propagated using the method of Barry and Sachs (1968). In early June 1994, we planted one rooted cutting of each clone in each root-box (128 ramets total). To aid in establishment, each plant received a single dose of N fertilizer in late June, consisting of 20 mg N in a 500-ml aqueous solution of $(NH_4)_2SO_4$. All plants were watered twice weekly (3 l per box) for the first 4 weeks. Subsequently, water was applied only twice in 1994 and twice in 1995, following dry periods lasting 1 week or longer. Five plants were replaced due to mortality in June of 1994, after which no further mortality occurred.

The daytime mean CO_2 partial pressures over both growing seasons (±SE) for ambient and elevated chambers were 36.7±0.1 Pa and 71.5±0.2 Pa, respectively. Blowers attached to each chamber exchanged two chamber volumes of air per minute (Dayton Electric Mfg. Co., Chicago, Ill., USA), and the flow rates of pure CO_2 to elevated chambers were adjusted manually (Curtis and Teeri 1992). Chamber CO_2 concentrations were monitored from sunrise to sunset every 15 min using a LI-COR 6252 infrared gas analyzer (LI-COR, Inc., Lincoln, Neb., USA) which was calibrated daily; CO_2 concentrations were recorded using a personal computer. Carbon dioxide fumigation was stopped and chambers were removed on 10 November 1994, after several hard frosts had occurred. Senesced leaves were removed as they fell, dried at 70°C for 48 h and weighed. By the end of the 1994 growing season, plants in the high-N soil had already outgrown cuvettes built for a ^{14}C pulse-labeling experiment that was planned for 1995. For this reason, all stems were removed 1 cm above the root collar in mid February 1995, dried at 70°C for 48 h and weighed. We replaced the chambers and re-initiated CO_2 fumigation on 14 May 1995, when signs of coppice regrowth began to appear.

Each of the 32 chambers was randomly assigned either to August or October harvest groups, so that each block of eight chambers contained four of each group. Chambers of the August group (16) were labeled with both ^{14}C and ^{15}N six days before being harvested in August 1995. October chambers were allowed to grow an additional 2 months in order to create a record of soil respiration rates over the entire growing season from June to October. The October chambers were then labeled with ^{15}N 6 days before being harvested in October of 1995.

Soil respiration

We determined soil respiration rates seven times from mid-June to early October of 1995. Between 1000 hours and 1400 hours on a given date, measurements were made from the same central location in each root box. The system consisted of a cylinder (4.4 cm inner diameter) made of FEP Teflon (du Pont de Nemours and Co., Wilmington, Del., USA) in line with a LI-COR 6262 infrared gas analyzer (IRGA) (LI-COR, Inc., Lincoln, Neb., USA) and a TD-4NA recirculating diaphragm air pump (Brailsford and Co., Rye, N.Y., USA). Air exchange through the cylinder was approximately 600 cm^3 min^{-1}. After inserting the sharpened bottom edge of the cylinder 2 cm into the soil surface, the system was flushed with ambient air for 10 min in order to purge excess soil CO_2. The system was then closed and CO_2 levels were monitored at 0.5-min intervals. A final reading was recorded when the rate of CO_2 increase in the chamber was constant for 3 min at a $p[CO_2]$ between 36.0 and 40.0 Pa. An atmospheric pressure transducer, periodically inserted through a rubber septum mounted on the cylinder, verified that no pressure differentials developed between the cylinder and ambient air.

^{15}N labeling

In the summer and fall of 1995, we conducted 6-day in situ ^{15}N pool-dilution experiments prior to the harvest of all 32 chambers. Sixteen chambers were labeled prior to budset in August (Julian dates 222, 223, 226, 227), and the remaining 16 chambers were labeled following budset in October (Julian dates 277, 278, 281, 282). Each day we labeled four root boxes from a single block, representing one replicate of each of the four soil-N×CO_2 treatment combinations. Our goal was to uniformly label the entire 0.637-m^3 volume of each root box to 6 μg NH_4-N g^{-1} soil and 2.5% ^{15}N. We chose an application rate of 6 μg g^{-1} to be near the lower end of the range of extractable NH_4-N (3–36 μg NH_4-N g^{-1}) found in surface soils of hardwood forests in northern Lower Michigan (Zak and Pregitzer 1990). Four channels (1.2 cm diameter) were punched into the soil of each box to a depth of approximately 1 m to facilitate infiltration of the ^{15}N solution. We added 40 l of $^{14+15}(NH_4)_2SO_4$ solution (0.0044 M, 2.5% ^{15}N) to the low-N and 60 l of solution to the high-N soil; these volumes were calculated to bring each soil to near field capacity. The labeling solutions infiltrated soil surfaces in less than 20 min. The soils remained undisturbed overnight (10 h) in order to allow soil water content throughout the root boxes to come to equilibrium.

The next morning we collected "initial" soil cores (4 per box; 5 cm diameter×1.3 m depth) to characterize ammonium and nitrate (NH_4^+ and NO_3^-) concentrations and ^{15}N enrichment for the calculation of gross N mineralization. Together, the four cores represented 1.6% of the soil volume of the entire root box. The cores were composited on a box basis and homogenized in plastic trays. Living coarse roots (>1 mm diameter) and fine roots (≤1 mm diameter) were removed by hand. Soil subsamples (4 per box; 50 g each) were extracted within 2 h with 2 M KCl, and inorganic N pools determined colorimetrically with an Alpkem 300 rapid flow analyzer (Astoria-Pacific International, Clackamas, Ore., USA). A Europa Scientific Integra-CN mass spectrometer (Europa Scientific Inc., Vandalia, Ohio, USA) was used to determine ^{15}N enrichment, after NH_4^+ was collected from soil extracts by a 10-day diffusion (Brooks et al. 1989).

Six days (144 h) after the initial cores were collected, we collected a second set of cores which were analyzed as described above. Rates of gross N mineralization were based on changes in the size and ^{15}N enrichment of NH_4^+ pools over 6 days (Davidson et al. 1991; Hart et al. 1994). In order to determine the total recovery of ^{15}N, soil subsamples were digested via a permanganate-reduced iron modification of the total Kjehldahl N (TKN) method that converts NO_3^- and NO_2^- in the conversion to NH_4^+ (Bremner and Mulvaney 1982). The digests were analyzed colorimetrically for NH_4^+ and diffused in preparation for analysis by mass spectrometry (MacKown et al. 1987). Total ^{15}N recovery was calculated as the sum of soil and plant ^{15}N at the time of harvest as a percentage of ^{15}N applied to each root box.

We tested the assumption that microbial N was not remineralized during the incubation period by labeling the NO_3^- pool of 200 g samples of low- and high N soils (n=8) to approximately 10% ^{15}N with an aqueous solution of $K^{15}NO_3$. These samples were incubated in sealed plastic beakers in the laboratory for 5 days at 20°C. The samples were extracted with KCl, and NH_4^+ from was collected via diffusion and analyzed for ^{15}N as described above. Incomplete N recovery and isotope fractionation are known to be potential problems in obtaining accurate measurements of ^{15}N enrichment using diffusion methods on high volume, low-N samples (Lory and Russelle 1994). For that reason, we also diffused a series of standards (50–200 μg NH_4-N in 70 ml of TKN digest solution, 0–10% ^{15}N) and analyzed via mass spectrometry.

Microbial N and N uptake

Microbial-biomass N was estimated using the chloroform fumigation/extraction (CFE) method (Brookes et al. 1985). Fumigated and non-fumigated control soils were extracted with 0.5 M K_2SO_4 (1:2 soil:extractant ratio) and subjected to TKN digestion (Bremner and Mulvaney 1982). The acid digests were analyzed for NH_4^+ and ^{15}N enrichment as described above. We report microbial N as the difference in N flushed from fumigated and control samples without correcting for extraction efficiency. The background ^{15}N enrichment of microbes was determined prior to ^{15}N application using the CFE method. Rates of microbial N immobilization were based on the appearance of ^{15}N in the

$CHCl_3$-labile pool over the 154-h period since the application of ^{15}N, using a model that assumes a non-linear decline in the ^{15}N enrichment of soil inorganic N (Davidson et al. 1991; Sheppard 1962).

Plant biomass, N, and N uptake

After the day-6 soil cores were removed, aboveground plant tissues were clipped and separated into leaf and stem fractions. Because the presence of ^{14}C prohibited extensive excavation of the root boxes, roots were harvested to a depth of approximately 20 cm, which appeared to contain the majority of roots. Plant tissues were frozen at –80°C until they could be hand-sorted. Coarse and fine roots were separated and washed to remove adhering soil. All tissues were weighed, and tissue subsamples were dried at 70°C for 48 h and reweighed to determine moisture content. Whole-chamber coarse- and fine-root biomass was calculated by summing the weight of directly harvested tissues (to 20 cm) and estimates based on roots contained in soil cores collected for ^{15}N analyses (1.3 m depth).

Plant tissues were chopped by hand into small pieces (1–4 mm) and subsampled for determination of total Kjehldahl N (Nelson and Sommers 1973) and for ^{14}C. Ammonium concentrations were determined as described above, and aliquots were diffused and analyzed for $\%^{15}N$ via mass spectrometry. Plant N-uptake rates over the 154-h labeling period were based on the appearance of ^{15}N in excess of the pre-labeling abundance of ^{15}N in plant tissues, assuming a non-linear decline in the ^{15}N enrichment of soil inorganic N, and negligible turnover of ^{15}N during the labeling period (Davidson et al. 1991; Sheppard 1962). The grinding of ^{14}C-labelled solid samples to fine powders may create a health hazard should the particulate matter be inhaled. As a radiation safety measure and for consistency, we used the older TKN/diffusion methods to prepare radio-labeled (August) and unlabeled (October) plants and soils for ^{15}N and ^{14}C analysis.

^{14}C labeling

From 12 to 16 August (Julian dates 223, 224, 227, 228), we pulse-labeled 16 chambers with $^{14}CO_2$, i.e., four replicates of each of the four CO_2×soil-N treatment combinations. Beginning approximately 2 h after sunrise (10 h after application of ^{15}N), and after removal of soil cores for ^{15}N analyses, each of the four units of one block were labeled with 18.5 MBq of ^{14}C. The labeling cuvettes consisted of clear acrylic chambers mounted on polyethylene bases enclosing each root box and forming an air-tight seal with the soil (Warembourg and Kummerow 1991). One small (0.71 m^3) and one large (0.96 m^3) labeling system were assembled in order to simultaneously label one low-N unit containing small-statured plants and one high-N unit containing taller plants. Chamber $p[CO_2]$ was monitored continuously using LCA-2 IRGAs (Analytical Development Co., Ltd., Hoddesdon, UK) in line with recirculating diaphragm air pumps (Brailsford and Co., Rye, N.Y., USA). Prior to the placement of labeling chambers and the introduction of $^{14}CO_2$, we determined the soil respiration rate of each chamber as previously described. With the labeling chambers in place, we then measured the net CO_2 uptake rates. These data were used to calculate labeling times and amounts of chase $^{12}CO_2$ required for total assimilation of the $^{14}CO_2$.

The $^{14}CO_2$-generating vessel consisted of a sidearm flask containing excess 1.0 M lactic acid. $^{14}CO_2$ was generated by injecting a solution of $NaH^{14}CO_3$ into the acid with a syringe through a rubber septum mounted in the top of the flask. A magnetic stirrer was used to mix the reactants and liberate the $^{14}CO_2$, which was introduced into the labeling chamber via the diaphragm pump. We maintained a constant $p[CO_2]$ of 50.0 Pa in the labeling chamber during the entire labeling period by periodically injecting a chase solution of $NaH^{12}CO_3$ into the generating flask. The common $p[CO_2]$ was used in order to give maximum rates of label uptake and to keep labeling times for ambient and elevated treatments as close as possible. Labeling the ambient-grown plants at 36 Pa would have led to unacceptably long labeling times for the low-N plants. Initial specific activities of $^{14}CO_2$ in the small and large labeling chambers were 103.6 and 77.7 MBq g^{-1} C, respectively. Temperatures inside the chambers were maintained at ambient levels (19–26°C) using AA-2000 cooling systems with fans mounted inside each unit (Hylan Inc., Kalamazoo, Mich., USA).

Labeling times and total ^{14}C recovery

The mean times calculated for label uptake for ambient and elevated chambers (±SE) were 148±22 and 125±27 min, respectively. Mean labeling times were 178±19 min for low-N chambers and 94±12 min for high-N chambers. We calculated that >90% (16.7 MBq) of the ^{14}C was assimilated by plants of both N treatments in the first 35 min following the introduction of $^{14}CO_2$. After all of the chase CO_2 had been assimilated, an air sample (50 cm^3) was removed from each chamber with a syringe and analyzed to quantify remaining ^{14}C. The samples were injected into a vial (200 cm^3) containing 10 cm^3 of Carb-asorb (Packard Instrument Co., Meriden, Conn., USA) and were allowed to equilibrate for 30 min. Aliquots of Carb-asorb were mixed with Permafluor (Packard Instrument Co., Meriden, Conn., USA) (1:4 v/v), and analyzed for ^{14}C with a model 1900TR liquid scintillation analyzer (Packard Instrument Co., Meriden, Conn., USA). Analysis of chamber atmospheres demonstrated that unassimilated $^{14}CO_2$ was not significantly affected by atmospheric CO_2 or soil N; it ranged from 0.06 to 0.70% of the 18.5 MBq applied to each chamber. Labeling chambers were removed at that point, replaced by open-top chambers, and normal CO_2 fumigation procedures resumed.

Soil ^{14}C respiration

For the 6 days between the application of $^{14}CO_2$ and harvest, static chambers were used to determine the daily ^{14}C efflux from soil (root+microbial) respiration. The openings of two polyethylene boxes were inserted into the soil in each chamber, enclosing an area of 94 cm^3 and a volume of approximately 850 cm^3. Inside the boxes were suspended jars containing 10 cm^3 of a solution of 10 M NaOH. The NaOH traps were replaced daily, and aliquots of NaOH were pipetted into Hi-ionic fluor (1:10 v/v) (Packard Instrument Co., Meriden, Conn., USA) and analyzed for ^{14}C using liquid scintillation counting. ^{14}C released from soil was expressed as MBq $chamber^{-1}$ day^{-1}.

Plant, soil, and microbial ^{14}C allocation

A Packard model 307 sample oxidizer and a Packard model 1900TR liquid scintillation analyzer were used to combust and analyze samples of each plant tissue (0.5–1.0 g each) and soil (0.8–1.0 g each) from each chamber (Packard Instrument Co., Meriden, Conn., USA). We processed two leaf, stem, coarse- and fine-root subsamples from every plant (a total of 8 per chamber of each tissue) and eight soil subsamples per chamber. Whole-chamber coarse- and fine-root ^{14}C (MBq $chamber^{-1}$) was calculated by multiplying chamber mean coarse- and fine-root specific activities (MBq g^{-1} tissue) by the estimates of whole-chamber root biomass described above. Similarly, whole-chamber leaf and stem ^{14}C was calculated by multiplying chamber mean leaf and stem specific activities by the measured tissue weights. Whole-chamber soil ^{14}C was calculated by multiplying mean soil specific activities by soil bulk density and root-box volume. Total soil ^{14}C represents the combined ^{14}C in microbial and non-microbial ^{14}C. ^{14}Carbon activities (MBq $chamber^{-1}$) were adjusted to account for daily oxidizer efficiencies ranging from 94 to 99%. Tissue and soil activities also were adjusted for the water content of the field-moist materials, and are expressed on a dry weight basis. The processing time of tissues and soils from thawing to oxidation was limited to approximately 1 h.

Microbial ^{14}C was determined using aliquots of the same samples generated by the $CHCl_3$ fumigation/direct extraction method

described above for N analyses. Aliquots of fumigated and non-fumigated extracts were analyzed via liquid scintillation counting. As ^{14}C extraction efficiency was unknown, microbial ^{14}C is reported as the difference in ^{14}C activities extracted from fumigated and control samples, without applying a correction factor.

Statistical analyses

A repeated measures analysis of variance (ANOVA) for unequal sampling intervals (Neter et al. 1990) was used to evaluate CO_2 and N effects on soil respiration over seven dates of the 1995 growing season. Fisher's LSD procedure was used to evaluate significant differences between interaction means on a given date. Effects of CO_2, soil N, and labeling date on plant, microbial, and N-cycling parameters were tested using a randomized complete block ANOVA. Effects of CO_2 and soil N on plant, soil, and microbial ^{14}C were tested using a two-factor ANOVA. In all ANOVA models, CO_2, soil N, and date (where present) were considered fixed effects and the block effect was random. Fisher's LSD procedure was used to evaluate significant differences where interactions were statistically significant. Single-factor regression analysis was used to describe the relationship between N uptake rates and soil respiration vs. root biomass. Proportions (%) of total biomass in leaves, stems, coarse roots, and fine roots were arcsine transformed prior to ANOVA to meet assumptions of normality.

Results

Plant biomass

There was a significant interaction between atmospheric CO_2 and soil N availability on total and coarse root biomass (Table 1). Relative to ambient CO_2, elevated CO_2 increased total biomass by 26% at low N and by 50% at high N, and increased coarse root biomass by 24% at low N and by 78% at high N. As a main effect, elevated CO_2 significantly increased the biomass of all components except for fine roots. Soil N also was a highly significant main effect, for all biomass components. Relative to low-N plants, increases in tissue weights in high-N soil ranged from 97% for coarse roots to 233% for stems. Relative to August biomass, plants harvested in October exhibited statistically significant gains in total biomass (+24%), and in coarse root biomass (+75%).

Atmospheric CO_2 did not significantly affect the distribution of plant biomass among plant tissues (data not shown). Both soil N availability and harvest date had significant main effects on biomass distribution. Relative to low-N plants, growth in high-N soil increased the percentage of biomass in stems (+35%) and decreased allocation to coarse roots (–15%). Relative to the August harvest, October plants contained significantly smaller percentages of biomass in leaves (–40%) and stems (–18%), and a significantly larger percentage in coarse roots (+48%).

Soil respiration

Carbon dioxide and soil N exhibited significant main and interactive effects on soil respiration (Table 2). Across sampling dates and N levels, elevated CO_2 stimulated mean soil respiration rates by 44%. The CO_2 response was consistently greater at high N that at low N throughout the 1995 growing season (Fig. 1). Across CO_2 and date, high soil N stimulated soil respiration by 85%. Date also was a significant main effect ($P<0.01$) on rates of soil respiration, with maximum rates occurring in mid- to late-June and declining to their lowest in early October. Mean soil respiration rates exhibited significant linear relationships with both fine- and coarse root biomass.

^{14}C allocation to plants, soil respiration, soil, and microbes

Within-plant allocation of ^{14}C across all $CO_2 \times N$ treatments closely resembled the distribution of biomass for

Table 1 Mean biomass (g m^{-2}) of *Populus tremuloides* plants and tissues after 2 growing seasons, across August and October harvest dates. SEs are listed in *parentheses* below each mean. Percentage response was calculated as the percentage increase or decrease of the 2nd mean relative to the first. *Letters* indicate that the soil-N×atmospheric-CO_2 interaction was significant at $P \le 0.05$; interaction means in a given *row* followed by the same letter were not statistically different at $P \le 0.05$. Statistical significance for main effect means is indicated as follows: *n.s.* no significant difference, $*P \le 0.05$, $**P \le 0.01$

	Interaction means				Main effect means					
	Low N		High N		Soil N			CO_2		
	Ambient CO_2	Elevated CO_2	Ambient CO_2	Elevated CO_2	Low	High	%Response	Ambient	Elevated	% Response
n	8	8	8	8	16	16		16	16	
Total	485a (47)	614a (51)	1047b (43)	1576c (104)	551 (47)	1312 (108)	(+138)**	767 (84)	1096 (151)	(+43)**
Leaves	86 (12)	124 (12)	218 (33)	306 (14)	104 (12)	263 (22)	(+153)**	151 (20)	216 (31)	(+41)**
Stems	100 (12)	129 (12)	322 (40)	439 (14)	114 (10)	380 (29)	(+232)**	210 (33)	283 (47)	(+35)*
Coarse roots	235a (10)	290a (16)	373b (20)	661c (35)	261 (35)	516 (65)	(+97)**	302 (35)	476 (73)	(+57)**
Fine roots	67 (6)	73 (18)	135 (29)	169 (43)	69 (8)	151 (16)	(+117)**	100 (18)	120 (16)	(+20)$^{n.s.}$

Table 2 Midday mean soil respiration rates (μmol m^{-2} s^{-1}) and ^{14}C distribution (MBq $chamber^{-1}$)following pulse labeling of *P. tremuloides*. Soil respiration rates are the means of 7 midday measurements taken from one set of 16 chambers from 14 June until harvest on 4 October 1995. In early August 1995, each of a second set of 16 chambers was pulse-labeled with 18.5 MBq of $^{14}CO_2$ and harvested after 6 days. SEs are listed in *parentheses* below each mean. Percentage response for main effects was calculated as the percentage increase or decrease of the second mean relative to the first. *Letters* indicate that the soil-N×atmospheric-CO_2 interaction was significant at $P \leq 0.05$. Means in a given *row* followed by the same letter were not statistically different at $P \leq 0.05$. Statistical significance for main effect means is indicated as follows: *n.s.* no significant difference, $*P \leq 0.05$, $**P \leq 0.01$

	Interaction means				Main effect means					
	Low N		High N		Soil N			CO_2		
	Ambient CO_2	Elevated CO_2	Ambient CO_2	Elevated CO_2	Low	High	%Response	Ambient	Elevated	% Response
n=	4	4	4	4	8	8		8	8	
Soil respiration	1.82a (0.12)	2.31a (0.16)	3.00b (0.24)	4.61c (0.33)	2.06 (0.10)	3.82 (0.22)	(+85)**	2.41 (0.16)	3.47 (0.24)	(+44)**
Total above ground ^{14}C	4.42 (0.62)	4.22 (0.72)	5.13 (0.58)	5.17 (0.49)	4.32 (0.44)	5.15 (0.35)	(+19) n.s.	4.78 (0.41)	4.69 (0.44)	(+2) n.s.
Leaves	2.08 (0.30)	1.85 (0.10)	2.86 (0.33)	3.19 (0.61)	1.96 (0.15)	3.03 (0.33)	(+54)*	2.47 (0.26)	2.52 (0.38)	(+2) n.s.
Stems	2.35 (0.46)	2.36 (0.66)	2.27 (0.64)	1.97 (0.23)	2.36 (0.37)	2.12 (0.32)	(–10) n.s.	2.31 (0.37)	2.17 (0.33)	(–6) n.s.
Total below ground ^{14}C	5.99 (0.62)	5.92 (0.79)	4.97 (0.46)	5.92 (0.93)	5.96 (0.47)	5.45 (0.52)	(–9) n.s.	5.48 (0.42)	5.92 (0.57)	(+8) n.s.
Coarse roots	2.18 (0.46)	2.4 (0.24)	2.66 (0.36)	3.64 (0.83)	2.33) (0.24	3.15 (0.46)	(+35) n.s.	2.42 (0.28)	3.07 (0.46)	(+27) n.s.
Fine roots	0.73 (0.20)	0.87 (0.33)	1.14 (0.40)	0.72 (0.07)	0.80 (0.18)	0.93 (0.20)	(+16) n.s.	0.93 (0.22)	0.79 (0.16)	(–15) n.s.
Soil ^{14}C	1.61 (0.44)	1.31 (0.29)	0.56 (0.06)	0.98 (0.14)	1.46) (0.25	0.77 (0.10)	(–47)**	1.08 (0.28)	1.15 (0.16)	(+6) n.s.
Microbial ^{14}C	0.04a (0.02)	0.06a (0.01)	0.08b (0.00)	0.15c (0.00)	0.05 (0.01)	0.12 (0.01)	(+140)**	0.06 (0.01)	0.11 (0.02)	(+83)**
Soil ^{14}C respiration	1.47 (0.28)	1.25 (0.21)	0.61 (0.09)	0.59 (0.07)	1.36 (0.18)	0.60 (0.05)	(–56)**	1.04 (0.21)	0.92 (0.10)	(–12) n.s.
Total ^{14}C recovered	10.29 (0.88)	9.97 (0.71)	9.73 (0.85)	10.49 (0.70)	10.13 (0.53)	10.26 (0.55)	(+1) n.s.	10.08 (0.58)	10.30 (0.49)	(+2) n.s.

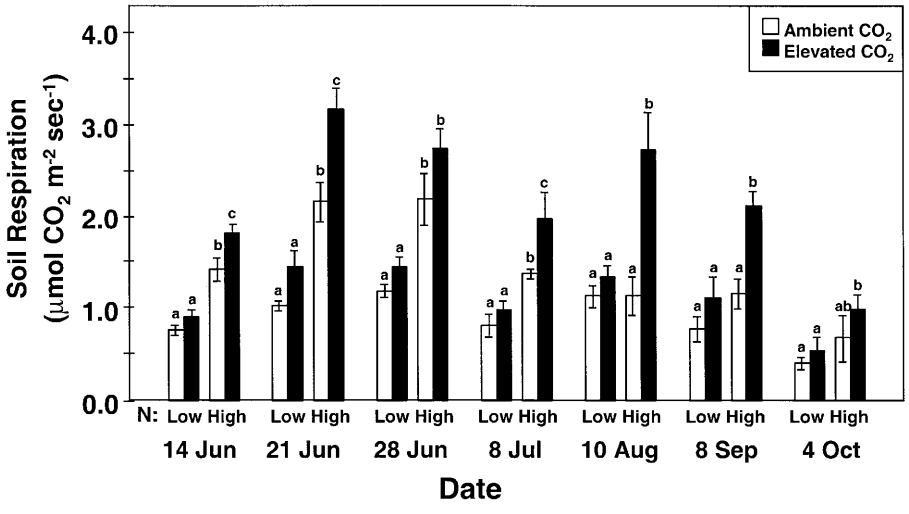

Fig. 1 Midday soil (root+microbial) respiration rates in trembling aspen mesocosms measured from mid-June to early October 1995. *Bars* represent the means (±SE) of 4 replicates of each factorial combination of N×CO_2. The N×CO_2 interaction was significant at $P \leq 0.05$. *Letters* above each bar represent multiple comparisons within a given date; bars of a given date denoted by the same letter were not significantly different at $P \leq 0.05$

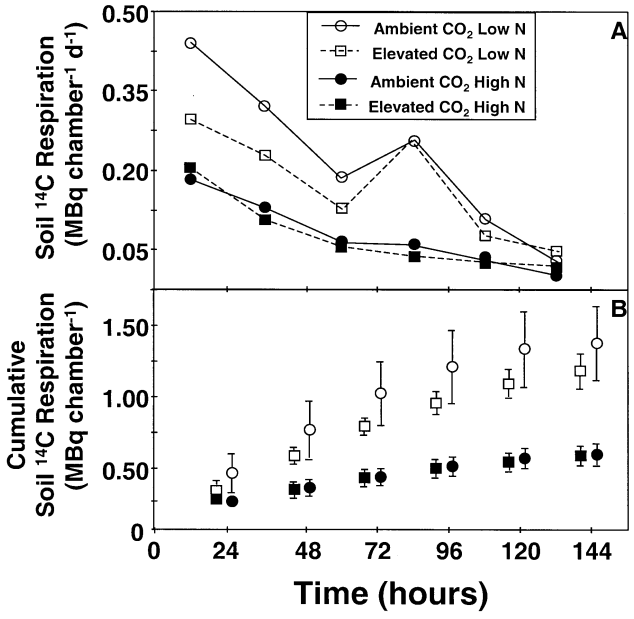

Fig. 2 A Daily soil ^{14}C respiration rates 24–144 h after pulse-labeling trembling aspen trees with $^{14}CO_2$, plotted at the mid-point of each 24-h period. **B** Cumulative $^{14}CO_2$ respired by the end of each 24-h period, shown with SEs. Each *point* represents the mean of 4 replicates of each factorial combination of N×CO_2. Each replicate chamber received 18.5 MBq of $^{14}CO_2$

the August harvest (data not shown). Leaves contained 30% of plant ^{14}C, and 26% of plant biomass. Similarly, stems contained 27% of plant ^{14}C, and 28% of plant biomass. Coarse roots contained 33% of plant ^{14}C and contained 33% of plant biomass. Fine roots contained 10% of plant ^{14}C and 13% of biomass.

Because each chamber assimilated the same amount of ^{14}C (18.5 MBq), differences in activity of a given pool, reported as MBq chamber^{-1}, also reflect differences in the relative distribution, or allocation, of ^{14}C 6 days after labeling (Table 2). Elevated CO_2 did not significantly affect the allocation of ^{14}C to any plant component, nor did it alter the total amounts remaining in soil or respired from soil over six days (Table 2). We observed a significant interaction between CO_2 and soil N with regard to the microbial fraction of soil ^{14}C, wherein elevated CO_2 increased microbial ^{14}C content by 50% at low N and by 88% at high N. As a main effect, elevated CO_2 increased microbial ^{14}C by 83% relative to microbial ^{14}C at ambient CO_2.

A trend toward greater allocation of ^{14}C to total aboveground components at high-N was not statistically significant (Table 2). High N availability significantly enhanced leaf ^{14}C by 54% relative to low N availability, and resulted in a non-significant 35% increase in coarse-root ^{14}C. ^{14}C in soil and evolved by soil respiration were significantly lower in high-N soil. Cumulative ^{14}C respired from high-N soils was 56% less than that respired from low-N soils, and the ^{14}C present in high-N soils was 47% less than that in low-N soils. In contrast, ^{14}C in the microbial biomass more than doubled from low- to high-N soil, a significant increase of 140%. Soil ^{14}C respiration rates were highest during the first 24 h of the chase period (Fig. 2A). Initial rates of ^{14}C respiration in low-N soil were markedly greater under ambient than elevated CO_2, but were little affected by CO_2 in high-N soil. The overall decline in rates of ^{14}C respiration was interrupted from 72 to 96 h by a sharp, temporary rate increase in both low-N soils. Rates of $^{14}CO_2$ emission from soil during the last sampling period from 120 to 144 h were 17% of their maximum rates in all treatments, and appeared to be rapidly approaching constant values.

Total ^{14}C recovery

Between 9.7 and 10.5 MBq of total ^{14}C could be accounted for in plants, soils, and cumulative soil respiration at harvest, representing 53–57% of the applied label (Table 2). Neither atmospheric CO_2 nor soil N affected total recoveries. Aboveground respiration was not quantified, but likely represented much of the unrecovered ^{14}C. High rates of leaf respiration have been noted in field studies following the assimilation of pulses of ^{14}C at high specific activities (Kuhns and Gjerstad 1991; Isebrands and Nelson 1983). Other potential losses of ^{14}C may have been ^{14}C in the soil atmosphere, leaching of dissolved ^{14}C in soil solutions below the coring depth of 1.3 m, and respiration of thawed plant tissues and soils during processing.

N pools and dynamics

The pre-labeling ^{15}N enrichments of both plants and microbes were not significantly different (n=4, P<0.05) from the background value of 0.3668% (dependent t-test), and did not differ with respect to CO_2 or soil N (2-factor ANOVA). The ^{15}N enrichments of NH_4 pools of soils labeled in the laboratory with $^{15}NO_3$ as a check against re-mineralization of microbial N were not significantly different than the background enrichment of unlabelled soils (dependent t-test, P<0.05). In addition, we found no significant deviations from expected ^{15}N enrichments of the diffused TKN standards and recovered 86–92% of N added to the diffusion vessels. Total N recovered from diffusions of KCl extracts ranged from 94–102% of N determined via rapid flow analysis.

Ten hours after the application of ^{15}N labeling solutions, there were no significant differences in initial soil inorganic N (NH_4^+ and NO_3^-) concentrations or %^{15}N related to CO_2, soil N, or labeling date. The mean initial inorganic N concentration across all treatments was 6.55±0.28 µg N g^{-1} soil, approximately equal to the amount of NH_4^+ applied to each root box (6 µg g^{-1}). Mean %^{15}N in the initial cores was 2.17±0.11%, close to the expected 2.5% ^{15}N of the labeling solutions. The small deviations from expected values in N concentration and ^{15}N enrichment were likely the result of over-

Table 3 Mean tissue and whole-plant N concentrations (%) of *P. tremuloides* plants after 2 growing seasons, across August and October ^{15}N-labelling/harvest dates. SEs are listed in *parentheses* below each mean. Percentage response was calculated as the percentage increase or decrease of the secondnd mean relative to the first. Soil N×atmospheric CO_2 interactions were not statistically significant at $P \leq 0.05$ for any parameters. Statistical significance for main effect means is indicated as follows: *n.s.* no significant difference, *$P \leq 0.05$, **$P \leq 0.01$

	Interaction means				Main effect means					
	Low N		High N		Soil N			CO_2		
	Ambient CO_2	Elevated CO_2	Ambient CO_2	Elevated CO_2	Low	High	% Response	Ambient	Elevated	% Response
n=	8	8	8	8	16	16		16	16	
Plant	0.85 (0.06)	0.71 (0.04)	1.24 (0.09)	1.00 (0.06)	0.78 (0.04)	1.12 (0.06)	(+44)**	1.04 (0.07)	0.86 (0.05)	(–17)**
Leaves	2.15 (0.14)	1.46 (0.13)	2.93 (0.13)	2.34 (0.20)	1.80 (0.13)	2.64 (0.14)	(+47)**	2.54 (0.14)	1.90 (0.16)	(–25)**
Stems	0.91 (0.13)	0.73 (0.09)	0.99 (0.12)	0.90 (0.10)	0.82 (0.08)	0.95 (0.07)	(+20)*	0.95 (0.08)	0.81 (0.07)	(–15)*
Coarse roots	0.80 (0.11)	0.59 (0.05)	1.20 (0.13)	0.92 (0.10)	0.70 (0.07)	1.06 (0.09)	(+51)**	1.00 (0.10)	0.76 (0.07)	(–24)*
Fine roots	0.83 (0.06)	0.93 (0.14)	1.12 (0.12)	1.03 (0.06)	0.88 (0.08)	1.07 (0.06)	(+22)*	0.97 (0.07)	0.98 (0.8)	(+1) n.s.

Table 4 Mean N pools (g m^{-2}) and dynamics (mineralization, immobilization, uptake: mg m^{-2} day^{-1}; uptake/biomass, immobilization/microbial N mg g^{-1} day^{-1}) under *P. tremuloides*, across August and October ^{15}N-labelling/harvest dates. SEs are listed in *parentheses* below each mean. Percentage response was calculated as the percentage increase or decrease of the 2nd mean relative to the first. *Letters* indicate that the soil N×atmospheric CO_2 interaction was significant at $P \leq 0.05$. Means in a given *row* followed by the same letter are not statistically different at $P \leq 0.05$. Statistical significance for main effect means is indicated as follows: *n.s.* no significant difference, **$P \leq 0.05$, ***$P \leq 0.01$

	Interaction means				Main effect means					
	Low N		High N		Soil N			CO_2		
	Ambient CO_2	Elevated CO_2	Ambient CO_2	Elevated CO_2	Low	High	% Response	Ambient	Elevated	% Response
n=	8	8	8	8	16	16		16	16	
Plant N	4.1 (0.5)	4.3 (0.5)	13.1 (1.5)	15.7 (1.6)	4.2 (0.3)	14.4 (1.1)	(+244)***	8.6 (1.4)	10.0 (1.7)	(+17) n.s.
Microbial N	7.3 (0.8)	8.3 (1.2)	26.9 (1.8)	28.6 (2.9)	7.8 (0.7)	27.7 (1.6)	(+257)***	17.2 (2.8)	18.4 (3.1)	(+7)n.s.
Gross N mineralization	96 (27)	98 (18)	239 (45)	267 (53)	96 (14)	253 (35)	(+164)***	167 (31)	182 (35)	(+9)n.s.
N immobilization	110a (14)	385b (40)	231c (25)	862d (116)	248 (39)	547 (100)	(+121)***	171 (23)	624 (92)	(+265)*
Plant N uptake	288a (53)	286a (33)	422b (88)	655c (104)	286 (31)	539 (71)	(+89)***	355 (51)	469 (69)	(+32)*
Plant N uptake/ Fine root biomass	2.1 (0.3)	2.4 (0.4)	1.8 (0.2)	1.7 (0.2)	2.3 (0.2)	1.8 (0.1)	(–22)**	1.9 (0.2)	2.1 (0.2)	(+11) n.s.
N immobilization/- microbial N	16.2 (2.6)	46.8 (3.7)	8.8 (1.3)	34.1 (7.1)	31.5 (4.5)	21.4 (4.8)	(–32)**	12.5 (1.7)	40.4 (4.2)	(+223)***

night mineralization of N contained in SOM and dilution by preexisting inorganic N. Mean total recoveries of ^{15}N at harvest ranged from 42% to 64% among all treatment combinations, with an overall mean of 53.8±1.8%. Potential losses of ^{15}N leading to incomplete recovery may have included nitrification followed by leaching below the coring depth of 1.3 m, or gaseous losses via denitrification. Recovery was unrelated to soil-N or CO_2 treatments, but was significantly related to labeling date (P=0.09); August and October mean total recoveries were 50.8 ±2.3% and 56.8±2.7%, respectively.

Tissue N concentrations were strongly influenced by N availability, with large increases in %N of all plant components in the high-N soil (Table 3). Carbon-dioxide enrichment caused significant decreases in the N concentrations of all components except for fine roots. Relative to ambient-grown plants, plant N capital was 17% greater under elevated CO_2, but the difference was not statistically significant (P=0.13; Table 4). Soil N had a significant positive effect on whole-plant N (Table 4), and on all individual plant components (data not shown). Harvest date also had a significant positive effect on whole-

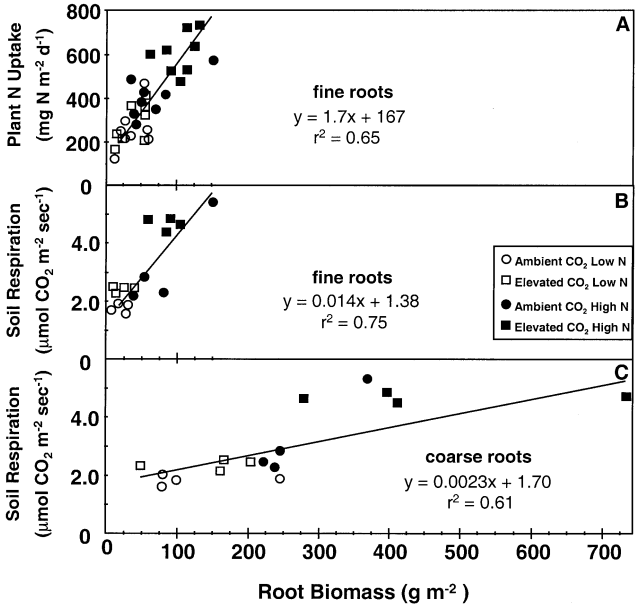

Fig. 3 **A** In situ N uptake rates versus fine-root biomass of 2nd-year trembling aspen trees during 6-day periods in August or October 1995. *Symbols* represent the individual data from 32 chambers (8 replicates of each of 4 factorial combinations of N×CO_2) labeled with $^{15}NH_4^+$. **B, C** Midday soil respiration rates versus biomass of **B** fine roots and **C** coarse roots of second-year trembling aspen trees. *Symbols* represent the seasonal means of 7 midday soil respiration measurements made from 16 chambers from mid-June to early October 1995, plotted against the October harvest biomass (4 replicates of each of 4 factorial combinations of N×CO_2)

plant N capital (+29% in October). A significant decrease in standing pools of leaf N (–30%) and significant increases in stem N (+79%) and coarse root N (+115%) from August to October may reflect in part the seasonal reallocation of N-containing compounds from photosynthetic tissues to storage.

As a main effect, elevated CO_2 did not significantly influence standing pools of microbial N or rates of gross N mineralization (Table 4). However, immobilization responded significantly to elevated CO_2 with an overall 265% increase relative to ambient. Elevated CO_2 also stimulated plant N-uptake rates by 32% (P=0.09). There were significant interactions between CO_2 and soil N on N uptake rates by both microbes and plants; elevated CO_2 stimulated N uptake to a greater degree at high N than at low N. Elevated CO_2 also significantly stimulated the specific N immobilization rate (mg N g^{-1} microbial N day^{-1}), but did not affect the fine-root-specific rate of plant N uptake (mg N g^{-1} fine root day^{-1}).

As expected, N-cycling pools and processes responded positively to soil N availability (Table 4); microbial N, gross N mineralization, immobilization, and plant N uptake exhibited large, highly significant increases from low to high soil N. By contrast, soil N had a significant negative effect on specific N uptake rates for both plants and microbes. Relative to low-N soils, plants at high N absorbed 22% less N per unit of fine root biomass (mg N g^{-1} fine-root biomass day^{-1}) and microbes at high N took up 32% less N per unit of microbial N. The regression analysis showed fine root biomass to be a highly significant predictor (P<0.01) of plant N uptake rate (Fig. 3A). Microbial biomass N, immobilization, and plant N uptake exhibited modest, non-significant declines (–8 to –20%) from August to October (data not shown). Gross N mineralization declined significantly by 31% from August to October (P=0.08). In addition, plant N uptake per unit of fine root biomass exhibited a significant 26% decline from August to October.

Discussion

Greater photosynthetic rates under elevated CO_2 may not result in equivalent increases in plant biomass; the additional C fixed by CO_2-enriched plants may be translocated below ground and lost via root respiration, root exudation, fine-root turnover, or allocation to mycorrhizal fungi (Norby et al. 1986; Fredeen et al. 1995; Pregitzer et al. 1995; Körner et al. 1996). In this study, elevated CO_2 stimulated maximum photosynthetic rates by 54% in low-N soil and 98% in high-N soil (Kubiske et al. 1997, 1998), but increased plant biomass by only 26% and 50% (Table 1). Higher rates of soil respiration may partly explain the difference, and confirm that elevated CO_2 increased C inputs to soil. Elevated CO_2 clearly enhanced the rates of C and N uptake by microbes. Our results are consistent with studies demonstrating that root-derived compounds can stimulate soil microbial activity after relatively short periods of exposure to elevated CO_2 (Diaz et al. 1993; Zak et al. 1993; Rice et al. 1994; Dhillion et al. 1996; Niklaus and Körner 1996; Cotrufo and Gorissen 1997; Hungate et al. 1997b).

In terms of absolute C flux, this study supports the hypothesis that proportional, i.e., isometric, growth responses of roots were the primary mechanism increasing total C inputs to soil under elevated CO_2 (Whipps 1985; Billes et al. 1993; Norby et al. 1987; Rattray et al. 1995). Neither the distribution of plant biomass after two growing seasons nor the short-term distribution of ^{14}C photosynthate to above- and belowground plant components, soils, and soil ^{14}C respiration were affected by elevated CO_2 (Table 1). This lack of allometric effects is consistent with recent reviews suggesting that elevated CO_2 can be expected to increase the allocation of biomass to roots only where soil conditions severely limit growth (Stulen and den Hertog 1993; Curtis and Wang 1998). The effect of elevated CO_2 on mean rates of soil respiration (+44%) corresponded closely to the increase in root biomass (+48%). Highly significant relationships between both coarse- and fine-root biomass and soil respiration further support the idea that soil C inputs increased in proportion to root growth under elevated CO_2 (Fig. 3B, C).

However, the magnitude of microbial responses to elevated CO_2 suggests that substrate availability to microbes, potentially a small fraction of total soil C inputs,

increased more than can reasonably be explained by a simple scaling up of root biomass. The percentage increase in microbial N uptake (+265%) was 5 times larger than increases in either root biomass or soil respiration under elevated CO_2. Similarly, microbes contained significantly more root-derived ^{14}C (+83%) under elevated CO_2; in the absence of above- and below-ground differences in ^{14}C distribution, this result implies that a larger *fraction* of root-derived C was accessible to or usable by soil microorganisms in CO_2-enriched microcosms. Although the CO_2 effect on soil respiration (+44%) represents a quantity of C ultimately of plant origin, it is difficult to separate direct root respiration from microbial respiration of C acquired from roots. If root respiration comprised a large portion of the total CO_2 produced in our soils, it would explain why the magnitude of increase in soil respiration was a relatively insensitive predictor of the increase in microbial substrate availability under elevated CO_2.

Beyond absolute effects of CO_2 on biomass and belowground C flow, the specific mechanism increasing microbial substrate availability is not clear. A shift in the chemical composition, or quality, of root-derived compounds may explain greater microbial fractions of soil ^{14}C under elevated CO_2. In other studies, increases in the non-structural carbohydrate content of fine roots suggest that the usable-energy content of rhizodeposition under elevated CO_2 may increase as well (van de Geijn and van Veen 1993; Darrah 1996). Whipps (1985) found that elevated CO_2 increased the solubility of root exudates in maize, a response that would enhance substrate availability to soil microbes. While increased C:N ratios of CO_2-enriched tissues are widely expected to slow microbial activity and litter decomposition rates (Rastetter et al. 1992; Schimel 1995), the opposite response seems likely if those tissues are predominantly composed of labile compounds, and if, as in this study, soil-solution N is sufficient to meet the demands of microbial biosynthesis.

Alternatively, a temporal shift in the release of a small but labile fraction of substrate into the rhizosphere may have increased microbial ^{14}C without affecting total soil ^{14}C contents under elevated CO_2. Production and release of the most labile root-derived compounds may be especially sensitive to higher rates of photosynthate translocation from source leaves to roots in CO_2-enriched plants (Rattray et al. 1995). It is also possible that, by increasing total root biomass and the area of root-soil contact, elevated CO_2 may have simply improved microbial access to an equivalent pool of root-derived ^{14}C.

It is important to note that pulse-labeling techniques of short duration, such as ours, trace the release of labile compounds that are rapidly assimilated by microorganisms. These labile compounds represent a potentially small fraction of total root-derived C entering the soil. With lengthening exposure to $^{14}CO_2$, increasing portions of ^{14}C assimilated by plants are found in structural C compounds that are deposited by dead roots (Meharg 1994). We found that elevated CO_2 increased the fraction of root-derived ^{14}C that was utilized by microorganisms, but others have found no effect (Cotrufo and Gorissen 1997; Griffiths et al. 1998), or a significant decrease (Rattray et al. 1995; Paterson et al. 1996). Beyond underlying experimental differences in species and soils, the range of reported CO_2 effects on microbial assimilation of ^{14}C reflects a variety of labeling techniques that examine different and largely undefined components of rhizodeposition. A longer pulse or chase period in this study, or the use of continuous ^{14}C labeling might have demonstrated differences in the allocation of ^{14}C with respect to CO_2, or elicited different patterns of ^{14}C utilization by microorganisms.

In the simplest analysis, inorganic N assimilated by microorganisms cannot be taken up by plants. Over short time scales, soil microorganisms are thought to have an advantage over plant roots through a higher substrate affinity for NH_4^+ (Rosswall 1976). Microbial N immobilization responded strongly to elevated CO_2 in both soils (+250% at low N, +273% at high N), whereas elevated CO_2 stimulated plant N uptake modestly (+32%), and only in the high-N soil (Table 4). Immobilization is only one link in a chain of processes governing N availability, however. Relative to soil microorganisms, plants remain competitive for N by being long-lived, assimilating N that is released during periods of microbial inactivity and death (Kaye and Hart 1997), or during active grazing by microfaunal predators such as protozoa and nematodes (Clarholm 1985). For N immobilized by microorganisms to remain inaccessible to plants, it must remain in the microbial biomass or be stabilized into humified forms.

Despite greater rates of N immobilization, standing pools of microbial N did not increase under elevated CO_2 (Table 4). One explanation is that elevated CO_2 may have begun to increase soil C availability and stimulate microbial demand for N only near the end of our study, in which case the effect on microbial N would be undetectable. It is reasonable to expect CO_2 effects on soil C and N dynamics to increase with time, as the influence of native SOM declines and that of incoming plant litter increases. If additional N had begun to accumulate in the biomass of CO_2-enriched microbes, temporal patterns of microbial N would have reflected this, since the CO_2 effect on N immobilization was highly significant on both dates. However, microbial N declined from August to October, and the decline was larger at elevated (-1.3 g N m^{-2}) than at ambient CO_2 (-0.28 g N m^{-2}), suggesting that microbes were not functioning as a long-term sink for N under elevated CO_2. As a rough index, the turnover time of microbial N (days=standing pool of microbial N/N immobilization rate) was markedly shorter under elevated (29 days) than ambient CO_2 (100 days). If microbial N were more rapidly re-mineralized under elevated CO_2, it may explain why more rapid rates of N immobilization did not increase microbial N, and why N availability to plants, inferred from plant N contents, did not decline under elevated CO_2. Greater microbial activity in the absence of corresponding changes in microbial C or N at elevated CO_2 has been interpreted in several recent studies as more rapid microbial turnover through

soil food webs, possibly via intensified predation from protozoa and soil fauna (Jones et al. 1998; Lussenhop et al. 1998; Niklaus and Körner 1996; Ross et al. 1995, 1996).

Our results suggest that elevated CO_2 may have begun to stimulate plant N-uptake capacity late in the study, possibly through increases in root biomass. The increase in whole-plant N (+17%; Table 4) was not statistically affected by elevated CO_2, but it is important to note that this measure integrates net changes in plant N that occurred over two growing seasons. By contrast, instantaneous rates of plant N uptake measured at the very end of the study exhibited a stronger response to elevated CO_2 (+32%; P=0.08), suggesting that the capacity of CO_2-enriched plants to acquire N may have increased with time. Fine-root biomass was a highly significant predictor of in situ rates of N uptake (Fig. 3A). Moreover, the relative responses to elevated CO_2 of whole-plant N (+5% and 20% at low and high N) closely corresponded to those of fine roots (+7% and +25% at low and high N). While these results are correlative, they are consistent with studies identifying larger root networks as the mechanism increasing N acquisition in trembling aspen (Rothstein et al. 2000) and in several other tree species exposed to elevated CO_2 (Luxmoore et al. 1986; Bassirirad et al. 1996; Vivin et al. 1996).

The allocation of ^{14}C with respect to N availability was consistent with functional equilibrium hypotheses predicting that C will be allocated preferentially to plant functions and tissues that promote uptake of limiting resources (Brouwer 1983). Significantly greater portions of the total ^{14}C fixed by plants were lost via respiration in low-N soils (Table 2). Previous research has demonstrated that the percentage of daily photosynthate respired by roots increases with decreasing nutrient availability, due to larger root systems in relation to leaf area and rates of photosynthesis (van der Werf et al. 1994; Lambers et al. 1996). Fine-root respiration rates also reflect the energetically costly processes of ion uptake (Bloom et al. 1992). Upon application of ^{15}N labeling solutions, the low-N plants of this study exhibited significantly greater in situ rates of fine-root specific N uptake (mg g^{-1} day^{-1}) relative to high-N plants, a common response of plants conditioned by nutrient deficiency (Chapin 1980).

Small increases in the metabolically active fungal biomass associated with fine roots can markedly increase C allocation to root and hyphal respiration rates in mycorrhizal tree seedlings (Norton et al. 1990; Rygiewicz and Andersen 1994). Plants grown in low-N soil of this study supported twice the length of extra-radical mycorrhizal hyphae (m g^{-1} soil) relative to those in high-N soil (Klironomos et al. 1997). The secondary maxima that we observed in the respiration of ^{14}C from low-N soils (Fig. 2A) were likely related to mycorrhizal C demand. A similar pattern was observed 10 d after pulse-labeling hybrid poplars (*P. euramericana* cv. Eugenei) (Horwath et al. 1994) and has been previously attributed to the acquisition of fine-root ^{14}C by mycorrhizal symbionts (Harris and Paul 1991). Taken together, our results suggest that relatively larger root systems, increased energetic demands of N acquisition, and a higher level of association with mycorrhizal symbionts increased the allocation of recent photosynthate to fine roots at low N availability.

In the spring, expanding *Populus* leaves are a strong C sink and import carbohydrates from stems and roots (Donnely 1974). By some point in mid-season, increasing photosynthate from mature leaves offsets the fixed C demands of apical tissues, resulting in net basipetal C flow. At budset, C is exported almost exlusively to active sinks in the lower stem and roots (Isebrands and Nelson 1983). By coppicing, we undoubtedly altered source-sink relationships between above- and belowground tissues, and likely delayed the onset of net C flow to roots. The removal of aboveground tissues would therefore certainly have decreased C inputs to soil. Treatment effects on soil C and N cycling are therefore likely conservative, i.e., would have been larger *without* coppicing. If anything, the fact that elevated CO_2 was stimulating soil respiration only 1 month after aboveground growth emerged (Fig. 1), only underscores the potential for elevated CO_2 to increase the availability of root-derived C and stimulate microbial activity.

In summary, exposure to elevated atmospheric CO_2 altered the in situ cycling of C and N at several levels of this experimental system. The plant growth response to elevated CO_2 was not matched by an equivalent dilution of plant N; CO_2 enrichment increased the absolute quantities of N incorporated by aspens over two growing seasons, especially in high-N soil. The ability of CO_2-enriched plants to forage for inorganic N may have been enhanced by larger root systems, as opposed to changes in root-specific rates of N uptake. The near doubling of microbial ^{14}C at elevated CO_2 amounts to a diversion of less than 1% of the labeled C assimilated by plants. This may be a small quantitative change in the cycling of C within our experimental system, but it represents an ecologically significant stimulation of microbial growth and N demand. Despite clear increases in N immobilization, standing pools of microbial N were not increased by CO_2 enrichment, suggesting that rates of N turnover through microbes increased. Soil N content was an important influence on several responses of this system to elevated CO_2. Both the absolute and relative responses of plant biomass and N capital, microbial N immobilization and plant N uptake rates to elevated CO_2 were greater in high-N soil. Effects of elevated CO_2 were significant both before and after budset, illustrating the importance of late season C gain not only to aspen biomass responses, but also to resulting feedback effects of CO_2 enrichment on belowground C and N dynamics.

Despite changes in the nature of N cycling, we cannot ascribe to these results an overall positive or negative effect of elevated atmospheric CO_2 on plant N availability. Greater whole-plant N contents may not be the result of

increased soil N availability, but they do illustrate the expansion of N-uptake capacity in CO_2-enriched plants. Similarly, the stimulation of N immobilization rates may not decrease plant access to N if sufficient inorganic N is present in soil, or if the additional N immobilized by microbes under elevated CO_2 is subsequently re-mineralized. These results may be most relevant to young, aggrading ecosystems, where soil resources are not yet fully exploited by plant roots, and microbial dynamics are not yet in equilibrium with inputs of above- and below-ground plant litter. In maturing forests, woody tissues will create additional sinks for N for as long as plant N uptake is enhanced by elevated CO_2. As N is removed from actively cycling pools, the N concentrations of physiologically active tissues will not decline indefinitely before eliciting a negative effect on growth. Whether faster turnover of N through the microbial biomass will alter N availability to plants growing under elevated CO_2 is not yet clear. The potential for sustained growth responses to elevated CO_2 may ultimately be determined by the balance between rates of gross N mineralization and the stabilization of N into recalcitrant forms of soil organic matter.

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