

Accelerated belowground C cycling in a managed agriforest ecosystem exposed to elevated carbon dioxide concentrations

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Abstract

We investigated the effects of three elevated atmospheric CO₂ levels on a *Populus deltoides* plantation at Biosphere 2 Laboratory in Oracle Arizona. Stable isotopes of carbon have been used as tracers to separate the carbon present before the CO₂ treatments started (old C), from that fixed after CO₂ treatments began (new C). Tree growth at elevated [CO₂] increased inputs to soil organic matter (SOM) by increasing the production of fine roots and accelerating the rate of root C turnover. However, soil carbon content decreased as [CO₂] in the atmosphere increased and inputs of new C were not found in SOM. Consequently, the rates of soil respiration increased by 141% and 176% in the 800 and 1200 µL L⁻¹ plantations, respectively, when compared with ambient [CO₂] after 4 years of exposure. However, the increase in decomposition of old SOM (i.e. already present when CO₂ treatments began) accounted for 72% and 69% of the increase in soil respiration seen under elevated [CO₂]. This resulted in a net loss of soil C at a rate that was between 10 and 20 times faster at elevated [CO₂] than at ambient conditions. The inability to retain new and old C in the soil may stem from the lack of stabilization of SOM, allowing for its rapid decomposition by soil heterotrophs.

Keywords: carbon, carbon sequestration, elevated CO₂, *Populus deltoides*, priming effect, root C turnover, soil respiration, stable isotopes

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Introduction

The levels of atmospheric [CO₂] are rapidly increasing. Terrestrial ecosystems exchange, about 120 Gt C yr⁻¹ from the atmosphere, through the processes of photosynthesis and ecosystem respiration (Schlesinger, 1997). Elevated atmospheric [CO₂] often stimulates net primary productivity (NPP) in many ecosystems, including forests (Korner & Arnone, 1992; DeLucia *et al.*, 1999; Norby *et al.*, 2001; Gill *et al.*, 2002). Increased NPP can lead to an increase in soil carbon in some cases (Parton *et al.*, 1996; Matamala *et al.*, 2003) but not in others (Van Kessel *et al.*, 2000; Schlesinger & Lichter, 2001; Van Groenigen *et al.*, 2002; Pendall *et al.*, 2004). Recently, Schlesinger and Lichter (2001) showed a lack of soil C accumulation in a *Pinus taeda* dominated forest after 3 years of elevated [CO₂] using free air CO₂ enrichment

(FACE). This finding appears to be inconsistent with the significant increases observed at the same site in photosynthesis (reviewed by Long *et al.*, 2004), standing biomass (Delucia *et al.*, 1999), and root (Matamala & Schlesinger, 2000) and litter productions (Finzi *et al.*, 2001) in response to high [CO₂]. In contrast, an increase in NPP in a *Liquidambar styraciflua* plantation exposed to elevated [CO₂] using FACE (Norby *et al.*, 2001, 2004) was accompanied by increases in soil C concentration in the upper horizons (Matamala *et al.*, 2003; J. Jastrow, personal communication). One potential explanation for this inconsistency may stem from the different responses of plant and soil respiration (R_{soil}) to elevated [CO₂] (Gonzalez-Meler *et al.*, 2004).

Responses of stand-level plant respiration to elevated [CO₂] may be proportional to increases in plant size and changes in carbon allocation patterns (reviewed in Gonzalez-Meler *et al.*, 2004). Responses of R_{soil} and its components to elevated [CO₂] are more complex because R_{soil} is a multi-organismal network of oxidation

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pathways that results in a major source of atmospheric C from land. Soil respiration has also been shown to be a major determinant of net ecosystem C budgets (Valentini *et al.*, 2000; Giardina & Ryan, 2000). The rates of R_{soil} of ecosystems exposed to elevated [CO₂] usually increase when compared with ambient controls (see Gonzalez-Meler & Taneva, 2004; King *et al.*, 2004). However the effects of high [CO₂] and other abiotic variables on the root/rhizosphere (defined as root respiration and microbial turnover of recent rhizodeposits), and heterotrophic components of R_{soil} , prevent the forecasting of the soil carbon sequestration potential (Knorr & Heimann, 2001).

The root/rhizosphere and heterotrophic components of R_{soil} may respond to high [CO₂] and climatic changes in contrasting ways. Part of the observed increase in R_{soil} of ecosystems exposed to elevated [CO₂] is because of recently photosynthetically fixed carbon (Andrews & Schlesinger, 2001; Pataki *et al.*, 2003a), because of increases in root/rhizosphere surface area, leaf litter inputs and decomposition and root production (Matamala & Schlesinger, 2000; Finzi *et al.*, 2001; Matamala *et al.*, 2003; Norby *et al.*, 2004). Increases in rates of root/rhizosphere respiration of plants exposed to elevated [CO₂], quickly returns plant photosynthate back to the atmosphere as CO₂ (Cheng & Johnson, 1998; Hamilton *et al.*, 2002) with no consequences for soil C accrual. The response of the heterotrophic component of R_{soil} to elevated [CO₂] is less known (Subke *et al.*, 2004; D. Lipson, personal communication). Increases in heterotrophic respiration in response to elevated [CO₂], however, may result in soils becoming a net source of CO₂ to the atmosphere if inputs of new C do not keep pace (Zak *et al.*, 1993; Hungate *et al.*, 1997). It has also been shown that decomposition of soil organic matter (SOM) can be stimulated in response to a substrate-induced priming effect (i.e. stimulation of microbial decomposition rates by enhanced availability of labile substrates) (Fontaine *et al.*, 2004) such as in soils of ecosystems exposed to elevated [CO₂] (Zak *et al.*, 1993).

Stocks of SOM are controlled by the balance between C inputs derived from plant production and outputs through decomposition processes mediated by heterotrophs (Jenny, 1941; Schlesinger, 1977). The unbalance between inputs and output to SOM is reflected by alterations of the elemental cycles of carbon and nitrogen (Jackson *et al.*, 2000; Lal, 2004). At the global scale, climate plays a significant role in the dynamics and storage of SOM (Nakane, 1975; Schlesinger, 1977; Sala *et al.*, 1988), however, at the regional scale, factors such as soil texture and vegetation type may be stronger determinants of variability in SOM content, as well as its rates of accumulation and decomposition. For instance, as clay content increases, C outputs

decrease because formation of organo-minerals slows decomposition (Paul, 1984). Therefore, responses of SOM turnover to perturbations such as rising atmospheric [CO₂] are confounded by the fact that C-cycling in physically or chemically protected SOM pools is not controlled by its inherent decomposability (Jastrow, 1996; Six *et al.*, 2002). Incorporation of otherwise mineralizable SOM to a stable protected form is accomplished by soil texture and by macro- and microorganisms, which promote soil aggregate formation (Tisdall & Oades, 1982; Jastrow, 1996).

In this study, we have characterized the short- and long-term dynamics of the root and SOM pools, and of R_{soil} in a *Populus deltoides* forest plantation exposed to elevated atmospheric [CO₂] in the Biosphere 2 Laboratory (B2L) for 4 years. Stable isotopes of C have been used as tracers of the photosynthetic C produced during the 4 years of the experiment in roots, soil, and R_{soil} . The plantations were also managed to restrict leaf litter inputs and had poorly developed macrofauna communities, limiting SOM stabilization.

Materials and methods

Site characteristics

The experiment was conducted at the B2L located in Oracle, Arizona, USA, approximately 30 km north of the city of Tucson (32°37.13'N; 110°47.05'W, 1200 m a.s.l.) in the intensively managed *P. deltoides* (Bartr.) genotype S7c8 forest plantation (IFP). Mid-day photosynthetic photon flux density (PPFD) consistently exceeded 1500 $\mu\text{mol m}^{-2}\text{s}^{-1}$ throughout the growing seasons although UV radiation was blocked by the glass structure (Barron-Gafford *et al.*, 2005). Each plantation had a volume of approximately 11 873 m³ with a soil area of 550 m². Plantation isolation within the IFP was accomplished with a thick translucent plastic curtain and double containment doors. Details on climate control and the structure of the IFP can be found elsewhere (Dempster, 1999).

Soils (1 m deep) were constructed as a silty loam rich in nutrients from initial fertilization in 1992 before any plant growth occurred. Soil texture consisted of 30% sand, 36% silt, and 24% clay (Torbert & Johnson, 2001). Soil texture is similar to that of soils in the San Pedro River Riparian area in Southeastern Arizona (D. Martens personal communication). From May 1992 until fall 1997 several soil sectors were always planted with *Triticum aestivum* L. All soil, flux, and isotope measurements were made in these wheat soil sectors to minimize variability. In May 1998, cuttings of *P. deltoides* clones were planted and grown at near-ambient [CO₂] for two growing seasons (Table 1). In May 2000,

Table 1 The history of plant species and treatments applied to the same soils in the AM/IFP of B2L since 1992

Plant type	Timeline	Manipulation
<i>Triticum aestivum</i>	May 1992– November 1997	Row crop-wheat
<i>Populus deltoides</i> Bartr.	May 1998	Seedlings planted
	January 1999	Coppiced
	March 1999	Temperature raised
	May 2000	CO ₂ treatment began
	January 2001	Coppiced
	March 2001	Temperature raised
	January 2002	Coppiced
	March 2002	Temperature raised
	November 2002	Induced drought
	January 2003	Dormancy
	March 2003	Temperature raised
	December 2003	Final total tree harvest

From 1999 to 2004 winter was induced by lowering the temperature to 20/12 °C (day/night) to send the trees into dormancy before coppicing. Each March, air *T* was increased to 25/20 °C (day/night) to allow the trees to resprout.

AM, agricultural mesocosm; IFP, intensively managed forest plantation; B2L, Biosphere 2 Laboratory.

elevated CO₂ treatments (400, 800, and 1200 µL L⁻¹ [CO₂]) were initiated. At the end of each growing season (December), dormancy was induced by lowering the air temperature (*T*) to 20/12 °C (day/night) and the main stem of each tree was coppiced to 30 cm above the soil. Growth was induced each spring by increasing air *T* to 25/20 °C (day/night). Resprouting began a few weeks after the temperature was changed, and by May all trees were pruned to have a single leader (Barron-Gafford *et al.*, 2005). Chamber temperature was maintained at 30/25 °C throughout the growing season. All aboveground biomass, i.e. leaf litter and fallen twigs, was removed from the soil surface throughout the growing season and after coppicing. Control of rain events and vapor pressure deficit (VPD) were accomplished by overhead irrigation and misting devices. Rain events occurred weekly irrigating with 11 356 L of water, thereby simulating 18.6 mm rainfall per event. Further details on the stand and management particulars can be found elsewhere (Murthy *et al.*, 2003; Barron-Gafford *et al.*, 2005).

CO₂ treatments and establishment of a carbon isotope tracer

In May 2000, CO₂ treatments began in each of the three bays of the IFP, reflecting near current (400 µL L⁻¹), 800, and 1200 µL L⁻¹ levels of atmospheric [CO₂]. The CO₂

was delivered from a tank reservoir to the plantations through large air handlers and mixing fans running at 60 Hz day and night to circulate the air. Use of tank CO₂ ceased at 10:00 hours each evening and began some minutes before sunrise. During the night-time the [CO₂]s were maintained by flushing the chambers with ambient air.

The tank CO₂ used to increase the [CO₂] in the IFP, had a constant and more negative isotopic signal than air. Changes in the air isotopic composition was used as an ecosystem C tracer after CO₂ treatments, were initiated (see Pataki *et al.*, 2003b). At the onset of the CO₂ treatments, the isotopic composition of atmospheric CO₂ during the photoperiod was decreased from a δ¹³C of -7.8 to a measured -10.5 ± 0.1‰, -19.4 ± 0.1‰, and -22.5 ± 0.2‰ for the 400, 800, and 1200 µL L⁻¹ [CO₂], respectively. (The tank source CO₂ used for increasing the [CO₂] in the IFP had a δ¹³C signature of -26.7 ± 0.3‰.) Unlike other elevated CO₂ field experiments (i.e. FACE), the three [CO₂] treatments had a detectable change in the isotopic signature of air CO₂ after CO₂ treatments began. Therefore, the new isotopic signature of atmospheric CO₂ affected the C-isotope composition of new vegetation in all three CO₂ treatments. The isotope tracer was used to track and separate the C fixed by photosynthesis after the CO₂ treatments began (new C) from C fixed before the CO₂ treatments started (old C) in May 2000.

Soil and root biomass collection

Soil cores were collected bimonthly from May 1998 until December 2003 and stored in a -80 °C freezer until processing. Soil cores from the IFP were taken randomly within three areas per CO₂ treatment. Soil samples were then sectioned in four depths 0–25, 25–50, 50–75 cm, and 75 cm–1 m. Live roots were carefully removed and separated into two size classes: fine roots ($\phi < 2$ mm) and coarse roots ($\phi > 2$ mm). Dead roots (as defined in Matamala & Schlesinger, 2000) were usually not found. Root samples were then rinsed with deionized water to remove adhered soil particles, and dried in a 65 °C air-force drying oven until constant dried weight was obtained. Root tissue was then ground to a fine powder for elemental and isotope analyses. Variance between root biomass collected from soil cores and that from the whole tree harvest varied by less than 0.01 kg m⁻².

Root free soil was rinsed with 0.1 M HCl to remove potential carbonates from the samples (soil in the top 25 cm did not contain carbonates). Washed soil samples were rinsed with deionized water under vacuum with the soil trapped on a glass microfiber filter. Soils were then placed in aluminum foil boats and dried in an

oven set to 80 °C (to preserve isotope composition) until constant dry weight was obtained. Dry samples were finely ground using a mortar and pestle and a soil subsample was loaded into a tin capsule and analyzed for C and N content and for stable isotope ratios.

The elemental analyses of roots and soils were made using a Costech Elemental Analyzer (Valencia, CA, USA) with a zero-blank autosampler with electronic actuator to eliminate air contamination in the samples. Stable isotope analyses of C and N were done on the same sample (pick jump) using a Finnigan Delta Plus XL ion ratio mass spectrometer (Bremen, Germany) operated in continuous flow mode and interfaced with conflo-III with a precision of 0.05‰ for ¹³C and 0.1‰ for ¹⁵N. Elemental and solid international and secondary isotope standards were used for instrument calibration and sample conversion to δ values.

Partitioning of carbon between old and new C

As indicated above, the source CO₂ used to maintain a [CO₂] in the three plantations had a distinct isotopic signal from that of normal air. This new air-CO₂ isotopic signal was used as an ecosystem C tracer after CO₂ treatments were initiated. The isotope tracer can be used to separate the amount of preexisting 'old' carbon in plant roots, soil and CO₂ fluxes (δ¹³C_{old}) from the 'new' carbon fixed by photosynthesis (δ¹³C_{new}) after the CO₂ treatments began (Pataki *et al.*, 2003a; Matamala *et al.*, 2003). The fraction of old carbon (*f*) in a given sample (δ¹³C_{sample}) will be measured as

$$\delta^{13}\text{C}_{\text{sample}} = (f)\delta^{13}\text{C}_{\text{old}} + (1 - f)\delta^{13}\text{C}_{\text{new}}.$$

The old C endmember (δ¹³C_{old}) was determined as the average isotopic composition of root or soils collected prior to the onset of CO₂ treatments. The new C endmember (δ¹³C_{new}) was determined as the root isotopic composition from ingrowth cores collected in 2003 (see Matamala *et al.*, 2003).

The isotope tracer in the three CO₂ treatments allowed for the determination of root C turnover. The standing crop of fine roots was progressively dominated by roots that reflected the new signature of the air CO₂ in each of the three CO₂ treatments. Root C turnover was determined by monitoring the change in the C isotopic composition of fine roots (diameter <2 mm) over time, following the procedures of Matamala *et al.* (2003). In brief, after determining the proportion of old C in roots over time (*t*) an exponential decay ($F(t) = ae^{-kt}$) was fitted to the data in which *a* is the initial amount of old C and *k* is the decay constant of old C. The mean residence time (MRT) of root C was then determined as $-1/k$.

Soil respiration and partitioning the isotopic composition of soil-respired CO₂

Soil respiration was determined by using a Li-Cor 6400-09 portable photosynthesis system (Lincoln, NE, USA) on 10 PVC collars placed in the same soil sectors as for root and soil sample collections. Collars were inserted 2 cm into the soil during dormancy and kept at the same place during the entire experiment. Soil respiration was measured on a transect during both the day and night. Soil respiration did not vary between day and night (less than 5% at the same collar). Each *R*_{soil} measurement was determined as the average of a three cycle measurement each consisting of at least 15 points.

Soil-respired CO₂ gas samples were collected into evacuated 150 mL flasks (glass plugs, PTFE tip O-rings, 63.4 mm outer diameter in the outlet arms) through a water trap connected to a soil chamber attached to a Li-Cor 6400-09 Portable Photosynthesis System. At the same time of sample collection, we measured its [CO₂]. An air-filled balloon replaced the volume of the sample taken from the 1.1 L chamber to avoid pressure changes and air leakage. Gas samples were collected when [CO₂] was at least 40 μL L⁻¹ apart from the previous samples. Samples collected at the same collars were at least 40 min apart to allow for reequilibration of soil [CO₂]. Previous trials had shown reequilibration occurred within 25 min (i.e. isotopic composition of respired CO₂ was the same in two consecutive samples made at the same [CO₂] on the same soil collar). Samples were shipped to the University of Illinois at Chicago for stable isotope analyses. Soil-respired CO₂ was purified using the low-temperature distillation method. Purified CO₂ samples were run through a 25 m Restek column (Gas Bench II) run at room temperature for N₂O separation before isotope analyses were made on a Finnigan Delta Plus XL operated in continuous flow mode against an Oztech CO₂ standard with a precision of 0.02%.

Model II regressions for Keeling plot analyses (Keeling, 1958) were used to determine the δ¹³C of soil-respired CO₂ in the absence of atmospheric air following the recommendations of Pataki *et al.* (2003b). All Keeling plots had [CO₂] spanning more than 200 μL L⁻¹ (Pataki *et al.*, 2003b). Keeling plots that yielded an *r*²-value of less than 0.95 were discarded.

Soil respiration was partitioned into new and old C fluxes as explained above. After the proportion of old C (*f*) was calculated from soil-respired CO₂, it was multiplied by *R*_{soil} from the same sampling period.

Statistical Analyses

The statistics were carried out using SAS software (SAS Institute, Cary, NC, USA), using paired mean *t*-tests

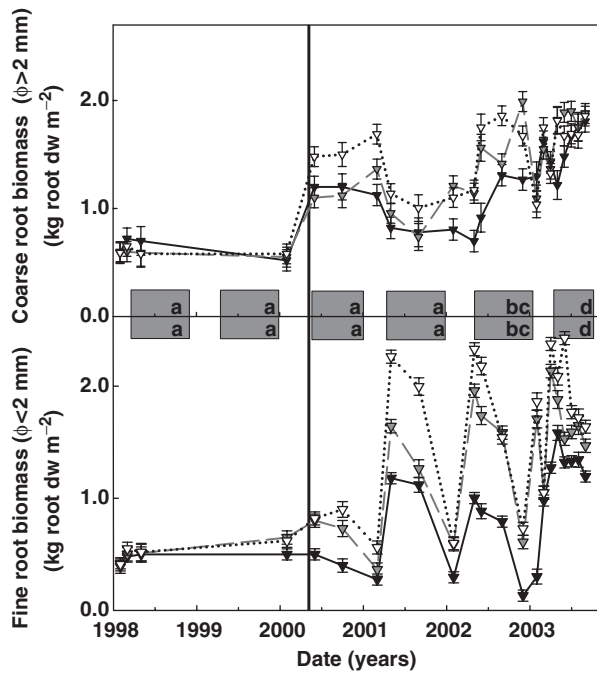


Fig. 1 Coarse root biomass (a, $\phi > 2$ mm) and fine root biomass (b, $\phi < 2$ mm) in the top 25 cm of soil indicated by squares for a forest plantation grown under 400 (solid), 800 (gray) or 1200 (hollow) $\mu\text{L L}^{-1}$ $[\text{CO}_2]$. Growing seasons are denoted by the gray boxes on the x-axis. Tree dormancy with coppicing (a), induced drought (b), dormancy without coppicing (c) and final harvest (d) are also indicated by arrows. The CO_2 treatments (denoted by the black bar) began in May 2000. During dormancy temperatures were dropped to 20/12 °C (day/night) from growing season temperatures (see Methods). Values are averages and standard errors of at least three samples collected at each time interval totaling over 200 samples.

and repeated measures analyses of variance (ANOVA). Curve fitting for the MRT was done by using the curve fit protocol from SigmaPlot graphic package (SPSS Inc., Chicago, IL, USA).

Results

Root biomass

P. deltooides trees produced more roots during the growing season as $[\text{CO}_2]$ increased in the atmosphere (Fig. 1a, b). Before the CO_2 treatments started in May 2000, the amount of coarse ($\phi > 2$ mm) and fine ($\phi < 2$ mm) roots were similar between the three plantations and averaged 0.6 ± 0.03 and 0.5 ± 0.01 kg dw m^{-2} for coarse ($\phi > 2$ mm, Fig. 1a) and fine root biomass ($\phi < 2$ mm, Fig. 1b), respectively. The initiation of the CO_2 treatments caused an increase in both coarse and fine roots. Coarse root biomass increased by 0.7 ± 0.1 ($P = 0.005$), 0.6 ± 0.1

($P = 0.006$), and 0.9 ± 0.1 ($P = 0.003$) kg dw m^{-2} at 400, 800, and 1200 $\mu\text{L L}^{-1}$ CO_2 , respectively from pre- CO_2 treatment levels. Despite an overall increase in coarse root biomass, there was no consistent CO_2 effect in stimulating coarse root biomass production during the 4 years of the study (Fig. 1a). In contrast, fine root biomass ($\phi < 2$ mm) was increased by tree growth at elevated $[\text{CO}_2]$ (Fig. 1b). During coppicing and regrowth cycles (Fig. 1a, 2000–2002), fine root production was increased by $64 \pm 7\%$ ($P = 0.001$) and $113 \pm 3\%$ ($P < 0.001$) at 800 and 1200 $\mu\text{L L}^{-1}$ CO_2 , respectively, when compared with controls. Coppicing of trees always resulted in a sharp decline in fine root biomass with average losses of 0.5 ± 0.3 ($P < 0.001$), 0.9 ± 0.3 ($P < 0.001$), and 1.0 ± 0.4 ($P < 0.001$) kg dw m^{-2} at 400, 800, and 1200 $\mu\text{L L}^{-1}$ CO_2 , respectively. In contrast, regrowth in trees that were dormant with no coppicing by 2003, reduced the CO_2 effect that elevated $[\text{CO}_2]$ had on fine root biomass during the previous 3 years (400–800 $P = 0.14$; 400–1200 $P = 0.06$) (Fig. 1b, c). Reduction in the CO_2 effect was because of a more than twofold ($P < 0.01$) increase in fine root biomass at ambient conditions over the coppicing years. For the 800 and 1200 $[\text{CO}_2]$ treatments, fine root biomass during the growing season of 2003 remained at similar values than in previous years.

After the induced drought from October 30th to December 10th, 2002 (Fig. 1b) (when trees lost a significant portion of their leaves (data not shown)) fine root biomass decreased by 0.7 ± 0.01 ($P < 0.001$), 1.0 ± 0.01 ($P < 0.001$), and 0.8 ± 0.01 ($P < 0.001$) kg dw m^{-2} at 400, 800, and 1200 $\mu\text{L L}^{-1}$ CO_2 , respectively, when compared with predrought conditions. Drought conditions induce a transient reduction of the CO_2 effect in fine root biomass among $[\text{CO}_2]$ treatments (400–800 $P = 0.3$; 400–1200 $P = 0.3$). Between the induced drought and the subsequent induced dormancy of 2003, trees were rewatered and reinitiated growth increasing fine root biomass by 0.2 ± 0.01 ($P = 0.02$), 1.1 ± 0.01 ($P = 0.003$), and 1.1 ± 0.01 ($P = 0.003$) kg dw m^{-2} at 400, 800, and 1200 $\mu\text{L L}^{-1}$ CO_2 , respectively when compared with the drought data (Fig. 1a, b compare (b) and (c)).

Root elemental composition was not altered by coppicing or growth at elevated $[\text{CO}_2]$. During the 4 years of the study root elemental composition remained constant at 36.7 ± 2.3 mg C g dw^{-1} and 0.8 ± 0.02 mg N g dw^{-1} . There was no significant change in root C-to-N ratio, 48.3 ± 4.2 with CO_2 treatment or coppicing, consistent with other studies (Gordon & Jackson, 2000; Olszyk *et al.*, 2003).

Root turnover

Root turnover was determined by examining the disappearance of the old C in the root population

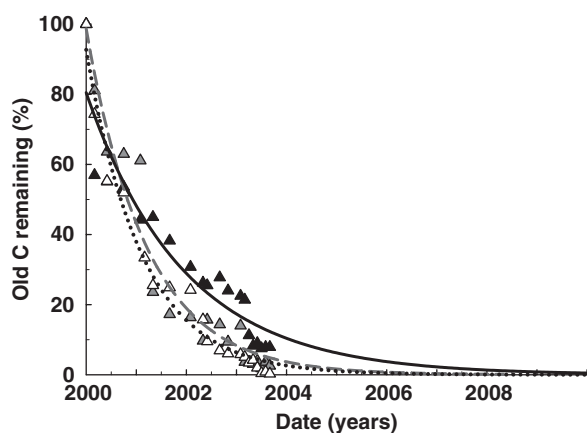


Fig. 2 Old C remaining in fine roots ($\phi < 2$ mm) in size from 0 to 25 cm soil depth for each CO₂ treatment. Using a SigmaPlot nonlinear exponential decay equation [$F(t) = a \exp^{-kt}$, where a is the initial amount of old C (%) and k is the rate of decay of the old C] that best explained the $\delta^{13}\text{C}$ changes in the root biomass over time. The decay of old C in roots was best fit by $F(t) = 0.99 \exp^{-0.0426t}$, $r^2 = 0.97$ and $P(k) = 0.004$ for the 400 $\mu\text{L L}^{-1}$ plantation (solid), $F(t) = 0.99 \exp^{-0.687t}$, $r^2 = 0.97$ and $P(k) = 0.006$ for the 800 $\mu\text{L L}^{-1}$ plantation (gray) and $F(t) = 0.99 \exp^{-0.0743t}$, $r^2 = 0.97$ $P(k) = 0.004$ for the 1200 $\mu\text{L L}^{-1}$ plantation (hollow).

following an exponential decay function (Fig. 2) (Luo, 2003; Matamala *et al.*, 2003). We estimated the MRT of root C as the average number of years that a molecule of C stays in the root system, once it has been first incorporated into root mass. This represents the exponential loss of C from roots within the forestry plantation. The MRT of C in fine roots (<2 mm in diameter) was 2.0 ± 0.2 , 1.2 ± 0.07 , and 1.1 ± 0.04 years for the 400, 800, and 1200 $\mu\text{L L}^{-1}$ CO₂ treatments, respectively.

Soil C and N dynamics

Soil C and N content decreased as [CO₂] in the atmosphere increased (Fig. 3). The initial presence of *P. deltoides*, planted in May 1998 (Table 1), did not affect the soil C and N content (Fig. 3). Soil C and N content were $2.8 \pm 0.2 \text{ kg C m}^{-2}$ and $0.3 \pm 0.02 \text{ kg N m}^{-2}$, respectively, with no differences among the three plantations before CO₂ treatments started. However, small variations in the soil isotopic composition of both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were seen associated to the growing season and coppicing events of the aboveground biomass (Fig. 4a, d – coppicing). When CO₂ treatments began in May 2000 (Figs 3 and 4), the soil C and N content generally decreased at the beginning of the growing season and increased during the later part of the growing season, and through coppicing for the 400 (Fig. 3a) and 800 $\mu\text{L L}^{-1}$ (Fig. 3b) CO₂ treatments. At 1200 $\mu\text{L L}^{-1}$

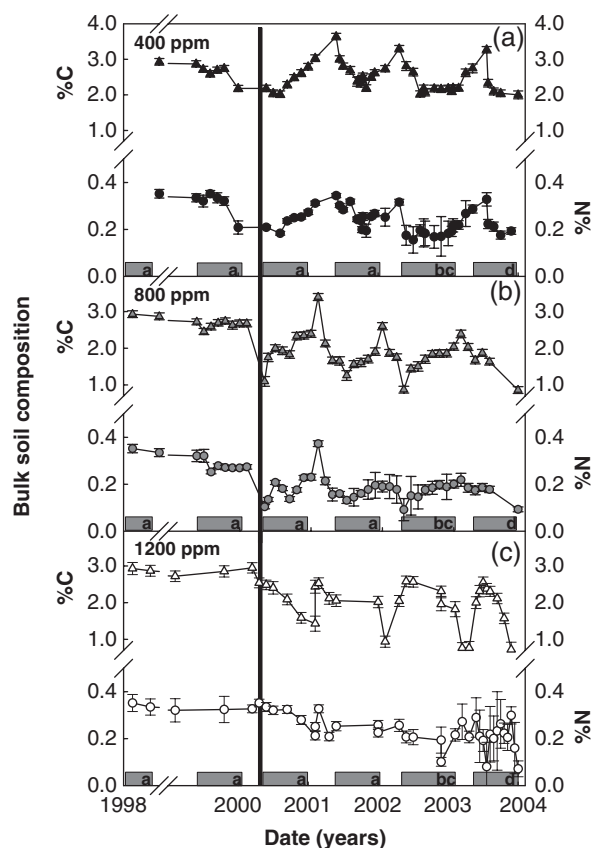


Fig. 3 Soil C and N in a managed forested plantation maintained at elevated [CO₂]. The graphs represent the monthly variation in the %C (triangles) and %N (circles) grown under 400 (solid), 800 (gray) or 1200 (hollow) $\mu\text{L L}^{-1}$ [CO₂]. Values are averages and standard errors of at least three samples collected at each time interval totaling over 200 samples. For other details see 'Materials and Methods' and Fig. 1.

[CO₂], however, soil C was significantly ($P = 0.001$) decreased during the first growing season, from 2.8 ± 0.1 to $1.4 \pm 0.1 \text{ kg C m}^{-2}$ (Fig. 3c) with moderate increases in soil C occurring soon after coppicing. The plantations maintained at 800 and 1200 $\mu\text{L L}^{-1}$ were increased $18 \pm 0.002\%$ ($P = 0.003$) over ambient [CO₂] because of coppicing.

The overall loss of C and N was faster as [CO₂] increased. At near ambient conditions (400 $\mu\text{L L}^{-1}$ CO₂), the overall net loss of soil C and N over the 4-year duration of the experiment occurred at an overall rate of $10 \pm 0.2 \text{ g C m}^{-2} \text{ yr}^{-1}$ and $1.9 \pm 0.1 \text{ g N m}^{-2} \text{ yr}^{-1}$ for C and N, respectively (Fig. 2a). At 800 $\mu\text{L L}^{-1}$ [CO₂], the overall decrease in soil C was $114 \pm 10 \text{ g C m}^{-2} \text{ yr}^{-1}$, about 12 times faster than at ambient conditions. Soil N losses were at a rate of $2.9 \pm 0.1 \text{ g N m}^{-2} \text{ yr}^{-1}$. At 1200 $\mu\text{L L}^{-1}$, soil C content never increased over pretreatment values, and soil C decreased at a rate of

$190 \pm 10 \text{ g C m}^{-2} \text{ yr}^{-1}$, 19 times faster than the rate of carbon loss at ambient conditions. Soil N losses for this treatment were at a rate of $19 \pm 0.8 \text{ g N m}^{-2} \text{ yr}^{-1}$ (Fig. 3c), roughly a rate that is 10 times higher than that at ambient conditions. These differential losses of C and N as influenced by the atmospheric $[\text{CO}_2]$ caused for the C/N ratio of the soil to increase at ambient conditions and to decrease at the elevated $[\text{CO}_2]$ (Table 2).

The increase in the air $[\text{CO}_2]$ decreased the $\delta^{13}\text{C}$ of the air CO_2 from $-7.8 \pm 0.1\text{‰}$ to $-19.4 \pm 0.1\text{‰}$, and $-22.5 \pm 0.1\text{‰}$ for the 800 and the 1200 $\mu\text{L L}^{-1}$ treatment, respectively, thereby changing the $\delta^{13}\text{C}$ of plant litter inputs to $-29.4 \pm 0.1\text{‰}$, $-37.8 \pm 0.1\text{‰}$, and $-40.8 \pm 0.1\text{‰}$. As new C was incorporated into SOM after each growing season as a result of replacement of old C and accrual of new C, then the isotopic composition of bulk soil will become more negative with respect to the pretreatment value and to ambient conditions (Table 3). Neither of the two CO_2 treatments showed a substantial retention of new plant C inputs (Fig. 4). During the first year of high CO_2 exposure, the two elevated CO_2 treatments had a different pattern on the fluctuation of the carbon isotopic composition of bulk soil when compared with the ambient CO_2 treatment (Fig. 4a–c). By the middle of the first growing season, and unlike the ambient treatment, the $\delta^{13}\text{C}$ of bulk soil became more positive reaching a maximum value of $-22.6 \pm 0.07\text{‰}$ by the time trees were first coppiced (Fig. 4). After the first growing season, the $\delta^{13}\text{C}$ of bulk soil at the 800 $\mu\text{L L}^{-1} \text{CO}_2$ treatment decreased throughout the subsequent growing seasons and increased about a month before each coppicing and dormancy periods (Fig. 4b), a pattern similar to that of the ambient treatment (Fig. 4a). The overall trend in the 800 $\mu\text{L L}^{-1}$ treatment was an isotopic depletion in $\delta^{13}\text{C}$ of bulk soil by $-0.14 \pm 0.08\text{‰ yr}^{-1}$ indicating that some new C was incorporated into the soil. In contrast, the soil $\delta^{13}\text{C}$ in the 1200 $\mu\text{L L}^{-1} \text{CO}_2$ treatment was more positive (reaching values similar to desert soils) during the growing season and depleted after coppicing or dormancy. This seasonal cycle varied in amplitude at around a mean value of $-23.8 \pm 0.6\text{‰}$ being maximal (-22.7‰) after the first growing season and minimal (-25.0‰) 2 months after fumigation started.

The isotopic composition of soil N also showed a seasonal variation before and after the CO_2 treatment began (Fig. 4d–f). Before the CO_2 treatments started, the soil $\delta^{15}\text{N}$ followed the same pattern as the soil $\delta^{13}\text{C}$. The soil $\delta^{15}\text{N}$ value increased right after the CO_2 treatment started to then decrease for the remainder of that first growing season at ambient and elevated $[\text{CO}_2]$. The average value of soil $\delta^{15}\text{N}$ was $7.6 \pm 0.3\text{‰}$ but varied from a maximal and minimal value of $9.4 \pm 0.1\text{‰}$ and $5.7 \pm 0.1\text{‰}$ for the all the CO_2 treatments.

Table 2 The bulk density (1998–1999) and the C/N ratios of the soils for 1998 and 2003

$[\text{CO}_2]$ ($\mu\text{L L}^{-1}$)	Bulk density g/cm ³	C/N 1998	C/N 2003
400	0.91 ± 0.1	10.4 ± 0.7	12.1 ± 0.5
800	0.97 ± 0.1	10.3 ± 0.8	9.5 ± 0.8
1200	0.95 ± 0.1	10.3 ± 0.8	6.2 ± 1.2

The C/N ratios were averaged from the carbon and nitrogen content shown in Figs 1 and 2. The C/N values varied from 12.9–8.1, 10.7–7.7 to 10.5–6.2 for the 400, 800, and 1200 $\mu\text{L L}^{-1}$ plantations, respectively. Values are averages of three replicates with standard errors. Each replicate unit is the average of more than 150 measurements over a 2-year period.

Soil respiration and the isotopic composition of soil-respired CO_2

The rates of R_{soil} were consistently increased by plant exposure to elevated $[\text{CO}_2]$ (Table 3). Late in the growing season in 2002 (October–November), R_{soil} was 5.9 ± 0.1 , 9.5 ± 0.3 , and $12.4 \pm 0.2 \mu\text{mol m}^{-2} \text{ s}^{-1}$ at 400, 800, and 1200 $\mu\text{L L}^{-1} \text{CO}_2$, respectively. This represented an increase in R_{soil} of 61% and 111% at 800 and 1200 $\mu\text{L L}^{-1} \text{CO}_2$, respectively when compared with the 400 $\mu\text{L L}^{-1} \text{CO}_2$ treatment ($P < 0.01$). During the most active plant growth part of the growing season in 2003 (June–September) R_{soil} was 7.6 ± 0.2 , 11.0 ± 0.1 , and $13.8 \pm 0.1 \mu\text{mol m}^{-2} \text{ s}^{-1}$ for the 400, 800, and 1200 $\mu\text{L L}^{-1} \text{CO}_2$ treatment, respectively. During this period, the relative increase in R_{soil} was 45% ($P = 0.001$) at the 800% and 82% ($P < 0.001$) at the 1200 $\mu\text{L L}^{-1} \text{CO}_2$ treatment, slightly less than during the end of the previous growing season. By the end of the growing season in 2003 (October–November), R_{soil} were $7.9 \pm 0.2 \mu\text{mol m}^{-2} \text{ s}^{-1}$ at 400, $11.1 \pm 0.2 \mu\text{mol m}^{-2} \text{ s}^{-1}$ at 800 and $14.1 \pm 0.1 \mu\text{mol m}^{-2} \text{ s}^{-1}$ at 1200 $\mu\text{L L}^{-1} \text{CO}_2$, which was an increase in R_{soil} of 18% ($P = 0.02$) and 89% ($P = 0.005$) over ambient levels for the 800 and the 1200 $\mu\text{L L}^{-1} \text{CO}_2$ treatment, respectively. R_{soil} was significantly higher at the end of the growing season of 2003 than at the end of the same period in 2002 across CO_2 treatments.

In order to determine the origin of carbon contributing to R_{soil} , we measured the isotopic composition of soil-respired CO_2 (Table 3). We determined the amount of old C (defined as C in R_{soil} that originated from soil C pools that were present before the CO_2 treatments started) and new C (defined as C in R_{soil} that originated from soil C pools that were formed after the CO_2 treatments began) as explained in Materials and Methods. By the end of the growing season in 2002 (October–November), the proportion of old C (f) in R_{soil}

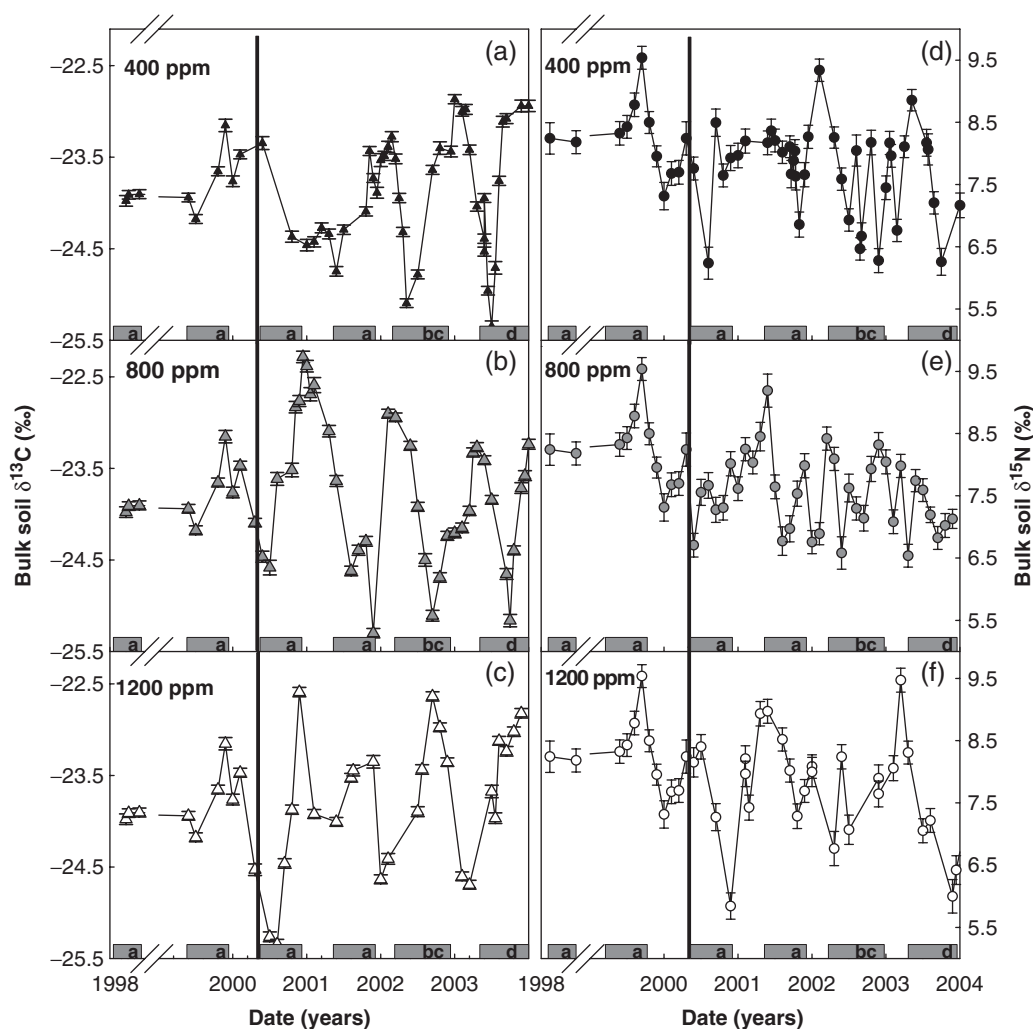


Fig. 4 The C (triangles) and N (circles) isotopic composition of bulk soil collected from the three plantations exposed to either 400 (solid), 800 (gray) or 1200 (hollow) $\mu\text{L L}^{-1}$ [CO₂]. Values are averages and standard errors of at least three samples collected at each time interval totaling over 200 samples. For other details see 'Materials and Methods' and Fig. 1.

was 0.32 ± 0.01 , 0.45 ± 0.01 , and 0.50 ± 0.01 in the 400, 800, and $1200 \mu\text{L L}^{-1}$ plantations. The amount of old C in R_{soil} was 1.9 ± 0.1 , 4.4 ± 0.1 , and $6.2 \pm 0.1 \mu\text{mol m}^{-2} \text{s}^{-1}$ at 400, 800, and $1200 \mu\text{L L}^{-1}$ [CO₂], respectively. This represented an increase in respiration of old C of 131% ($P = 0.002$) and 226% ($P = 0.001$) at 800 and $1200 \mu\text{L L}^{-1}$ CO₂, respectively, over the $400 \mu\text{L L}^{-1}$ CO₂ treatment. During the most active plant growth part of the growing season in 2003 (June–September) the proportion of old C (f) in R_{soil} was 0.36 ± 0.01 , 0.47 ± 0.01 , and 0.53 ± 0.02 and the amount of old C in R_{soil} was 3.2 ± 0.1 , 5.4 ± 0.2 , and $7.2 \pm 0.1 \mu\text{mol m}^{-2} \text{s}^{-1}$ for the 400, 800, and $1200 \mu\text{L L}^{-1}$ CO₂ treatment, respectively. During this period, the relative increase of old C in R_{soil} was 68% ($P = 0.008$) at the 800% and 125% ($P < 0.001$) at the $1200 \mu\text{L L}^{-1}$ CO₂ treatment. By the end of the growing

season in 2003 (October–November), f was 0.40 ± 0.01 , 0.48 ± 0.01 , and 0.52 ± 0.01 while the amount of old C in R_{soil} was 3.0 ± 0.1 , 5.5 ± 0.2 , and $7.4 \pm 0.1 \mu\text{mol m}^{-2} \text{s}^{-1}$ at 400, 800, and $1200 \mu\text{L L}^{-1}$ CO₂, respectively. This is an increase of old C in R_{soil} of 53% ($P = 0.01$) and 150% ($P = 0.001$) over ambient levels for the 800 and the $1200 \mu\text{L L}^{-1}$ CO₂ treatment, respectively. Old C in R_{soil} was slightly higher at the end of the growing season of 2003 than at the end of the same period in 2002 across CO₂ treatments.

The rates of R_{soil} 10 days after coppicing at the end of 2003 were markedly reduced and similar across CO₂ treatments (Fig. 5). R_{soil} switched from a mix of new and old C sources before coppicing to primarily new C after aboveground biomass was removed. Rates of old C in R_{soil} decreased by 2.8 ± 0.2 , 4.7 ± 0.2 , and $6.7 \pm 0.2 \mu\text{mol m}^{-2} \text{s}^{-1}$ at 400, 800, and $1200 \mu\text{L L}^{-1}$ CO₂,

respectively, whereas the rates of new C were decreased by 3.6 ± 0.2 , 2.5 ± 0.3 , and $3.6 \pm 0.2 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively.

Discussion

The growth of *P. deltoides* at elevated atmospheric $[\text{CO}_2]$ increased belowground carbon inputs by increasing fine root biomass and the root C turnover. However, and despite increased plant carbon inputs under elevated atmospheric $[\text{CO}_2]$, the amount of carbon in the soil decreased. The soils of the IFP may not have had the capacity to protect and stabilize SOM and, consequently, new C inputs were released back to the atmosphere via respiration by soil heterotrophs. As a result, the rates of R_{soil} were higher under elevated than under ambient conditions. Moreover, increased C inputs to soils in plantations exposed to elevated $[\text{CO}_2]$ resulted in an increase in the rate at which decomposers oxidized old C. The net loss of soil C occurred at a rate that was between 10 and 20 times faster at elevated $[\text{CO}_2]$ than at ambient conditions.

Fine root biomass markedly increased during the growing season in trees grown under elevated $[\text{CO}_2]$ when compared with the ambient $[\text{CO}_2]$ (Fig. 1). Increases in fine root biomass are generally seen in plants and ecosystems exposed to elevated $[\text{CO}_2]$ although the level of response varies (Curtis & Wang, 1998). *P. deltoides* experienced 64% and 113% enhancement in fine root biomass after 4 years at 800 and $1200 \mu\text{L L}^{-1}$, comparable with other elevated $[\text{CO}_2]$ studies. In a *P. taeda* dominated forest exposed to elevated $[\text{CO}_2]$ (ambient + $200 \mu\text{L L}^{-1}$) using FACE, fine root biomass increased by 86% over ambient levels after 2 years of study (Matamala & Schlesinger, 2000). Similar results were seen in other FACE sites (Norby *et al.*, 2004) and for other *Populus* species under FACE (King *et al.*, 2001). For instance, *P. alba* L., *P. nigra* L., and *P. deltoides euramericana* showed fine root biomass increases of 35%, 84% and 53% after 3 years of a $200 \mu\text{L L}^{-1}$ increase in atmospheric $[\text{CO}_2]$ enrichment (Lukac *et al.*, 2003). Fine root biomass has also been shown to increase at comparable levels at a higher atmospheric $[\text{CO}_2]$ of $700 \mu\text{L L}^{-1}$ (Pregitzer *et al.*, 2000; Dilustro *et al.*, 2002). Our results are also consistent with the root excavation data done at the same site (Barron-Gafford *et al.*, 2005). Barron-Gafford *et al.* (2005), reported fine root biomass increases of 0.45, 0.52, and $0.68 \text{ kg tree}^{-1}$ at 400, 800, and $1200 \mu\text{L L}^{-1} \text{CO}_2$, respectively. This increase in plant C allocation to roots under elevated $[\text{CO}_2]$ has been proposed as an important mechanism to increase soil C storage (Singh *et al.*, 2003). Considering the importance of root biomass and turnover in regulating belowground ecosystem C cycling,

Table 3 The isotopic composition of soil-respired $[\text{CO}_2]$ the amount of old (pre- CO_2 treatment) and new C (post- CO_2 treatment) in R_{soil} and the old and new C end members for a forest plantation exposed to either 400, 800 or $1200 \mu\text{L L}^{-1} [\text{CO}_2]$ for 4 years

Date (M/D/Y)	400 $\mu\text{L L}^{-1}$			800 $\mu\text{L L}^{-1}$			1200 $\mu\text{L L}^{-1}$		
	$R_{\text{soil}} \delta^{13}\text{C}$ (‰)	$R_{\text{soil}} \text{old C}$ ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	$R_{\text{soil}} \text{new C}$ ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	$R_{\text{soil}} \delta^{13}\text{C}$ (‰)	$R_{\text{soil}} \text{old C}$ ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	$R_{\text{soil}} \text{new C}$ ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	$R_{\text{soil}} \delta^{13}\text{C}$ (‰)	$R_{\text{soil}} \text{old C}$ ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	$R_{\text{soil}} \text{new C}$ ($\mu\text{mol m}^{-2} \text{s}^{-1}$)
Oct 15, 2002	-23.7 ± 0.1	1.8 ± 0.3	4.0 ± 0.3	-30.1 ± 0.1	4.5 ± 0.4	5.1 ± 0.4	-32.1 ± 0.1	6.3 ± 0.4	6.2 ± 0.4
Nov 12, 2002	-25.2 ± 0.1	1.9 ± 0.4	4.0 ± 0.4	-30.0 ± 0.1	4.2 ± 0.4	5.0 ± 0.4	-31.9 ± 0.1	6.0 ± 0.4	6.2 ± 0.4
June 9, 2003	-25.7 ± 0.1	3.1 ± 0.3	4.8 ± 0.3	-30.3 ± 0.1	4.8 ± 0.4	5.2 ± 0.4	-32.6 ± 0.1	6.8 ± 0.4	6.8 ± 0.4
July 04, 2003	-25.5 ± 0.1	2.9 ± 0.4	5.1 ± 0.4	-30.7 ± 0.2	5.3 ± 0.3	5.1 ± 0.3	-32.6 ± 0.1	7.2 ± 0.4	6.5 ± 0.4
July 25, 2003	-25.8 ± 0.1	3.4 ± 0.4	5.2 ± 0.4	-30.7 ± 0.3	5.4 ± 0.3	5.2 ± 0.3	-32.4 ± 0.1	7.3 ± 0.4	6.4 ± 0.4
Aug 26, 2003	-25.9 ± 0.1	3.2 ± 0.3	4.5 ± 0.3	-30.4 ± 0.1	5.8 ± 0.3	6.1 ± 0.3	-32.7 ± 0.1	7.4 ± 0.4	6.5 ± 0.4
Sept 13, 2003	-26.1 ± 0.1	3.4 ± 0.3	4.1 ± 0.3	-30.3 ± 0.1	5.5 ± 0.4	6.0 ± 0.4	-32.6 ± 0.1	7.4 ± 0.3	6.5 ± 0.3
Sept 30, 2003	-26.0 ± 0.1	3.2 ± 0.4	4.1 ± 0.3	-30.1 ± 0.1	5.5 ± 0.4	6.3 ± 0.4	-32.6 ± 0.1	7.4 ± 0.4	6.6 ± 0.4
Oct 15, 2003	-25.8 ± 0.1	2.9 ± 0.3	4.3 ± 0.3	-30.3 ± 0.1	5.6 ± 0.4	6.0 ± 0.4	-32.4 ± 0.1	7.3 ± 0.3	6.7 ± 0.3
Oct 30, 2003	-25.6 ± 0.1	3.0 ± 0.3	5.2 ± 0.3	-31.0 ± 0.2	5.9 ± 0.4	5.3 ± 0.4	-32.7 ± 0.1	7.6 ± 0.3	6.6 ± 0.3
Nov 10, 2003	-25.6 ± 0.1	3.1 ± 0.2	5.3 ± 0.3	-30.4 ± 0.1	5.3 ± 0.4	5.6 ± 0.4	-32.6 ± 0.1	7.3 ± 0.4	6.6 ± 0.4
Nov 25, 2003	-25.6 ± 0.1	2.9 ± 0.3	5.0 ± 0.3	-30.3 ± 0.1	5.3 ± 0.4	5.7 ± 0.4	-32.5 ± 0.2	7.3 ± 0.4	6.7 ± 0.4
Pretreatment old soil C				-23.4 ± 0.2					
Treatment new C				-37.8 ± 0.1					

Values are averages and standard errors (determined from model II regressions) using the Keeling plot approach.

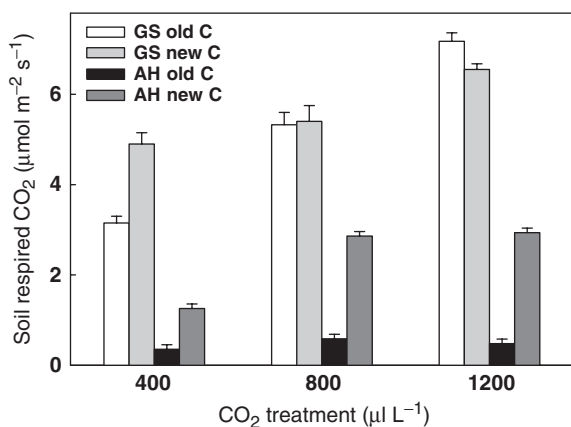


Fig. 5 Old and new C in soil-respired CO₂ when aboveground biomass is present or harvested. Old C (white bar, $f = 0.37 \pm 0.02$, 0.49 ± 0.01 , 0.52 ± 0.01 in the 400, 800 and 1200 $\mu\text{L L}^{-1}$ plantations) and new C (light gray bar, $f = 0.63 \pm 0.01$, 0.51 ± 0.01 , 0.48 ± 0.02 in the 400, 800 and 1200 $\mu\text{L L}^{-1}$ plantations) in soil-respired CO₂ during the growing season (GS) in 2003 and old C (black bars, $f = 0.22 \pm 0.02$, 0.17 ± 0.02 , 0.14 ± 0.01 in the 400, 800 and 1200 $\mu\text{L L}^{-1}$ plantations) and new C (dark gray, $f = 0.78 \pm 0.01$, 0.83 ± 0.01 , 0.86 ± 0.01 in the 400, 800 and 1200 $\mu\text{L L}^{-1}$ plantations) after the final aboveground harvest (AH) in 2003 for the three plantations maintained at 400, 800 and 1200 $\mu\text{L L}^{-1}$.

estimates of seasonal and interannual fluctuations in root biomass are essential (Pendall *et al.*, 2004). However, only few studies have examined seasonal cycles of root dynamics in forests (Matamala & Schlesinger, 2000), often because of the destructive nature of soil sampling and restrictions on collections at FACE sites. Oscillations in fine root biomass during the growing season were affected by both, exposure to elevated atmospheric [CO₂] and aboveground biomass (Fig. 1). The amplitude of the seasonal fine root dynamics appears to be enhanced by exposure to elevated [CO₂] (Fig. 1b) (Matamala & Schlesinger, 2000). The coppicing of aboveground biomass resulted in live root biomass losses while root regrowth occurred rapidly after dormancy (Fig. 1). Because photosynthetic tissues were not fully developed at the time root regrowth started, nonstructural carbohydrate reserves in rhizomes and coarse roots could have supported belowground and aboveground growth (Loescher *et al.*, 1990). Therefore, elevated [CO₂] may increase the turnover of belowground plant carbon.

Plants grown at elevated [CO₂] had accelerated rates of fine root C turnover as evidenced by the isotopic composition of roots (Fig. 2). Root C turnover was increased by 45% and 55% at 800 and 1200 $\mu\text{L L}^{-1}$, respectively, over ambient levels. In addition to increases in root biomass, root turnover has been

hypothesized as a major mechanism to increase C inputs to soils under elevated atmospheric [CO₂] (Berntson & Bazzaz, 1997; Fitter *et al.*, 1997; Pregitzer *et al.*, 2000; Wan *et al.*, 2004). Jackson *et al.* (1996) calculated that root turnover accounted for about a third of global NPP. However, recent experimental evidence suggests that the MRT of root C is longer than previously thought (Gaudinski *et al.*, 2000; Langley *et al.*, 2002; Matamala *et al.*, 2003; Pendall *et al.*, 2004) reducing the impact that root turnover may have on soil C sequestration. Fine root C turnover values in our study were between 1 and 2 years, similar to root C turnover values for *L. styraciflua* (Matamala *et al.*, 2003; Norby *et al.*, 2004) and considerably shorter than for *P. taeda* (Matamala *et al.*, 2003) or other hardwood or grass species (Gaudinski *et al.*, 2000; Milchunas & Lauenroth, 2001; Pendall *et al.*, 2004). Using the ¹³C tracer method for elevated and the radiocarbon method for ambient conditions, Matamala *et al.* (2003) concluded that root C turnover was not affected in a *P. taeda* forests when atmospheric CO₂ was elevated by 200 $\mu\text{L L}^{-1}$. Unfortunately, the lack of isotope tracers under ambient conditions has prevented the identification of CO₂ effects on root C turnover in other studies using the isotope tracer method. Taking advantage of the addition of an isotope label at the three [CO₂], we were able to detect changes in the MRT root C turnover: an increase in 400 $\mu\text{L L}^{-1}$ in atmospheric [CO₂] reduce the MRT (i.e. increased turnover) of root C from 2 to 1.2 years. Further increases in atmospheric [CO₂] did not affect the MRT of root C, suggesting a CO₂ saturation response. Increases in root turnover have been seen in other tree species using other methods at increases in [CO₂] comparable with this study (Norby *et al.*, 1992; Pregitzer *et al.*, 1995; Berntson & Bazzaz, 1997) but not in FACE experiments (Matamala & Schlesinger, 2000; Norby *et al.*, 2004) perhaps because of the lower increase in [CO₂]. An exception are other *Populus* species exposed to elevated [CO₂] under FACE in which fine root turnover was enhanced between 27% and 55% after 3 years of exposure (Lukac *et al.*, 2003). Luo (2003) argue that isotope dilution from the use of nonstructural carbohydrate reserves for new root growth could result in overestimation of root MRT and longevity. However, a distinction should be made between the turnover of the root structure and that of the C contained in it. The isotope tracer method measures the dynamics of C in the plant tissues and includes the use of storage C for replacing structural C in existing tissue as well as any reabsorption of carbon before the decay of tissue takes place. Because these processes (storage and reabsorption) are very difficult to measure in root tissues, we argue that root C turnover best represents the dynamics of plant C in root systems.

Although, increase root biomass was not supported by storage at the Duke FACE site (Matamala *et al.*, 2003, 2004; Luo *et al.*, 2004) the seasonal dynamics seen in root biomass during coppicing events in our study (Fig. 1), suggests that substantial fine root growth (as much as 50%) was supported by carbohydrate reservoirs. Storage of nonstructural carbohydrates is often seen in species prone to defoliation (Iwasa & Kubo, 1997) and Langley *et al.* (2002), found that 33% of new fine roots produced each year can be derived from stored C in a fire-dominated ecosystem exposed to elevated $[\text{CO}_2]$. The increases in root C turnover under elevated $[\text{CO}_2]$ seen in this study may be due in part to a faster utilization and turnover of nonstructural carbohydrates for growth at the beginning of the growing season after each coppicing event.

Seasonal oscillations of soil C (Fig. 3) are coupled to the seasonality seen in fine roots. However, exposure to elevated $[\text{CO}_2]$ decreased soil C content during tree dormancy (Fig. 3). The current expectation is that soils at elevated $[\text{CO}_2]$ have a greater C sequestration potential (Parton *et al.*, 1996; Pregitzer *et al.*, 2000; Pendall *et al.*, 2004). However, this is highly dependant on the response of the soil heterotrophic community to changes in environmental conditions, the soil type, and the type of plant inputs. Harvesting aboveground biomass resulted in a decreased supply of C to roots and cessation of water and nutrient movement (Barron-Gafford *et al.*, 2005). Irrespective of the $[\text{CO}_2]$ concentration soil C increased soon after coppicing (Fig. 3) when live root biomass decreased (Fig. 1), but soil C subsequently decrease, especially when air temperature was increased to induce tree regrowth (Fig. 3). In fact, there is strong correlation between the amount of fine root biomass and the soil C content from the same soil core (Fig. 6) suggesting that variations in soil C are largely explained by variations in live root biomass. The relationships also suggest that at higher $[\text{CO}_2]$, fewer rhizodeposits are stabilized into SOM. The isotopic composition of soil C is a good indicator of incorporation of new C into soils (Fig. 4). Using the ^{13}C isotope tracer method, C from roots to soils can be monitored (i.e. Leavitt *et al.*, 2001). We found little evidence of new C being incorporated into SOM as $[\text{CO}_2]$ increased in the atmosphere. A possible explanation for the loss of C incorporated from soils at elevated $[\text{CO}_2]$ s could be the lack of stabilization of new C inputs into SOM in the soils of these plantations. Protection of SOM typically occurs via chemical attraction to clay particles, both promoting soil aggregate formation (Tisdall & Oades, 1982; Jastrow, 1996) and it is enhanced by the presence of soil macro- and microorganisms (Haynes & Fraser, 1998; Zaller & Arnone, 1999). Increased rhizodeposits under elevated $[\text{CO}_2]$ can also stimulate the formation

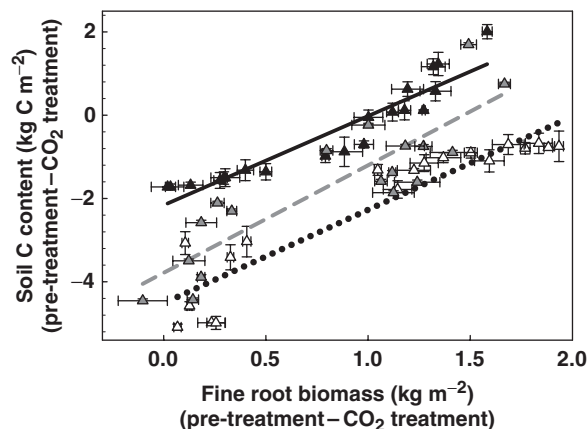


Fig. 6 The relationship between the net difference in pre and post-treatment values for soil C and fine root biomass in the forest plantations exposed to elevated $[\text{CO}_2]$ over a 4-year period. Values are soil C-root biomass pairs from the same soil cores coupled several times during the growing season over the 4 years of the study. The regression between soil C and root biomass is best fit to linear regressions of $y = mx + b$. The equations were $y = 2.1x - 2.2$, $r^2 = 0.9$, $P < 0.0001$ for $400 \mu\text{L L}^{-1}$ (solid triangles), $y = 2.6x - 3.8$, $r^2 = 0.8$, $P = 0.0002$ for $800 \mu\text{L L}^{-1}$ (gray triangles), and $y = 2.2x - 4.5$, $r^2 = 0.9$, $P < 0.0001$ for $1200 \mu\text{L L}^{-1}$ (hollow triangles).

and stabilization of soil aggregates through the chemical interaction of clay minerals, metal oxides and SOM (Brady & Weil, 1999). The high sand content of the soils, and a limited presence of macrofaunal communities during the length of the study may have prevented the stabilization of SOM in this study. Unprotected SOM could be readily available to decomposers resulting in higher rates of R_{soil} (Table 3).

Growth at elevated $[\text{CO}_2]$ enhanced both R_{soil} and the proportion of old C in R_{soil} (Table 3; Fig. 5) resulting in substantial losses of C from soils (Figs 3 and 4). Increased recent plant inputs are believed to be the main driver of increased rates of R_{soil} at elevated $[\text{CO}_2]$ (reviewed by Gonzalez-Meler & Taneva, 2004; King *et al.*, 2004) and it might be expected that new C inputs would be responsible for the observed increase in R_{soil} (Hungate *et al.*, 1997). However, during 2003 and when root biomass was not different among the CO_2 treatments, up to 90% of the increase in R_{soil} at $800 \mu\text{L L}^{-1} \text{CO}_2$ when compared with ambient, was because of the oxidation of old C (i.e. C existing prior to the onset of CO_2 treatments - Fig. 5). Increase belowground carbon inputs as a result of increased root mass and root C turnover can therefore alleviate microbial C limitations in these soils under elevated $[\text{CO}_2]$, which can prompt the decomposition of previously unavailable recalcitrant SOM (Fontaine *et al.*,

2004). This so-called priming effect (Bingeman *et al.*, 1953) occurs when recent C is made available as substrate to stimulate the growth and respiration of the soil heterotrophic communities. Subke *et al.* (2004) observed that microbial and fungal biomass and activity were enhanced when exogenous addition of fresh C were made in a Norway spruce stand. Similarly, the soil microbial biomass increased linearly as atmospheric [CO₂] increased in the same soils of this study (D. Lipson, personal communication). Further evidence of the coupling between recent C inputs and the priming effect of stored C is provided by the reductions in old and new C in R_{soil} a few weeks after the coppicing of trees at the end of 2003 (Fig. 5). Oxidation of new C was roughly reduced by 50–60% after trees were coppiced as a result of reduced root respiration, the remaining rate likely to represent decomposition of rhizodeposits. In contrast, the respiration of old C was reduced by more than 90%, suggesting that the rapid oxidation of more stable SOM is dependent on the availability of a continuous supply of labile C to heterotrophs.

The interplay between plant C inputs, soil texture, soil C saturation, and microbial community structure appear to be crucial players in the stabilization of SOM, and its rate of turnover (Six *et al.*, 2002). The interactions of these factors and their direct and indirect responses to climate change will determine the carbon sequestration strength of the terrestrial biosphere at future atmospheric CO₂ concentrations.

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