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Fine-root biomass and fluxes of soil carbon in young stands of paper birch and trembling aspen as affected by elevated atmospheric CO_2 and tropospheric O_3

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Abstract Rising atmospheric CO_2 may stimulate future forest productivity, possibly increasing carbon storage in terrestrial ecosystems, but how tropospheric ozone will modify this response is unknown. Because of the importance of fine roots to the belowground C cycle, we monitored fine-root biomass and associated C fluxes in regenerating stands of trembling aspen, and mixed stands of trembling aspen and paper birch at FACTS-II, the Aspen FACE project in Rhinelander, Wisconsin. Free-air CO_2 enrichment (FACE) was used to elevate concentrations of CO_2 (average enrichment concentration 535 µl l^{-1}) and O_3 (53 nl l^{-1}) in developing forest stands in 1998 and 1999. Soil respiration, soil pCO_2, and dissolved organic carbon in soil solution (DOC) were monitored biweekly. Soil respiration was measured with a portable infrared gas analyzer. Soil pCO_2 and DOC samples were collected from soil gas wells and tension lysimeters, respectively, at depths of 15, 30, and 125 cm. Fine-root biomass averaged 263 g m^{-2} in control plots and increased 96% under elevated CO_2. The increased root biomass was accompanied by a 39% increase in soil respiration and a 27% increase in soil pCO_2. Both soil respiration and pCO_2 exhibited a strong seasonal signal, which was positively correlated with soil temperature. DOC concentrations in soil solution averaged ~12 mg l^{-1} in surface horizons, declined with depth, and were little affected by the treatments. A simplified belowground C budget for the site indicated that native soil organic matter still dominated the system, and that soil respiration was by far the largest flux. Ozone decreased the above responses to elevated CO_2, but effects were rarely statistically significant. We conclude that regenerating stands of northern hardwoods have the potential for substantially greater C input to soil due to greater fine-root production under elevated CO_2. Greater fine-root biomass will be accompanied by greater soil C efflux as soil respiration, but leaching losses of C will probably be unaffected.

Keywords Northern forests · Global change · Carbon sequestration · Soil respiration · Dissolved organic carbon · Soil pCO_2

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Introduction

Decades of experimentation using growth chambers, glasshouses, and open-top chambers (OTCs) have provided evidence that rising atmospheric CO_2 will increase tree productivity in the absence of strong limitation by other resources (Ceulemans and Mousseau 1994; Curtis 1996; Wulschleger et al. 1997; Curtis and Wang 1998; Ceulemans et al. 1999). Productivity may be further stimulated by nitrogen (N) deposition (Galloway et al. 1995; Vitousek et al. 1997), warmer global temperatures, and a more vigorous hydrologic cycle (Houghton et al. 1996). Less attention has been given to gaseous pollutants that may dampen or even reverse the stimulating effect of elevated CO_2.

Coincident with the rise in atmospheric CO_2 over the past 150 years, ambient levels of O_3 have risen from <10 nl l^{-1} to the current 30–40 nl l^{-1} background levels today (Hough and Derwent 1990; Levy et al. 1997; Crutzen 1998; Percy et al. 2000). Elevated O_3 experiments using charcoal-filtered air as a treatment have repeatedly demonstrated that plant growth is currently constrained by ambient levels of ozone (Pye 1988; Baker et al. 1994; Taylor et al. 1994; Karnosky et al. 1996), and atmospheric O_3 concentrations are expected to continue to rise (Hough and Derwent 1990). Highly reactive O_3 binds to plasma membranes resulting in poor regulation of stomatal aperatures and damage to thylakoids, thereby inhibiting photosynthesis (Taiz and Zeiger 1991). These effects are directly antagonistic to those of elevated CO_2. Therefore, experiments (and models) that seek to provide insight into future forest productivity should explicitly examine the influence of these interacting gases.

Extending results from previous research in controlled environments to actual field conditions is a challenging but necessary advancement in ecology. Recently, investigators working at the FACTS-I free-air carbon dioxide enrichment (FACE) experiment reported a 25% stimulation of total net primary production in a young loblolly pine stand after 2 years of fumigation with elevated CO_2 (Delucia et al. 1999). This degree of stimulation falls within the 16–31% increase in total biomass summarized from over 500 studies performed in growth chambers, glasshouses, and OTCs in a recent meta-analysis of the CO_2 literature (Curtis and Wang 1998). The consistency of results from different experimental scales lends confidence to our interpretation of tree responses to elevated CO_2, but knowledge of how rising tropospheric O_3 will affect the CO_2 growth response is still limited. In an earlier review of the literature, Pye (1988) reported reductions in growth from 2 to 69% (average 23%) for a variety of coniferous and deciduous tree species exposed to elevated O_3, although most studies were of seedlings and of short duration. More recently, rising tropospheric O_3 has been recognized as a possibly potent modifier of forest ecosystem responses to elevated atmospheric CO_2 (Bortier et al. 2000). The few OTC studies that have examined the interaction of elevated CO_2 and O_3 on woody perennial biomass (Dickson et al. 1998; Volin et al. 1998; Loats and Rebbeck 1999) show that elevated CO_2 tends to ameliorate the negative effects of O_3 on photosynthesis and growth (or conversely that O_3 decreased the stimulation due to elevated CO_2), but this is not always the case (Kull et al. 1996).

The importance of forests to the global carbon (C) cycle, particularly the potential to sequester C from that accumulating in the atmosphere, has been the focus of much ecophysiological science (Kramer 1981; Strain and Bazzaz 1983; Eamus and Jarvis 1989; Schimel 1995; Koch and Mooney 1996; Schlesinger 1997; Jarvis 1998). Of particular importance is how the capacity for long-term C storage of forest soils will be affected by the growth dynamics and chemical composition of ephemeral tissues (fine roots and foliage) (Allen et al. 2000; Martens 2000; Rosenzweig and Hillel 2000). Soil is the largest, most persistent reservoir of C in forests (Dixon et al. 1994; Schlesinger 1997), and turnover of ephemeral tissues provides the greatest annual input of C to that reservoir (Waring and Schlesinger 1985; Vogt et al. 1986). Quantification of belowground pools and fluxes of C has proven to be an exceedingly difficult task, however, because of high spatial heterogeneity and difficulty of observation within the soil. Although our knowledge of fine-root dynamics and responses to environmental change is improving (e.g., Hendrick and Pregitzer 1992; Pregitzer et al. 1995, 2000; Berntson and Bazzaz 1996; Reuss et al. 1996; Kubiske et al. 1998), the fate of C once allocated belowground is still poorly understood (Zak et al. 2000). By constraining estimates of C entering and exiting the system, and elucidating the transformations of C as it is converted from plant litter to stable soil organic matter, we should be able to arrive at a mechanistic understanding of the belowground C cycle.

To investigate the interactive effects of elevated CO_2 and O_3 on the belowground C cycle of an aggrading north-temperate forest, we monitored C pools and fluxes in soil during the first 2 years of fumigation at the FACTS-II, Aspen FACE project. This project uses FACE technology (Dickson et al. 2000) similar to that of the FACTS-I project in Durham, N.C.„ to enrich the air with CO_2 and O_3 in forest ecosystems while minimizing artifacts due to the fumigation hardware (Hendrey et al. 1999). In addition to the O_3 treatment, another factor that distinguishes this site from FACTS-I is the use of communities dominated by single (aspen) and multiple (aspen-birch) deciduous tree species. Trembling aspen (*Populus tremuloides* Michx.) is an early successional tree with high rates of photosynthesis and growth, and responds vigorously to disturbance. It has the widest distribution of any tree species in North America and is commercially important across its range (Perala 1990). Paper birch (*Betula papyrifera* Marsh.) is also an early successional species that co-occurs with trembling aspen across much of its range, and can be a strong competitor for resources when both species occupy the same sites (Barnes and Wagner 1981). We hypothesized that forest communities developing under elevated atmospheric CO_2 would exhibit greater soil C inputs due to greater production of fine roots, and that these responses would be decreased by elevated O_3. We expected the greater fine-root biomass under elevated CO_2 to stimulate soil C efflux as soil respiration and the production of dissolved organic C (DOC), but that these responses would be dampened with the addition of O_3. Finally, we reasoned that competition would be minimal at this early stage of stand development and did not expect to see large differences in response between the two community types.

Materials and methods

FACTS-II: the Aspen FACE project

The Forest-Atmosphere Carbon Transfer and Storage (FACTS-II) Aspen FACE research project (Karnosky et al. 1999; Dickson et al. 2000) is located at the USDA Forest Service, North Central Research Station, near Rhinelander, Wis. (45°40.5′ N, 89°37.5′ E, 490 m elevation). The 32-ha facility is a randomized complete-block design, with three replicates (blocks) of four treatments: control, elevated CO_2, elevated O_3, and elevated CO_2 plus elevated O_3. A PVC toroidal plenum carries diluted fumigation gases to 32 vertical vent pipes spaced uniformly around each 30-m plot. Vent pipes on the upwind side of the plot release fumigation gases in proportion to the difference between target and measured gas concentration. Monitoring and control equipment are stored in small sheds next to each plot, and maintain target CO_2 and O_3 concentrations by means of real-time computer algorithms linked to the analyzers by fiber-optic connections. In 1998, the exposure period lasted 165 days beginning 1 May, and the mean (±SD) daytime CO_2 concentration measured at the top of the canopy at the center of elevated-CO_2 plots was 522.7±76.1) µl l^{-1}. The ambient O_3 concentration averaged 34.5±6.2 nl l^{-1} (seasonal sum 0, 65.3 µl l^{-1}) while the elevated O_3 concentration averaged 54.5±8.4 nl l^{-1} (seasonal sum 0, 97.8 µl l^{-1}). The 1999 exposure period lasted 144 days beginning 10 May, and ambient and elevated CO_2 concentrations averaged 346.5±22.2 and 547.8±70.9 µl l^{-1}, respectively. Ambient and elevated O_3 concentrations averaged 36.9±6.0 nl l^{-1} (season sum 0, 61.9 µl l^{-1}) and 51.7±6.8 nl l^{-1}, respectively (seasonal sum 0, 89.0 µl l^{-1}). For a complete description of the hardware and performance data of the Aspen FACE project see the USDA Forest Service Research Report (Dickson et al. 2000).

Soils at the site are classified as mixed, frigid, coarse loamy Alfic Haplorthods. The sandy loam topsoil (~15 cm thick) grades into a plow-layer clay accumulation layer (~30 cm thick), then grades back into a sandy loam stratified sand and gravel substratum. Clay lenses, 30–60 cm deep, are found throughout the field, but primarily in the northern 16 ha. Soil characteristics were quantified at the beginning of the study as a basis for future comparison (Table 1). In general, soil properties varied little across the 32-ha field, but total soil C and N averaged slightly higher in elevated-CO_2 and elevated-CO_2+O_3 plots.

The experiment was planted with three community types during the summer of 1997. One-half of each plot contains five clones of aspen of varying sensitivity to O_3, and early or late leaf phenology. One quarter of each plot was planted with pairs of sugar maple (*Acer saccharum* Marsh.) (seed source: several trees in Alberta, Mich.) and a single aspen clone ("216"). The final quarter was planted with pairs of mixed-stock paper birch and a single aspen clone ("216"). Each plot has a total of 670 trees that currently measure up to 5 m tall. A central "core" area within the plots has been determined to have the most uniform exposure to the fumigation gases (Dickson et al. 2000), resulting in sharply decreased variance in plant height and diameter (E. MacDonald, personal communication). All measurements for belowground C cycling studies are taken within this uniform inner area. We report here results from the aspen-only and aspen-birch community types.

Instrumentation

Instrumentation needed to monitor fluxes of the belowground C cycle was installed in all plots during the summer of 1998, and consists of soil respiration collars, soil pCO_2 gas wells, and tension lysimeters. The soil respiration collars are made of segments of PVC pipe 10 cm in diameter and 6 cm in length. One edge was beveled, and the collar seated approximately 2.5 cm into the soil surface at ten random locations within a community type in each plot (30 collars per plot=360 sample locations total). The collars enable repeated sampling of the same location over time using PP Systems EGM-2 soil respiration monitors (PP Systems, Haverhill, Mass.). Collar locations were rerandomized at the beginning of each field season to avoid cumulative systematic bias that might be associated with the collars. Soil respiration monitors were adjusted for changes in atmospheric pressure and calibrated with certified gas traceable to National Institute of Standards and Technology (NIST) each day of sampling. The cuvette volume used to cal-

Table 1 Summary of physical and chemical soil properties[a] from samples collected to a depth of 10 cm on 22 July 1997 at the Aspen FACE project in Rhinelander, Wis. Values are treatment means (*n*=3) with SDs in *parentheses* (*WHC* water-holding capacity, D_b bulk density)

	Ambient O_3		Elevated O_3	
	Ambient CO_2	Elevated CO_2	Ambient CO_2	Elevated CO_2
Soil texture				
% sand	55.1 (3.58)	53.9 (2.60)	58.3 (1.98)	55.0 (2.94)
% silt	36.1 (3.15)	37.8 (2.30)	35.3 (3.74)	37.4 (2.68)
% clay	8.7 (1.31)	8.4 (1.04)	6.4 (1.87)	7.7 (0.72)
Gravimentric measurements				
Moisture content (WHC)	0.10 (0.02)	0.11 (0.01)	0.10 (0.002)	0.11 (0.005)
D_b (Mg m^{-3})	1.27 (0.14)	1.30 (0.13)	1.32 (0.15)	1.43 (0.10)
Total C (%)	1.54 (0.27)	1.68 (0.33)	1.60 (0.32)	1.31 (0.20)
Total N (%)	0.12 (0.02)	0.13 (0.03)	0.12 (0.03)	0.10 (0.0)
C:N	12.9 (0.77)	12.4 (0.44)	13.5 (0.70)	12.8 (0.65)
Extractable P (µg P g^{-1})	124.0 (28.1)	155.0 (23.5)	132.0 (22.4)	136.0 (17.3)

[a] On 22 July 1997, ten soil samples were collected per plot using random azimuths and distances from the center. Soils were collected to a depth of 10 cm using a 3.27-cm-diameter core (volume=218 cm^3). Samples were packed on ice and returned for analysis at the University of Michigan Terrestrial Ecosystem Laboratory. Soil cores were weighed and subsamples were oven-dried for determination of bulk density. Pairs of cores from each ring were composited to yield five samples per ring. Subsamples were used for measurement of pH (1:2 deionized water), KCl-extractable ammonium and nitrate (Alpkem RFA 300, Clackamas, Ore.), dilute acid-fluoride-extractable P (Alpkem RFA 300), exchangeable bases (Perkin-Elmer 403, Norwalk, Conn.), and exchangeable acidity. Soil samples were ground in a roller mill prior to analysis of total soil carbon and nitrogen (NA 2500 Elemental Analyzer; CE Elantech, Lakewood, N.J.). Subsamples of each of the five samples per ring were composited (equal weight) prior to determination of soil texture (hydrometer method). Soil moisture desorption curves were constructed using a ceramic pressure plate extractor (Soil Moisture Corp., Santa Barbara, Calif.)

culate the respiration rate can be adjusted with software, and tests early in the study indicated that use of the collars did not affect our measurements. Soil temperature integrated over the top 10-cm depth was recorded adjacent to each soil respiration measurement with CheckTemp digital soil thermometers (Hanna Instruments, Woonsocket, R.I.) at the time of measurement.

The pCO_2 wells are designed to sample soil atmosphere, and for our study were installed at depths of 15, 30, and 125 cm in each community type in all plots for a total of 108 sample locations. They consist of stainless steel tubes 2 or 4 mm in diameter, depending on depth, with small slots at the appropriate depths to allow the tubes to come into equilibrium with soil atmosphere. The tubes extend approximately 5 cm above the surface and are capped with Luer-lok screw top valves. They are sampled by withdrawing a gas sample in an airtight syringe and injecting it into evacuated 3-ml serum vials sealed with butyl rubber septa. The vials were tested and found not to leak for a period of at least 44 days, providing ample time to transport the samples back to the laboratory. Samples were analyzed by gas chromatography with a Tracor 540 gas chromatograph (Tremetrics, Austin, Tex.) equipped with a Poropak Q column and thermal conductivity detector. Certified gas traceable to NIST was used to develop standard curves for each run and check standards were run approximately every ten samples.

To sample DOC in soil solution, tension lysimeters (Soil Moisture Inc., Santa Barbara, Calif.) were installed at depths of 15, 30, and 125 cm adjacent to each pCO_2 gas well. These consist of 5-cm-diameter PVC tubes of appropriate length capped at the bottom with a porous ceramic cup (pore size 2 µm). The tops are sealed with rubber caps and the tubes are placed under a vacuum approaching the matric potential of the soils (–0.05 to –0.06 MPa) for a given sampling interval. Soil solution that had collected was withdrawn, acidified to ~pH 2.0 with concentrated HCl, filtered (0.45 µm), and analyzed for DOC using a Shimadzu TOC-5000A (Wooddale, Ill.). The TOC-5000A oxidizes the sample with a Pt on alumina catalyst (680°C) to quantify total C after removing all inorganic C. Potassium hydrogen phthalate ($KH_5C_8O_4$) was used to develop standard curves for each run, and check standards were run approximately every ten samples. As with the pCO_2 gas wells, there are three lysimeters per community type in all 12 plots for a total of 108 sample locations.

Sampling

Sampling was conducted on a biweekly basis during the growing season. Sampling commenced in 1998 by measuring soil respiration in control and elevated-CO_2 plots. We attempted to collect soil solution as well, but since it was an unusually dry year, only a small amount of sample from some but not all lysimeters was recovered. In 1999, we sampled biweekly in all plots for the entire growing season. Precipitation occurred throughout the year allowing us to collect a full set of soil solution samples. In addition, on 30 August 1999, ten soil cores, 5.5 cm diameter×10 cm deep, were collected from random locations within each plot to estimate fine-root biomass (<0.5 mm and 0.5–1.0 mm diameter). Holes left by coring were filled with sieved soil collected adjacent to each plot and marked with pin flags. Cores were immediately placed on ice and transported to the laboratory where they were stored at –20°C. Roots were extracted from the cores by careful hand-sorting after the mineral soil had been washed away in a Gillison's hydropneumatic elutriator (Benzonia, Mich.). During processing, dead roots were identified (black, lack of succulent cortex) and quantified and are presented here with the size classes combined.

Statistical analyses

All data were analyzed with a fixed-effects model in an analysis of variance (ANOVA) for a randomized complete-block design. Experimental block is considered a fixed effect to account for a gradient in *Populus* productivity from south to north across the field, identified in previous studies. In addition, sampling is performed on a block-by-block basis so that possible differences in response variables due to the time of day when samples were collected can be accounted for by the block term in the model. Repeated measurement of the same experimental units over time (soil respiration, pCO_2, DOC) required repeated-measures ANOVA, which was accomplished by treating time as an additional splitting factor (Steel and Torrie 1980). The model used in the analysis was as follows:

$$Y_{ijklm}=\mu+\rho_i+\alpha_j+\beta_k+(\alpha\beta)_{jk}+\gamma_{ijk}+\chi_l+(\alpha\chi)_{jl}+(\beta\chi)_{kl}+(\alpha\beta\chi)_{jkl}+\zeta_{ijkl}+\delta_{m(l)}+(\alpha\delta)_{jm}+(\beta\delta)_{km}+(\chi\delta)_{(l)m}+(\alpha\beta\delta)_{(jk)m}+(\alpha\chi\delta)_{(jl)m}+(\beta\chi\delta)_{(kl)m}+(\alpha\beta\chi\delta)_{(jkl)m}+\varepsilon_{(ijkl)m}.$$

Where:

- ρ=effect due to block (i=3)
- α, β=effects due to CO_2 and O_3, respectively (j, k=2), and γ is the random component associated with whole units
- χ=effect due to community type (l=2), and ζ is the random component associated with the split-plot effect
- δ=effect due to depth (m=3), or time (m=17 in 1998, and m=10 in 1999) and ε is the random component associated with split-split plot units

The split-plot ANOVA (Appendix) was adapted from Steel and Torrie (1980) and appropriate error terms for F-tests were specified using test statements in the GLM procedure of the SAS (Cary, N.C.) statistical software package using type III sums of squares. To account for effects of diurnal and seasonal variation in soil temperature on temperature-sensitive biological processes (e.g., respiration), soil temperature was used as a covariate in the analysis of soil respiration and pCO_2 data. Similarly, to normalize the DOC data for variation in soil organic matter (SOM) across the experimental site, initial SOM was used as a covariate in the analysis of the DOC data. Finally, to control for variation in initial plant size at the time of planting, initial mean plot D^2H (diameter squared×height) was used as a covariate in the analysis of the fine-root biomass data. Inspection of residuals and normal probability plots revealed heterogeneity of variance, so data were log (biomass, DOC, pCO_2) or square-root (soil respiration) transformed before analysis, which satisfied the assumptions of ANOVA. Data are presented here as means and standard errors calculated for split-plot design (Steel and Torrie 1980). To decrease the complexity of the plotted data, means were calculated by averaging over non-significant factors. Treatment effects were considered significant if $P \leq 0.05$.

Carbon budget

To construct the belowground C budget for our site, we calculated pool sizes and net fluxes for May through October 1999. Since the soils at our site are cold and covered with snow for most of the time for which we lack measurements, we feel our budget captures most of the C dynamics of our system. Future efforts, however, will be directed at augmenting the length of our seasonal curves. The exception to this is DOC, for which most of the flux (leaching) occurs during the non-growing season because of decreased evapotranspiration. Accordingly, the flux for DOC in Fig. 5 is based on the water budget for the site for the entire year.

Calculations are based on a soil pedon 1.0×1.0×0.1 m deep, and all units have been converted to g C m^{-2}. The pool size for fine-root biomass (and associated mycorrhizae) was calculated directly from the soil cores by summing the mass for live and dead roots ≤1 mm diameter. Microbial biomass was estimated by the chloroform fumigation-incubation technique on soil samples collected in July (Larson 2000). Total soil organic C was determined directly from soil samples collected in 1997 (Table 1). The DOC pool size was calculated by averaging DOC concentrations of the 15-cm-depth lysimeters for the season and multiplying this value by the seasonal average amount of water contained in the pedon as

determined by volumetric soil moisture measurements using time domain reflectometry (TDR) (Topp and Davis 1985). Pool size for soil pCO_2 was determined by multiplying the seasonal average CO_2 concentration from the 15-cm pCO_2 gas wells by the average volume of pore space not saturated with water (determined from the TDR measurements) times the mass-volume relationship for CO_2. The net leaching flux of DOC was calculated by determining a simple annual water budget for the site (Dunne and Leopold 1978; Thornthwaite 1948) using meteorological data from the National Weather Service for Rhinelander, Wis. (www.crh.noaa.gov/grb/climate.html). This method allowed estimation of the volume of soil water leached from the site, which was multiplied by the seasonal average DOC concentration from the 125-cm-depth lysimeters, and assumes that all of the C measured at this depth leaves the system in groundwater. Finally, the net flux of C leaving the system as soil respiration was calculated by assuming that the average rate measured in a treatment on a given day represented the 24-h mean rate for that day. The hourly rate was multiplied by 24 for the net daily efflux of CO_2 (g m^{-2}), and a seasonal curve was constructed from these values. By integrating the area under this curve, we were able to estimate the total C efflux from the soil for the period of measurement.

Results

Fine-root biomass

Fine-root biomass (<0.5 mm diameter) was similar for both aspen and aspen-birch communities and averaged 217 g m^{-2} in control plots (Fig. 1A). Elevated CO_2 significantly increased fine-root biomass (Table 2) to 555 and 317 g m^{-2} for aspen (113% increase) and aspen-birch (83% increase) communities, respectively, compared to controls. Under elevated O_3, fine-root biomass was similar to that of control plots, and the combination of elevated CO_2 and O_3 resulted in a fine-root biomass intermediate between that of control and elevated CO_2 plots, at approximately 361 g m^{-2} (Fig. 1A). Ozone main effects and interactions were not statistically significant, however (Table 2). Biomass of larger-diameter roots (0.5–1.0 mm) was much less than that of the fine roots (Fig. 1B), averaging 46 g m^{-2} in control plots, and was not significantly affected by community type (Table 2). Elevated CO_2 resulted in a 72% increase in larger-root biomass compared to controls with an average standing crop of 84 g m^{-2}, while ozone main effects and interactions were not significant (Table 2). Finally, dead-root biomass of the combined size classes exhibited a similar response to that of the live roots (Table 2). Control plots averaged 89 g m^{-2} (34% of total live roots in control plots), while elevated-CO_2 plots averaged 214 g C m^{-2}, a 140% increase. As with live roots, ozone main effects and interactions were not statistically significant.

Soil respiration

For both years of the study, the seasonal pattern of soil respiration closely followed that of soil temperature (Fig. 2). The spring of 1998 was cooler than that of 1999, the soils warming to a maximum of 25°C approximately 2 weeks earlier in 1999 (Fig. 2C, D). In contrast, the fall of 1999 was cooler than that of 1998, and the soil began cooling in late July, whereas it remained warm until early September in 1998. Soil respiration in 1998 started at approximately 0.3 g CO_2 m^{-2} h^{-1}in the spring, reached a plateau of approximately 0.9 g CO_2 m^{-2} h^{-1} for most of the growing season, then declined back to the former level in the fall (Fig. 2A). Elevated CO_2 significantly increased the rate of soil respiration for the entire growing season (Table 2), and the significant Community×CO_2 interaction indicated that the de-

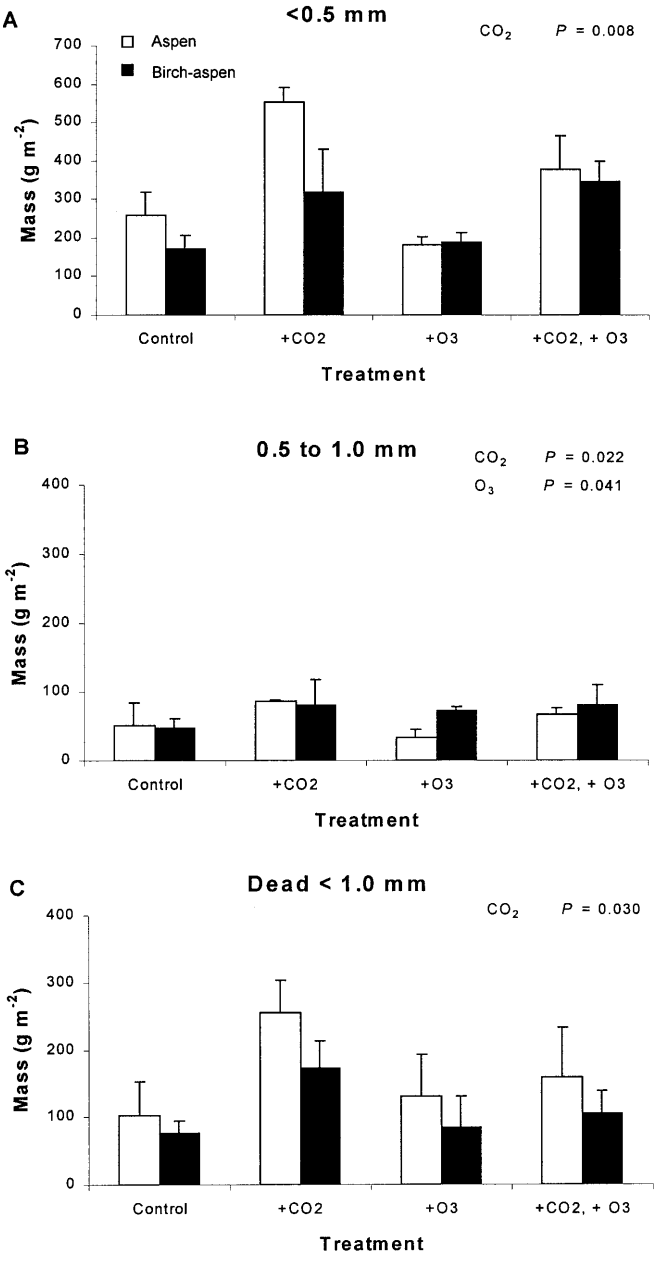

Fig. 1 Fine-root biomass of two diameter classes [<0.5 mm (**A**), 0.5–1.0 mm (**B**)] and dead root biomass (<1.0 mm) (**C**) sampled on 30 August 1999 at the Aspen FACE project in Rhinelander, Wis. Values are means (n=3) and *bars* are SEs calculated for a split-plot ANOVA

Table 2 *P*-values for responses of belowground pools and fluxes of carbon to the experimental treatments at the Aspen FACE project in Rhinelander, Wis. *F*-test error terms appropriate for testing main- and split-plot effects were selected for a randomized complete-block design with splits in space and time (Appendix) (*Rs* respiration; *NS* not significant ($P>0.05$), *NA* not applicable)

Source	Very fine roots (<0.5 mm)	Fine roots (0.5–1 mm)	Dead roots (≤1 mm)	Soil Rs 1998	Soil Rs 1999	Soil pCO_2	DOC
CO_2	0.0081	0.0358	0.0302	0.0125	0.0072	0.0224	NS
O_3	NS	NS	NS	NA	NS	NS	NS
CO_2×O_3	NS	NS	NS	NA	NS	NS	NS
Community	NS	NS	NS	0.0206	NS	NS	NS
CO_2×Community	NS	NS	NS	0.0021	NS	NS	NS
O_3×Community	NS	NS	NS	NA	NS	NS	NS
CO_2×O_3×Community	NS	NS	NS	NA	NS	NS	NS
Time	NA	NA	NA	0.0016	0.0001	0.0001	0.0001
CO_2×Time	NA	NA	NA	NS	0.0513	NS	NS
O_3×Time	NA	NA	NA	NS	0.0116	0.0295	NS
CO_2×O_3×Time	NA	NA	NA	NS	NS	NS	NS
Depth	NA	NA	NA	NA	NA	0.0031	0.0002
CO_2×Depth	NA	NA	NA	NA	NA	NS	NS
O_3×Depth	NA	NA	NA	NA	NA	NS	NS
CO_2×O_3×Depth	NA	NA	NA	NA	NA	NS	NS
Community×Time	NA	NA	NA	NS	NS	0.0213	NS
CO_2×Community×Time	NA	NA	NA	NS	NS	NS	0.0146
O_3×Community×Time	NA	NA	NA	NS	NS	0.0225	NS
CO_2×O_3×Community×Time	NA	NA	NA	NS	NS	NS	NS
Community×Depth	NA	NA	NA	NA	NA	NS	NS
CO_2×Community×Depth	NA	NA	NA	NA	NA	NS	NS
O_3×Community×Depth	NA	NA	NA	NA	NA	NS	NS
CO_2×O_3×Community×Depth	NA	NA	NA	NA	NA	NS	NS

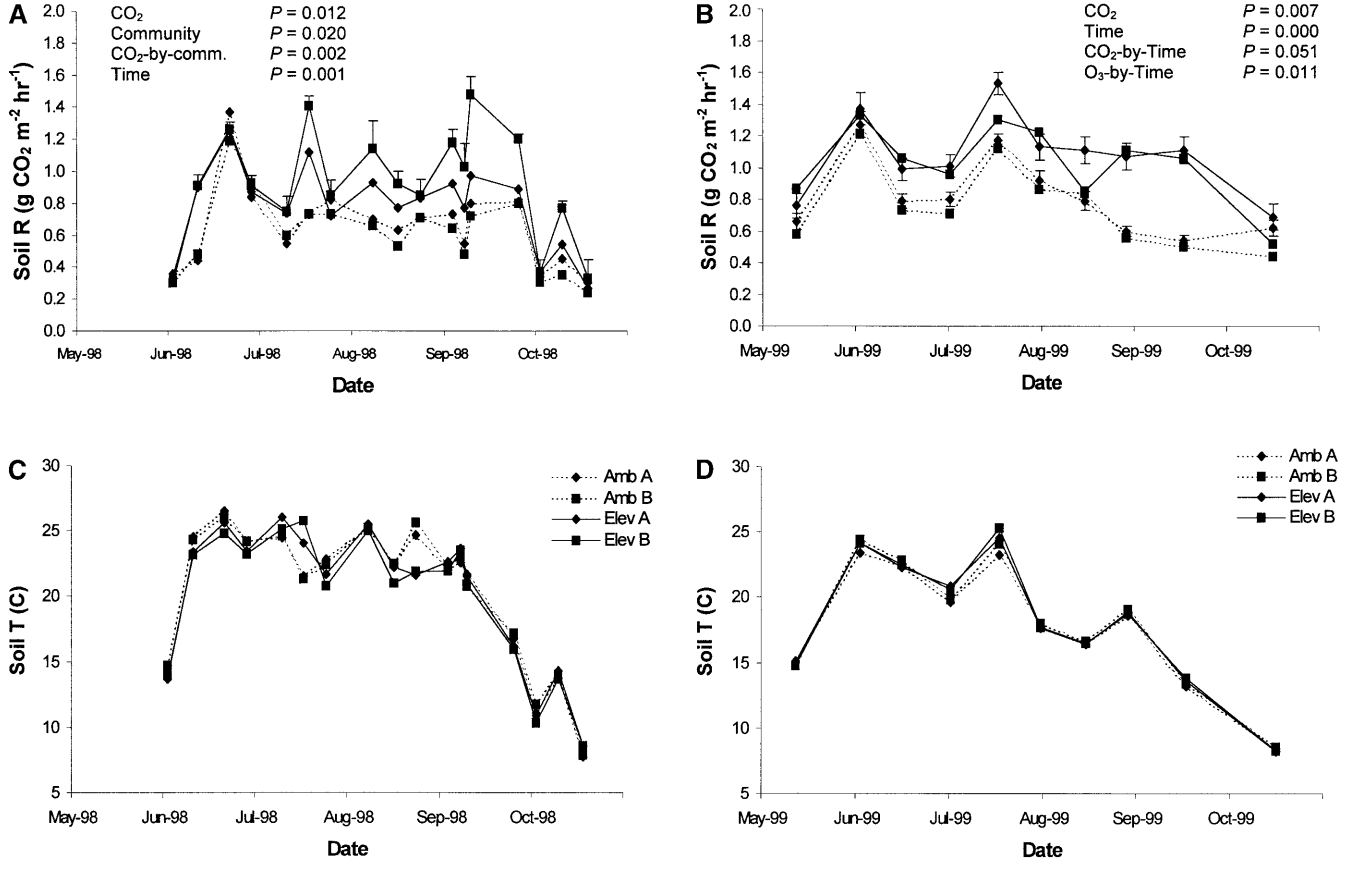

Fig 2 Soil respiration (**A,B**) and soil temperature (**C,D**) to 10 cm depth for 1998 (**A,C**) and 1999 (**B,D**) at the Aspen FACE project in Rhinelander, Wis. Values are means (n=6) and *bars* are SEs calculated for a split-plot ANOVA (*Amb A* ambient CO_2, aspen-only community; *Amb B* ambient CO_2, aspen-birch community; *Elev A* elevated CO_2, aspen-only community; *Elev B* elevated CO_2, aspen-birch community)

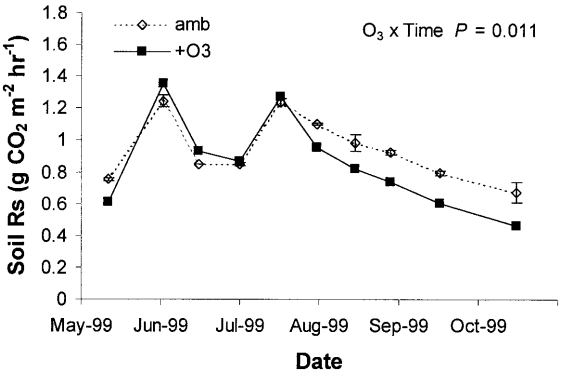

Fig. 3 Soil respiration for 1999 showing the O_3×Time interaction at the Aspen FACE project in Rhinelander, Wis. Values are means (*n*=6) and *bars* are SEs calculated for a split-plot ANOVA (*amb* ambient O_3, *+O3* elevated O_3)

gree of stimulation was greater for the aspen-birch (56% increase in season average) than for the pure aspen (21% increase in season average) community. In 1999, soil respiration rates reached an average 0.7 g CO_2 m^{-2} h^{-1} by early May and increased to a maximum of 1.5 g CO_2 m^{-2} h^{-1} by mid-July (Fig. 2B). Rates then declined for the remainder of the season as soil temperature dropped. A significant CO_2×Time interaction was due to the seasonal change in temperature, with little stimulation early and late in the season, but a large response at mid-season. The average 1999 growing season stimulation of soil respiration due to elevated CO_2 was 40%, and in contrast to 1998, both community types responded similarly. The significant O_3×Time interaction was due to an average 20% decrease in soil respiration under elevated O_3 late in the growing season (Fig. 3). As there were no significant interactions of O_3 with community type or CO_2, the late-season decrease in soil respiration under elevated O_3 was uniform across these other treatments.

Soil pCO_2

In 1999, pCO_2 in the soil atmosphere displayed a pattern similar to that of soil respiration (Fig. 4). Soil pCO_2 varied little by community type; however, time, depth, elevated CO_2, and elevated O_3 all had significant effects (Table 2). Average soil pCO_2 was approximately 0.5 kPa (5,000 ppm) early in the season, increased to a summer peak of 1.8 kPa in mid-August, then declined to 0.7 kPa by mid-October, accounting for the significant time effect. Soil pCO_2 increased with increasing soil depth (Fig. 4), and elevated CO_2 resulted in average increases of 32, 12, and 22% at depths of 15, 30, and 125 cm, respectively. The significant interactions between O_3, time, and community type (Table 2) resulted from very slight, inconsistent, and transient variation in pCO_2, making interpretation of these higher-order responses tenuous at this time.

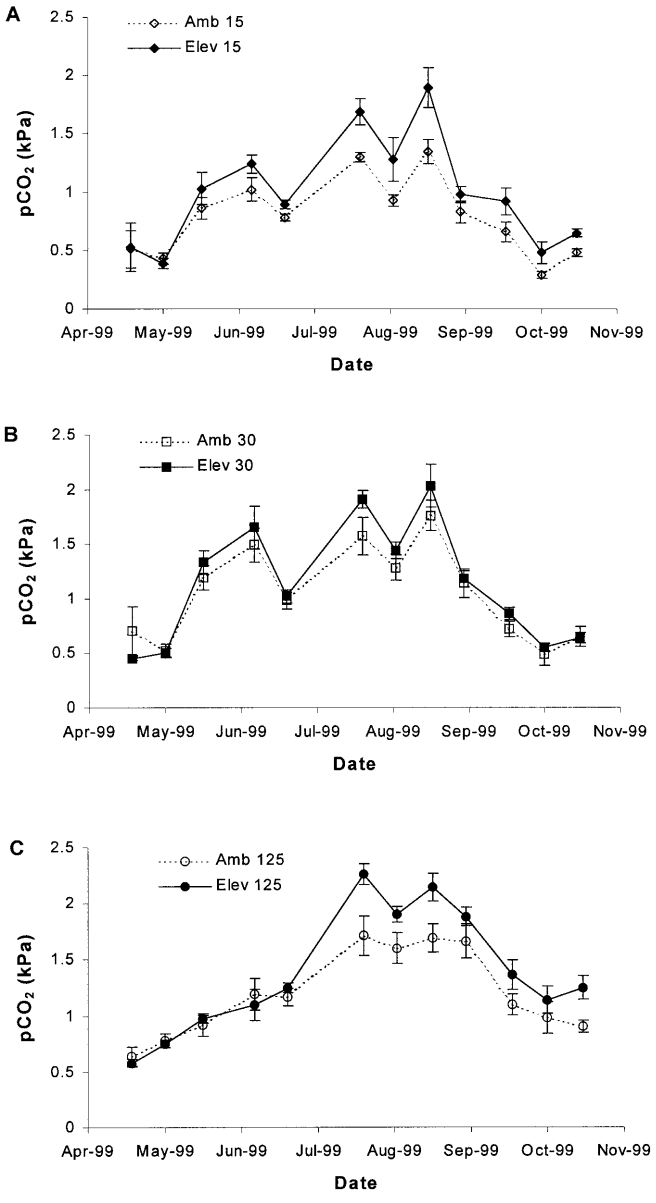

Fig. 4 Partial pressure of CO_2 (pCO_2) in soil atmosphere at depths of 15 (**A**), 30 (**B**), and 125 (**C**) cm under aspen and aspen-birch communities in 1999 at the Aspen FACE project in Rhinelander, Wis. Values are means (*n*=6) and *bars* are SEs calculated for a split-plot ANOVA (*Amb*, *Elev* ambient and elevated CO_2, respectively)

Dissolved organic carbon

DOC in soil solution was most strongly affected by time and depth (Table 2). DOC concentrations were similar for lysimeters at 15- and 30-cm depths, ranging from 8.5 to 22.7 mg l^{-1}, and there was a significant increase over the growing season (Fig. 5). DOC concentrations at 125-cm depth ranged from 3.2 to 10.5 mg l^{-1}, and also increased over the course of the growing season. A significant CO_2×Community×Time interaction indicates that CO_2 and community type were also important factors contributing to DOC concentrations in soil solution.

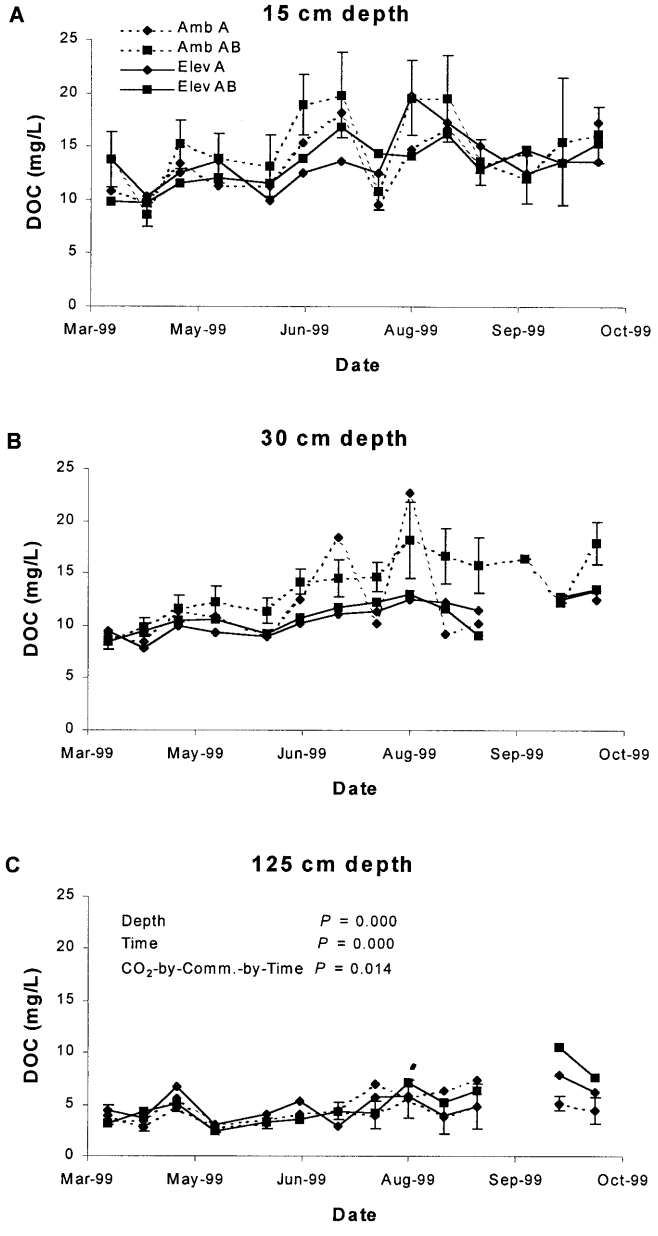

Fig. 5 Dissolved organic C (*DOC*) in soil solution at depths of 15 (**A**), 30 (**B**), and 125 (**C**) cm under aspen and aspen-birch communities in 1999 at the Aspen FACE project in Rhinelander, Wis. Values are means (n=6) and *bars* are SEs calculated for a split-plot ANOVA (*Amb*, *Elev* ambient and elevated CO_2, respectively; *A*, *AB* aspen-only and aspen-birch communities, respectively)

Under elevated CO_2, the aspen-birch community appears to have produced less DOC late in the growing season in the shallow lysimeters (especially at 30 cm); however, this effect did not persist to the lysimeters at 125 cm. High variance and transitory responses indicate caution when interpreting the DOC data at this time.

Carbon budget

Because community and ozone effects on the belowground C cycle were rarely significant in 1999, we constructed the C budget for control and elevated-CO_2 plots averaged over community type (Fig. 6). By far the largest pool of C was that in soil organic C, at 2,039 g C m^{-2} in control plots. Live and dead roots (and associated mycorrhizae) created the next largest pool, having 178 g C m^{-2} in control plots that increased to 367 g C m^{-2} under elevated CO_2. Microbial biomass contained 2.4 g C m^{-2} and was unaffected by elevated atmospheric CO_2 (Larson 2000). The average C content as DOC was 0.155 g C m^{-2}, while soil pCO_2 contained 1.3 g C m^{-2}, which increased to 1.6 g C m^{-2} under elevated CO_2. The major flux of C from the site was as soil respiration, at 760 g C m^{-2} over the study period, and this increased to 1,028 g C m^{-2} under the elevated-CO_2 treatment. Finally, leaching losses of C were minimal, estimated at only 0.9 g C m^{-2}.

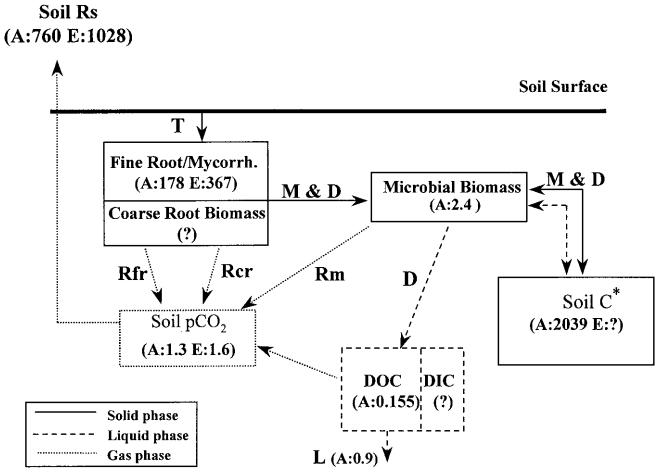

Fig. 6 Pools and fluxes of the belowground C cycle for 1999 at the Aspen FACE project in Rhinelander, Wis. Calculations are described in Materials and methods. All units are g C m^{-2}. *Due to the spatial variability in soil organic C, we feel it is premature to comment on changes in soil C at this time, although it is being monitored continuously (*A* value at ambient CO_2, *D* decomposition, *E* value at elevated CO_2, *L* leaching, *M* mortality, *Rcr* coarse-root respiration, *Rfr* fine-root respiration, *Rm* microbial respiration, *Rs* soil respiration, *T* translocation from shoots, *DOC* dissolved organic carbon, *DIC* dissolved inorganic carbon)

Discussion

Taken together, our results provide a first glimpse at the emerging response of the belowground C cycle to the elevated CO_2 and O_3 treatments at the Aspen FACE project. Aboveground studies of the aspen and aspen-birch communities (Isebrands et al. in press; Noormets et al., in press; Sober et al., in press; M.E. Kubiske, personal communication), indicate that in 1999, photosynthesis was stimulated 7–58% under elevated CO_2, and D^2H increased on average 38% in both communities. Reductions in photosynthetic rates due to elevated O_3 ranged from –1 to –11% compared to controls, and D^2H was reduced approximately –18% for the aspen community and –13% in aspen-birch. Application of elevated CO_2 along

with O_3 ameliorated the negative effects of O_3 on growth in the aspen-birch communities (+5% stimulation compared to controls), but not in aspen-only (–18% decrease compared to controls). As the belowground C cycle is dependent on C assimilation in the canopy, we expected to see greater fine-root biomass under elevated CO_2, with greater associated C fluxes from soil respiration and DOC production, and that elevated O_3 would dampen these responses. While we did observe significant and consistent stimulation of fine-root biomass, soil respiration, and soil pCO_2 under elevated CO_2, DOC responses and all responses to O_3 were small or inconsistent.

Fine-root biomass and associated C fluxes

At the end of the second year of growth, live fine-root biomass (≤1 mm) in the control plots averaged over community type was 263 g m^{-2} (2,60 kg/ha), of which 81% was composed of roots <0.5 mm diameter. Fine-root biomass at our site was in the middle of the range (260–8,320 kg/ha) reported for a variety of northern forests (Steele et al. 1997). In support of our hypothesis, elevated CO_2 stimulated fine-root production, almost doubling standing crop (96% increase) compared to the control treatment. This is consistent with Pregitzer et al. (2000), who reported a 52% increase in *Populus* fine-root biomass under elevated CO_2 in open-top chambers. Virtually all studies reviewed by Rogers et al. (1994) reported increased root biomass in response to elevated CO_2. The increase in fine-root biomass is likely due to proportional increases in all plant parts (i.e., bigger plants), because elevated CO_2 has been shown to have little effect on partitioning of biomass between root fractions (King et al. 1996) or other plant parts (Gebauer et al. 1996; Curtis and Wang 1998). Matamala and Schlesinger (in press) reported an 86% stimulation of fine-root increment in loblolly pine after 2 years fumigation with elevated CO_2, providing good agreement between the two forest FACE experiments. The loblolly pine stand at the FACTS-I site was 13 years old at the time of the reported measurements and in a stage of stand development much in advance of our 3-year-old stands. Therefore, we might expect to see greater fine-root biomass under elevated CO_2 at our site for some time to come, even though the soil will become increasingly occupied with roots as the stands develop.

Consistent with aboveground growth, elevated tropospheric O_3 decreased the stimulation of fine-root growth due to elevated CO_2, although O_3 main effects and interactions were not statistically significant. Although not statistically significant, we feel the negative effects of ozone on root growth are real because plots receiving both elevated CO_2 and O_3 exhibited a fine-root biomass intermediate between that of control and elevated-CO_2 plots (64% stimulation compared to controls). Amelioration of negative ozone effects on growth by elevated CO_2 is consistent with the findings of Dickson et al. (1998). In addition, decreased root growth in response to elevated O_3 agrees with studies that have reported proportionately greater reductions in root growth relative to shoots under elevated O_3 (Cooley and Manning 1987; Coleman et al. 1996; Wulff et al. 1996; Andersen et al. 1997). Reduced relative root growth is thought to be due to allocation of C for the repair of damaged photosynthetic tissues under high O_3 stress (Bortier et al. 2000). Further study is required, however, before we can say this is occurring at the Aspen FACE project.

Although we did not explicitly quantify fine-root turnover in this study, the 139% increase in dead-root standing crop under elevated CO_2 demonstrates greater C input to soil due to greater rates of fine-root production and mortality (Pregitzer et al. 1995). This finding is consistent with the growing body of literature (using glass-wall or minirhizotron techniques) documenting greater fine-root turnover under elevated CO_2 (Pregitzer et al. 1995, 2000; Berntson and Bazzaz 1996; Fitter et al. 1997; Kubiske et al. 1998). Using a modified compartment flow model, Allen et al. (2000) reported a non-significant 25% increase in annual fine-root turnover in loblolly pine after 2 years exposure to elevated CO_2 at FACTS-I. As soil microbial communities are generally C limited (Smith and Paul 1990; Zak and Pregitzer 1998), the greater input of soil C from root turnover under elevated CO_2 is likely to have large effects on microbial dynamics, nutrient cycling, and future forest productivity (Zak et al. 1993, 2000). Contrary to the effects of O_3 on live-root biomass, dead-root biomass actually increased under elevated O_3 (20%) and elevated CO_2 plus O_3 (47%) relative to controls, although this was not statistically significant. Given the apparent decrease in live fine-root biomass under elevated O_3, it will be interesting to see if the increase in dead root biomass will persist over time.

In support of our hypothesis, soil respiration was closely correlated to changes in fine-root biomass under the experimental treatments. High correlation between soil respiration and root biomass has been reported in a number of elevated-CO_2 studies (Johnson et al. 1994; Vose et al. 1995; Luo et al. 1996; Pregitzer et al. 2000). This finding supports the hypothesis that autotrophic respiration exerts a dominant influence on soil CO_2 efflux, especially in forests with poorly developed litter layers (Johnson et al. 1994 and references therein). In both years of our study, soil respiration was strongly influenced by the seasonal progression of soil temperature (r=0.79), illustrating the contemporaneous link between C uptake and delivery to soil (Horwath et al. 1994). Elevated CO_2 resulted in a 39% stimulation of soil respiration, averaged over species and time, which is in the middle of the range of stimulation (15–70%) reported for a variety of ecosystems exposed to elevated atmospheric CO_2 (Johnson et al. 1994; Vose et al. 1995; Luo et al. 1996; Ball and Drake 1998; Pregitzer et al. 2000). The strong, consistent stimulation of soil respiration under elevated CO_2 suggests forests of the future will rapidly cycle much of the additional CO_2 from enhanced photosynthesis through short-term belowground pools (Hungate et

al. 1997). This does not preclude greater long-term C storage in forest soils of the future, but is, rather, a by-product of enhanced biological activity in soil under elevated CO_2.

The significant CO_2×Community interaction in 1998 was due to much greater stimulation of soil respiration in the aspen-birch compared to the aspen-only community. This effect disappeared in 1999, possibly due to slightly greater root biomass in the aspen community (e.g., Fig. 1), which may have offset any differences in rates of specific root respiration between the two communities. Interestingly, the significant O_3×Time interaction resulted from steadily decreasing soil respiration under elevated O_3 late in the growing season. Elevated O_3 has been shown to stimulate leaf senescence in aspen (Karnosky et al. 1996), and if fine-root longevity is similarly reduced, this could account for the decrease in soil respiration late in the season. Coleman et al. (1996) observed decreased root system respiration of aspen exposed to elevated O_3 and attributed it to reduced root growth rather to than changes in specific respiration. Elevated O_3 is known to increase the susceptibility of roots to disease (Bonello et al. 1993), which could be a mechanism for decreased longevity. These hypotheses need to be tested in the future.

Increased belowground respiration under elevated CO_2 was significantly correlated with higher concentrations of CO_2 in the soil atmosphere (r=0.70), and this effect persisted at all depths of the soil profile. As with soil respiration, pCO_2 concentrations were strongly correlated with soil temperature (r=0.60), indicating dependence on contemporaneous biological activity. These results are consistent with Johnson et al. (1994), who reported increased root biomass in ponderosa pine after several years of exposure to elevated CO_2, which was highly correlated to greater soil pCO_2 at 15- and 30-cm depth, and ultimately greater soil CO_2 efflux as soil respiration. The authors also found increasing pCO_2 with depth, and incubations of root-free experimental soil demonstrated that root respiration was the main source of CO_2 in the soil atmosphere (Johnson et al. 1994). In contrast, Allen et al. (2000) reported few significant effects of elevated CO_2 on soil respiration or soil pCO_2, even though they observed enhanced root growth. Higher soil pCO_2 could have implications for mineral weathering and nutrient leaching, as well as for greater C export from the site as dissolved inorganic C (DIC) in soil solution. At our site, elevated CO_2 has resulted in approximately 27% higher pCO_2 concentrations (averaged over depth and time), causing higher concentrations of carbonic acid in soil solution. The carbonic acid system involves deprotonation reactions that displace nutrient cations from exchange sites and primary minerals (Richter and Markewitz 1995; Richter et al. 1995). Soil solution from our deep lysimeters will be analyzed periodically for concentrations of cations and DIC to test for these effects.

We expected to see higher concentrations of DOC in soil solution under elevated CO_2 due to greater fine-root turnover and decomposition, and lower concentrations under O_3. Average DOC concentrations for each depth of the Aspen FACE project fell within the ranges reported for A, B, and C horizons in a variety of forest soils (Herbert and Bertsch 1995). Although we observed what appeared to be decreased DOC production in surface horizons under elevated CO_2 in the aspen-birch communities, the high degree of variability within and between treatments requires caution in interpreting this response. Furthermore, DOC concentrations were similar in all treatments at 125-cm depth, suggesting little effect on C export from the system.

High DOC concentrations and variance in surface horizons that decline with depth are commonly reported in the literature (Meyer and Tate 1983; McDowell and Wood 1984; Cronan and Aiken 1985; Herbert and Bertsch 1995). Reduction in DOC concentration with depth is attributed to sorption of hydrophobic fractions to mineral surfaces (Herbert and Bertsch 1995), and degradation by soil microbial communities has been shown to play a minor role in removal of DOC from soil solution (Qualls and Haines 1991, 1992). The increase in DOC concentrations we observed over the course of the growing season is consistent with seasonal variation reported by others (Cronan and Aiken 1985), and is evidence for the cumulative effect of contemporaneous biological activity on organic C in soil solution. DOC concentrations in soil solution from the deep lysimeters at our site are close to the range (1.38–7 mg l^{-1}) reported for groundwater and forested streams (Herbert and Bertsch 1995), so much of the C that reaches this depth is likely exported from the ecosystem. We are aware of only two other studies that examined the effect of elevated CO_2 on DOC production in decomposing tree litters (Cotrufo et al. 1991; King et al. 2001), and ours is the only one evaluating the impact on C export from intact forest ecosystems. To date, all studies have found a minimal effect of elevated CO_2 on the quantity of DOC produced.

Belowground C cycle in a changing atmosphere

For our site at the peak of the growing season in 1999, soil organic C contained over 91% of the C in the solid phase, while fine roots/mycorrhizae (live and dead) contained 8%, and microbial biomass approximately 0.1%. Such a small fraction of the total soil C in microbial biomass is one reason why assessing the response of soil microbial communities (N transformations) to elevated atmospheric CO_2 has been difficult (Zak et al. 2000). Under elevated CO_2, fine-root/mycorrhizal biomass increased to 15% of the total. At this point we do not have estimates of coarse-root biomass, but this fraction is much less dynamic than fine roots and therefore inputs to soil C are probably insignificant at this stage of stand development. The average amount of organic C contained in soil water in surface horizons was only 0.155 g

m^{-2} and the total calculated annual leaching loss was 0.9 g C m^{-2}, representing a minor flux from the system, and one apparently not affected by elevated CO_2 or O_3. Qualls et al. (1991) observed an annual DOC flux of 40.5 g C m^{-2} from a southern Appalachian watershed and McDowell and Likens (1988) reported a flux of 26.3 g C m^{-2} from Hubbard Brook. Edwards and Harris (1977) reported an annual DOC loss of 1.25 g C m^{-2} from a southern deciduous forest dominated by *Liriodendron tulipifera*. All of these reports were from closed-canopy forests with well-developed litter layers, so our estimate of 0.9 g C m^{-2} $year^{-1}$ is perhaps not surprising for a young stand with little forest floor and incomplete root development. More C was actually contained in the soil atmosphere as CO_2, 1.3 g m^{-2} on average, which increased to 1.6 g m^{-2} under elevated atmospheric CO_2. The increase in pCO_2 under elevated CO_2 led to proportionate increases in soil respiration from 760 to 1028 g C m^{-2} $year^{-1}$, by far the largest flux in the system. Our estimates of C flux from soil respiration should be considered maximum rates because they are based on average daily values and do not account for diurnal variation. Nor do our estimates account for short-term pulses, but we feel they are representative of the system because the seasonal curves for 1998 and 1999 are consistent with one another, and are strongly reliant on the seasonal progression of soil temperature. Hence our estimates are based on the overall seasonal signal in soil respiration, and have few qualifying assumptions. Our estimate of annual soil CO_2 efflux for ambient plots at our site agrees well with the ~800 g C m^{-2} $year^{-1}$ reported for forest soils of our latitude (Schlesinger 1977). Furthermore, the magnitude of the soil respiration flux with respect to the other pools and fluxes of the system supports the contention that respiration is a major controller of C storage in forest ecosystems (Valentini et al. 2000).

Conclusions

We found that growth under elevated atmospheric CO_2 increased fine-root biomass in intact communities of aspen and aspen-birch, and this stimulated higher concentrations of CO_2 in the soil atmosphere causing higher rates of soil respiration. These responses appeared to be dampened by elevated O_3, but O_3 effects to date were rarely significant and we feel require further observation in order to rigorously test our hypotheses. Fine-root and microbial biomass are relatively small compared to native soil organic C, hence some treatment responses (N transformations) may be highly buffered by the soil for some time to come. We found very low C losses from the system due to leaching of DOC, which appears to be insensitive to elevated CO_2 or O_3. Finally, soil respiration was by far the greatest flux of C from the belowground system, which, in balance with long-term soil C inputs from root and leaf litter will determine the ability of these regenerating forests to store C in a CO_2-enriched world.

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Appendix

Analysis of variance for the split plot in space and time for the randomized complete-block design at the Aspen FACE project in Rhinelander, Wis. Adapted from Steel and Torrie (1980)

Reference	Source	*df*	Sum of squares
I	Blocks, *R*	*r*–1	$\Sigma_i Y^2_{...}/abcd$–CT (correction term)
	CO_2, *A*	*a*–1	$\Sigma_j Y^2_{.j..}/rbcd$–CT
	O_3, *B*	*b*–1	$\Sigma_k Y^2_{..k.}/racd$–CT
	$CO_2 \times O_3$, *AB*	(*a*–1)(*b*–1)	$\Sigma_{j,k} Y^2_{.jk.}/rcd$–CT
	Error (*a*), *RAB*	(*r*–1)(*a*–1)(*b*–1)	$\Sigma_{i,j,k} Y^2_{ijk.}/cd$–CT–SS(*R*)–SS(*A*)–SS(*B*)–SS(*AB*)
	Subtotal I	*rab*–1	$\Sigma_{i,j,k} Y^2_{ijk.}/cd$–CT
II	Community, *C*	*c*–1	$\Sigma_l Y^2_{...l.}/rabd$–CT
	$CO_2 \times$Community, *AC*	(*a*–1)(*c*–1)	$\Sigma_{j,l} Y^2_{.j.l.}/rbd$–CT–SS(*A*)–SS(*C*)
	$O_3 \times$Community, *BC*	(*b*–1)(*c*–1)	$\Sigma_{k,l} Y^2_{..kl.}/rad$–CT–SS(*B*)–SS(*C*)
	$CO_2 \times O_3 \times$Community, *ABC*	(*a*–1)(*b*–1)(*c*–1)	$\Sigma_{j,k,l} Y^2_{.jkl.}/rd$–CT–SS(*A*)–SS(*B*)–SS(*C*)
	Error (*b*), *RC+RABC*	(*r*–1)*ab*(*c*–1)	$\Sigma_{i,j,k,l} Y^2_{ijkl.}/d$–CT–SS(*I*)–SS(*C*)–SS(*AC*)–SS(*BC*)–SS(*ABC*)
	Subtotal I+II	*rabc*–1	$\Sigma_{i,j,k,l} Y^2_{ijkl.}/d$–CT
III	Time, *D*	(*d*–1)	$\Sigma_m Y^2_{....m}/rabc$–CT
	Error (*c*), *RD*	(*r*–1)(*d*–1)	$\Sigma_{i,m} Y^2_{i...m}/rabc$–CT–SS(*R*)–SS(*D*)
	Subtotal III	*rd*–1	$\Sigma_{i,m} Y^2_{i...m}/rabc$–CT

Appendix (continued)

Reference	Source	*df*	Sum of squares
IV	CO_2×Time, *AD*	(*a*–1)(*d*–1)	$\Sigma_{j,m}Y^2_{.j..m}/rbc$–CT–SS(*A*)–SS(*D*)
	O_3×Time, *BD*	(*b*–1)(*d*–1)	$\Sigma_{k,m}Y^2_{..k.m}/rac$–CT–SS(*B*)–SS(*D*)
	CO_2×O_3×Time, *ABD*	(*a*–1)(*b*–1)(*d*–1)	$\Sigma_{j,k,m}Y^2_{.jk.m}/c$–CT–SS(*A*)–SS(*B*)–SS(*ABD*)
	Error (*d*), *RABD*	(*r*–1)(*a*–1)(*b*–1)(*d*–1)	$\Sigma_{i,j,k,m}Y^2_{ijk.m}/c$–CT–SS(*I*)–SS(*C*)–SS($E_c$)–SS(*AC*)–SS(*BC*)–SS(*ABC*)
	Subtotal I+III+IV	(*rabd*–1)	$\Sigma_{i,j,k,m}Y^2_{ijk.m}/c$–CT
V	Community×Time, *CD*	(*c*–1)(*d*–1)	$\Sigma_{l,m}Y^2_{...lm}/rab$–CT–SS(*C*)–SS(*D*)
	CO_2×Community×Time, *ACD*	(*a*–1)(*c*–1)(*d*–1)	$\Sigma_{j,l,m}Y^2_{.j.lm}/rb$–CT–SS(*A*)–*SS*(*C*)–SS(*D*)
	O_3×Community×Time, *BCD*	(*b*–1)(*c*–1)(*d*–1)	$\Sigma_{k,l,m}Y^2_{..klm}/ra$–CT–SS(*B*)–SS(*C*)–SS(*D*)
	CO_2×O_3×Community×Time, *ABCD*	(*a*–1)(*b*–1)(*c*–1)(*d*–1)	$\Sigma_{j,k,l,m}Y^2_{.jklm}/r$–CT–SS(*A*)–SS(*B*)–SS(*C*)–SS(*D*)
	Error (*e*), *RCD*+*RABCD*	(*r*–1)*ab*(*c*–1)(*d*–1)	$\Sigma_{i,j,k,l,m}Y^2_{ijklm}$–CT–SS(I+II+III+IV)–SS(*CD*)–SS(*ABCD*)
	Grand total	*rabcd*–1	

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