

# Fine root responses of mature deciduous forest trees to free air carbon dioxide enrichment (FACE)

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## Summary

1. Elevated atmospheric carbon dioxide (CO<sub>2</sub>) concentrations have often been reported to increase carbon allocation below-ground, particularly to fine root production. However, for trees these responses have primarily been studied in young expanding systems while the evidence for late successional systems that have reached steady state above- and below-ground is very limited.
2. At the Swiss Canopy Crane (SCC) experimental site, we assessed whether elevated CO<sub>2</sub> affects fine root biomass, fine root expansion and fine root C and N concentration under mature deciduous trees (c. 100 years) exposed to 7 years of free air CO<sub>2</sub> enrichment (FACE) in a typical near-natural central European forest.
3. After 5 and 6 years of CO<sub>2</sub> enrichment, both, the soil core and ingrowth core method yielded similar reductions in biomass of c. –30% under elevated CO<sub>2</sub> for live fine roots < 1 mm diameter. In year 7 of the experiment, when fine root biomass was re-assessed at peak season, there was no significant CO<sub>2</sub>-effect detectable. C and N concentrations in newly produced fine roots remained unaffected by elevated CO<sub>2</sub>. Soil moisture under CO<sub>2</sub>-exposed trees was significantly increased during rainless periods.
4. The isotopic label introduced into the system by canopy enrichment with <sup>13</sup>C-depleted CO<sub>2</sub> allowed us to trace the newly assimilated carbon. After 6 years of growth at 550 ppm CO<sub>2</sub>, recent fine roots (< 1 mm, ingrowth cores) of CO<sub>2</sub>-enriched trees consisted of 51% new carbon, suggesting a rather slow root turnover and/or slow mixing of old and new carbon in these trees.
5. Reduced tree water consumption under elevated CO<sub>2</sub> and resultant soil water savings might cause these trees to reduce their fine root investments in a future CO<sub>2</sub>-enriched atmosphere.
6. Our findings and those from other multi-year experiments indicate that fine root mass in late successional systems may also be unaffected or even suppressed instead of being stimulated by elevated CO<sub>2</sub>.

**Key words:** carbon cycle, carbon sequestration, elevated CO<sub>2</sub>, fine root turnover, soil moisture

## Introduction

Fossil fuel burning and rigorous land use change caused the atmospheric carbon dioxide (CO<sub>2</sub>) concentration to rise from pre-industrial 280 ppm to its current 385 ppm and this increase is projected to exceed 700 ppm in the late 21st century (IPCC 2007; Tans 2008). Thus, plant life on earth is directly challenged by carbon enriched nutrition in addition to various indirect CO<sub>2</sub> effects via the climate system (e.g. warming).

Given the role of forests as major terrestrial biomass carbon stores, tree responses to the ongoing rise in atmospheric CO<sub>2</sub> are crucial for the future global carbon cycle (Schimel 1995; Schlesinger 1997). A considerable number of studies suggest

that carbon assimilation of woody plants is substantially enhanced in response to increasing atmospheric CO<sub>2</sub> (Rogers *et al.* 1994; Curtis & Wang 1998; Norby *et al.* 1999; Sholtis *et al.* 2004; Zotz, Pepin & Körner 2005). However, this stimulation in photosynthesis does not necessarily result in above-ground biomass increment (Körner & Arnone 1992; Drake, Gonzalezmeier & Long 1997; Körner *et al.* 2005), but often leads to increased carbon allocation to roots (Norby & O'Neill 1991; Körner & Arnone 1992; Rogers *et al.* 1994; Curtis & Wang 1998; Hättenschwiler & Körner 1998; Matamala *et al.* 2003; Norby *et al.* 2004). According to the functional equilibrium concept (Brouwer 1962) this carbon investment in root systems under elevated CO<sub>2</sub> is presumably driven by the need to acquire more nutrients in order to match the enhanced atmospheric C supply.

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Fine roots ( $\leq 2$  mm) link autotrophic plant parts with the rhizosphere and provide the principal pathway for both water and nutrient uptake from the soil, and the input of carbon and nutrients via exudates and turnover to the soil. Although fine roots contribute less than 1.5% to the total biomass in forests (Körner 1994; Perruchoud 1999; Brunner & Godbold 2007), up to one-third of the global annual net primary production may flow below-ground for maintenance and new growth of fine roots (assuming  $< 1$  year turnover, Jackson, Mooney & Schulze 1997). As a consequence, fine roots may supply equal or even larger annual carbon and nutrient inputs to the soil than leaves (Nadelhoffer & Raich 1992; Hendricks, Nadelhoffer & Aber 1993).

At the Swiss Canopy Crane FACE site we did not observe consistent above-ground biomass responses to elevated  $\text{CO}_2$  – neither in basal area nor in leaf litter (LAI) – while light-saturated photosynthesis per unit leaf area was 40–52% enhanced during years 3 and 8 of  $\text{CO}_2$  enrichment (Körner *et al.* 2005; Zotz *et al.* 2005; Bader *et al.* unpublished data). This paradox was reported previously for various ecosystems exposed to elevated  $\text{CO}_2$  and is not yet fully resolved (Pitelka 1994; Niklaus *et al.* 2001; Nowak, Ellsworth & Smith 2004; Körner 2006). However, enhanced soil air  $\text{CO}_2$  concentration and stable isotope data both indicated increased carbon flux to the soil under elevated  $\text{CO}_2$  during the early years of this experiment (Steinmann *et al.* 2004; Keel, Siegwolf & Körner 2006). Therefore, we hypothesized greater below-ground C allocation under elevated compared to ambient  $\text{CO}_2$  assessable in enhanced fine root biomass and fine root expansion into previously unexplored soil volume. Furthermore, we asked whether elevated  $\text{CO}_2$  affects fine root quality through shifts in C and N concentrations.

## Materials and methods

### STUDY SITE

The Swiss Canopy Crane (SCC) site is situated in a diverse mixed forest roughly 15 km south of Basel, Switzerland (47°28' N, 7°30' E, 550 m a.s.l.). The c. 100-year-old forest reaches canopy heights ranging from 30 to 35 m and the leaf area index at peak season (LAI) is around 5. Tree density is 415 trees  $\text{ha}^{-1}$  (breast height diameter  $\geq 0.1$  m) and stem basal area amounts to 46  $\text{m}^2 \text{ha}^{-1}$ . *Fagus sylvatica* L. (European beech), *Quercus petraea* (Matt.) Liebl. (Sessile oak) and *Carpinus betulus* L. (Hornbeam) dominate the stand, which is interspersed with less abundant tree species such as *Tilia platyphyllos* Scop. (Largeleaf linden), *Acer campestre* L. (Field maple), *Prunus avium* L. (cherry) and 4 species of conifers (*Picea abies* (L.) Karst., *Larix decidua* Mill., *Pinus sylvestris* L., *Abies alba* Mill.).

The understorey vegetation (which does not receive  $\text{CO}_2$  enrichment) is highly diverse and dominated by tree seedlings and saplings, the liana *Hedera helix* and shrubs such as *Rubus fruticosus* agg., and *Lonicera periclymenum*. Among the most abundant herb species appear *Galium odoratum*, *Anemone nemorosa*, *Mercurialis perennis*, *Paris quadrifolia*, *Circaea lutetiana* and *Sanicula europaea*. The forest grows on a Rendzic Leptosol (WRB) (Rendzina (FAO), Lithic Rendoll (USDA)) with a very low accessible profile depth between 10 and 20 cm (maximal 25 cm) followed by extremely rocky subsoil merging into the calcareous bedrock at depths of 20–90 cm. As

Swiss forests growing on deeper soils had largely been converted to farmland centuries ago, this type of shallow soil is typical for many Swiss and other European forests, which nevertheless prosper vigorously. According to finger probe estimates we classified the soil as silty loam and the pH determination in 1 molar KCl yielded a value of  $5.8 \pm 0.2$  (Mean  $\pm$  SE,  $n = 25$ ) in the top 10 cm of the profile.

The temperate climate at the study site is characterized by mild winters and moderately warm summers with mean air temperatures in January and July of 2 °C and 19 °C, respectively. Long-term mean annual precipitation at the study site is 990 mm, two-thirds of which falls during the 6-month growing season (Pepin & Körner 2002).

### PRECIPITATION AND SOIL MOISTURE

Precipitation was recorded at a nearby weather station (Flüh, 2 km air-line distance from the SCC) at 2-min intervals and was averaged on a weekly basis. Soil volumetric water content during the growing seasons 2004–2005 was recorded continuously at hourly intervals using eight (ambient  $\text{CO}_2$ :  $n = 5$ , elevated  $\text{CO}_2$ :  $n = 3$ ) TDR probes (ML2x probes, Delta-T, Cambridge, UK) complemented by spot measurements with a hand-held TDR device (Trime-FM, Imko, Ettlingen, Germany). Due to technical failure, there was only discontinuous data available for 2006, which was not sufficient for statistical analysis. In March 2007, we started recording soil water content at 0–10 cm depth 4 times daily at 6-h intervals using 'ECH<sub>2</sub>O Probes' (EC-10, Decagon Devices Ltd., Pullman, Washington, DC; ambient  $\text{CO}_2$ :  $n = 20$ , elevated  $\text{CO}_2$ :  $n = 15$ ). Each of the 'ECH<sub>2</sub>O Probe' sensors was standardized to its own maximal value. We also used a hydrological model (input variables: daily precipitation sum, daily mean temperature, potential radiative energy input, potential evapotranspiration, and transpiration estimated from sapflow measurements under ambient and elevated  $\text{CO}_2$ ) to estimate soil moisture differences under ambient and elevated  $\text{CO}_2$  (Leuzinger & Körner 2009, in press).

### FREE AIR $\text{CO}_2$ ENRICHMENT SYSTEM

At the SCC site, a future,  $\text{CO}_2$ -enriched atmosphere in tree canopies was realized by means of a particular free air  $\text{CO}_2$  enrichment (FACE) technique called web-FACE (Pepin & Körner 2002). In this experiment we applied a step increase from ambient to 550 ppm atmospheric  $\text{CO}_2$ , corresponding to an elevation to approximately twice the pre-industrial  $\text{CO}_2$ -level. In brief, pure  $\text{CO}_2$  was pulse-released through a fine web of perforated tubes woven into tree crowns (20–35 m above-ground) with the aid of a construction crane. Computer-controlled magnetic valves governed the  $\text{CO}_2$ -supply to the canopies to sustain the setpoint of 550 ppm as accurately as possible. The understorey vegetation was not exposed to elevated  $\text{CO}_2$ , hence any isotopic signal originated exclusively from the forest canopy (Keel *et al.* 2006). Canopy  $\text{CO}_2$ -concentration served as the main control signal, which was monitored by an air sampling system consisting of several suction heads per tree feeding canopy air through sampling lines into infra-red gas analysers (LI-800 GasHound and LI-820, Licor, Lincoln, NE). We also used isometers (*C<sub>4</sub>* grass, *Echinochloa crus-galli*) growing in 50 mL containers (sand-clay mixture) fixed in the tree crowns to monitor the abundance of  $^{13}\text{C}$  in the canopy  $\text{CO}_2$  (Keel *et al.* 2006; Körner *et al.* 2005).  $\text{CO}_2$  enrichment was confined to daylight hours (PPFD  $> 100 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) of the growing season and was discontinued from the time of leaf shedding until next season's bud break (end of October to mid-April). Out of the 62 trees growing in the operating range of the crane (60 m), 12 deciduous

trees (three *F. sylvatica*, three *Q. petraea*, three *C. betulus*, one *T. platyphyllos*, one *A. campestris* and one *P. avium*) received elevated atmospheric CO<sub>2</sub> since autumn 2000. A larger number of control trees were available in the remaining crane area at sufficient distance to the CO<sub>2</sub>-enriched zone.

#### FINE ROOT BIOMASS

Given the shallow and rocky soil at the SCC site, we chose soil cores and root ingrowth cores rather than minirhizotrons for assessing fine root biomass. In our experiment, the methodological drawbacks involved with the use of ingrowth cores were of minor concern as this method may allow relative comparisons of new fine root growth between CO<sub>2</sub>-enriched and control trees. Moreover, under CO<sub>2</sub> enrichment, the expansion of new roots into unexplored soil volume is likely to produce a signal more sensitive to increased C supply than bulk fine root sampling using soil corers. This is because soil sampled with corers, includes a substantial fraction of older (and dead) fine roots, which perhaps originated prior to the start of CO<sub>2</sub> enrichment and thus, may conceal potential CO<sub>2</sub>-induced signals in fine root biomass.

In March 2005, a total of 84 soil cores were taken to a depth of 15 cm using a soil corer (3.5 cm diameter). Soil cores were taken in triplicate at each study tree (trees under ambient CO<sub>2</sub>:  $n = 16$ , trees under elevated CO<sub>2</sub>:  $n = 12$ ). In the remaining holes left from the soil core removal, we immediately installed ingrowth cores (12 cm high and 3.6 cm in diameter, 2 mm mesh), filled with root-free soil from fresh molehills in the respective plots. At the time of installation, bulk soil density in ingrowth cores was similar to bulk soil density (on-site adjustment of the mass to volume ratio of freshly stuffed ingrowth cores to that of soil cores). The ingrowth cores were inserted 3 cm below the soil surface, covered with topsoil and their locations were labelled for recovery. At the end of the growing season, 6 months after installation, the ingrowth of freshly formed fine roots was checked in six ingrowth cores that were harvested and directly analysed. These cores showed little ingrowth of fine roots from the surrounding soil. Therefore, the remaining 78 ingrowth cores were harvested after two growing seasons in November 2006, 20 months after installation. In July 2007, we re-assessed fine root biomass but restricted the sampling to the three replicated dominant tree species in this FACE experiment (*F. sylvatica*, *C. betulus*, *Q. petraea*). There were three trees of each species available in both CO<sub>2</sub> treatments ( $n = 9$ ) and we collected 3 soil cores per tree. Soil cores were taken at 1–2 m distance (depending on the penetrability of the soil due to the large number of rocks) around the stem of a given sample tree to a depth of 18 cm using a soil corer (5 cm diameter). Attempts to core beyond this soil depth were not successful.

All soil samples were stored on ice until arrival at the lab where they were kept at 4 °C. Soil cores from 2005 were frozen and processed in autumn 2006 while all other samples were processed within 3 weeks after removal from the forest soil. Fine roots were picked by hand, separated from bulk soil using tweezers and then rinsed with water. By means of a fine root reference collection, shrub and herbal roots were distinguishable from tree roots and were excluded from further analysis as CO<sub>2</sub> enrichment was confined to the tree crowns. In soil cores taken in 2007, a refined reference collection of roots sampled from trees growing on site, allowed us to distinguish between fine roots of different tree species. Roots from soil cores in 2005 and ingrowth cores were sorted into two diameter size classes < 1 mm and 1 ≤ 2 mm, whereas roots from soil cores in 2007 were separated in three size classes < 0.5 mm, 0.5 < 1 mm and 1 ≤ 2 mm. Tensile strength and white vascular tissue served as

vitality indicators to distinguish between live and dead roots (Matamala *et al.* 2003). The sorted fine roots were dried at 80 °C for at least 48 h and then weighed for biomass determination.

#### C AND N ANALYSIS OF FINE ROOTS

Washed live fine roots (< 1 mm diameter) obtained from ingrowth cores were oven-dried as described above and ground using a steel ball mill (Retsch MM 2000, Haan, Germany). Samples of 2–3 mg of the dry fine root powder were analysed for C and N using a CHN-analyser (Vario EL III, Elementar Analysensysteme GmbH, Hanau, Germany).

#### CARBON-ISOTOPE ANALYSIS OF FINE ROOTS

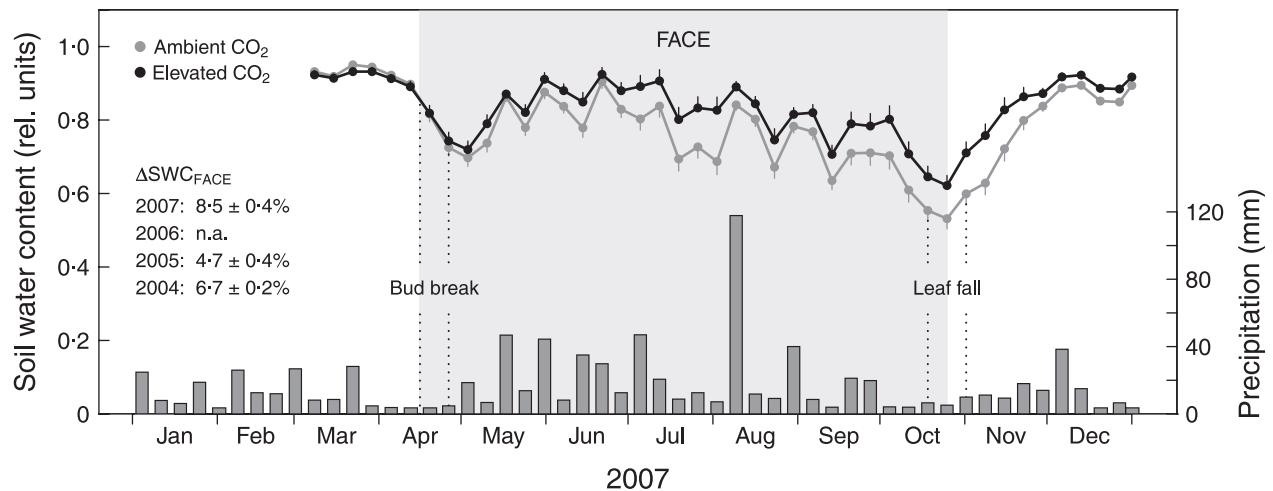
Aliquots of 0.6–0.8 mg of the dried and ground fine root powder were filled in tin capsules for δ<sup>13</sup>C analysis. After combustion in an elemental analyser (EA-1110, Carlo Erba Thermoquest, Milan, Italy) the gas samples passed a variable open-slit interface (Conflo II, Thermo Finnigan Mat, Bremen, Germany) leading to the mass spectrometer (Delta S, Thermo Finnigan Mat, Bremen, Germany), which was operated in continuous flow mode. The precision of δ<sup>13</sup>C analyses was < 0.1‰. The isotope values are expressed in the δ-notation: δ<sup>13</sup>C = ( $R_{\text{sample}}/R_{\text{standard}} - 1$ ) × 1000 (‰) where  $R$  is the molar ratio of <sup>13</sup>C to <sup>12</sup>C for the sample and standard, respectively. The difference in δ<sup>13</sup>C between isometers grown in ambient and elevated CO<sub>2</sub> (Mean ± SE for 2001–2006: 5.7 ± 0.6‰) was assumed to reflect the isotopic signal imposed on the canopy, because the C<sub>4</sub> grass biomass was exclusively formed of C that originated from newly assimilated CO<sub>2</sub>, free of the influence of old C reserves. The fraction of new C in fine roots was calculated using the rule of proportion where the isometer signal of 5.7‰ refers to 100%.

#### STATISTICAL ANALYSIS

As our experiment ran on a tree scale, individual trees growing under ambient and elevated CO<sub>2</sub> were the statistical units of replication. Each replicate consisted of three soil samples (soil cores, ingrowth cores), which were averaged prior to statistical testing to give a robust value for each study tree.

For the analysis of fine root biomass data from soil cores taken in 2005 and ingrowth cores we applied a linear mixed effects-model including the fixed factors 'CO<sub>2</sub>', 'living status' (dead or alive), and 'diameter' and the random factor 'tree'. We used Student's *t*-test to analyse soil water content 2007 as well as fine root C and N and stable isotope data obtained from ingrowth cores (only live fine roots < 1 mm diameter).

Analysis of covariance (ANCOVA) was performed to test the influence of the previous fine root biomass estimated from soil cores (covariate) on newly produced fine root biomass in ingrowth cores. In soil cores taken in 2007, a reference collection of roots sampled from plants growing on-site, allowed us to distinguish between fine roots of different species. Therefore, fine root biomass data from these soil cores as well as the related stable isotope data were analysed in a linear mixed effects-model, which included the fixed factors 'CO<sub>2</sub>', 'living status', 'diameter', and 'species' and the random factor 'tree'. Heteroscedasticity of within-group errors was modelled using power and constant variance functions. Quantile–quantile plots were applied for normality testing of residuals and random effects. Homogeneity of variances was checked with Bartlett's test. All statistical computations were performed using R, version 2.8.0 <[www.r-project.org](http://www.r-project.org)>.



**Fig. 1.** Precipitation and soil water content (SWC) under trees exposed to ambient or elevated  $\text{CO}_2$  in 2007. The  $\text{CO}_2$  enrichment period is grey-shaded and the dotted lines indicate the bud break or leaf fall period, respectively.  $\Delta\text{SWC}_{\text{FACE}}$  values give the mean difference in SWC between the elevated (E) and the ambient  $\text{CO}_2$  area (A) during the FACE period (growing season) from 2004, 2005 and 2007, calculated as  $E/A \times 100\% - 100$ . Due to technical failure there was insufficient data for 2006 (n.a. = not available). Hydrological model data for the study years: 2004: 5.5%, 2005: 5.9%, 2006: 5.6%, 2007: 10.1% (Leuzinger, in revision).

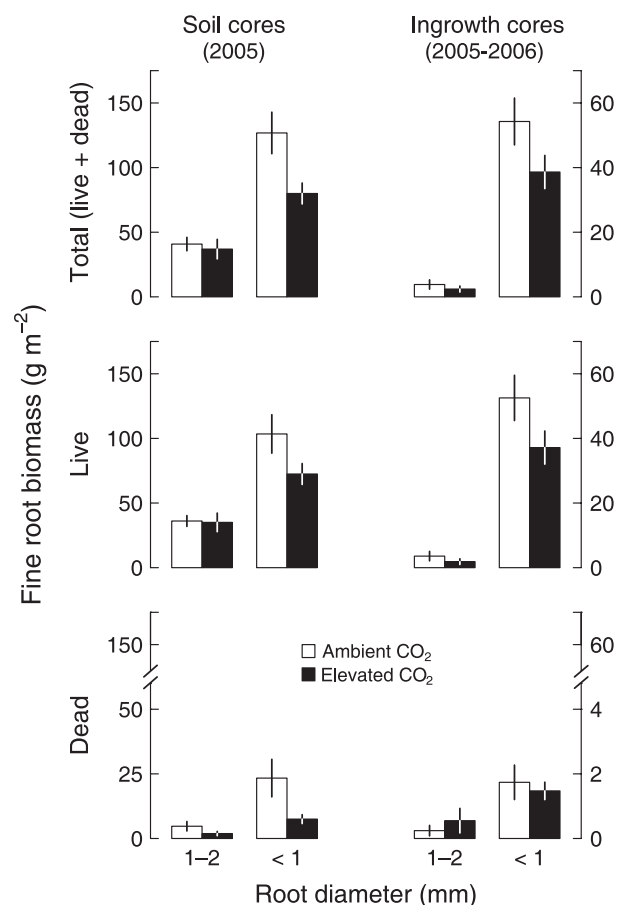
## Results

### SOIL MOISTURE

Mean volumetric water content during growing seasons was significantly higher in soil under trees exposed to elevated  $\text{CO}_2$  than under control trees, resulting from reduced sap flow (i.e. reduced transpiration) in  $\text{CO}_2$ -enriched trees, which positively fed back to soil water supplies ( $t = -10.27$ ,  $P < 0.001$ , Cech, Pepin & Körner 2003; Leuzinger & Körner 2007; Fig. 1). In 2007, prior to bud break and during early leaf development when transpirative demands were low, soil moisture in the control and  $\text{CO}_2$  enriched plot was identical (Fig. 1). Parallel to progressing leaf maturation and thus, increasing canopy transpiration, ambient soil moisture was reduced compared to soil moisture under elevated  $\text{CO}_2$  and remained lower throughout the entire growing season. During replenishment at high or continuous precipitation, soil water resources in the control and  $\text{CO}_2$ -enriched area transiently approached each other but rapidly diverged again afterwards. Following leaf abscission, soil moisture in control soil gradually converged with soil moisture in the  $\text{CO}_2$ -enriched plot towards the end of the year.

### TREE FINE ROOT BIOMASS

The original soil cores removed for the installation of ingrowth cores showed less fine root biomass of *c.* 30% in live fine roots < 1 mm diameter in sample areas under trees receiving 550 ppm  $\text{CO}_2$  compared to conspecific control trees (Fig. 2). Statistically this is supported by a significant  $\text{CO}_2$  effect and a  $\text{CO}_2 \times \text{diameter}$  interaction (Table 1). Similarly, the biomass of new fine roots < 1 mm that had expanded into ingrowth cores over two growing seasons,



**Fig. 2.** Fine root biomass of mature deciduous forest trees growing under elevated and ambient  $\text{CO}_2$ . Soil cores taken in winter 2005 (left panel) represent the soil volume removed for the installation of the ingrowth cores (right panel). Means  $\pm$  SE,  $n = 14$  in ambient and  $n = 12$  in elevated  $\text{CO}_2$ .

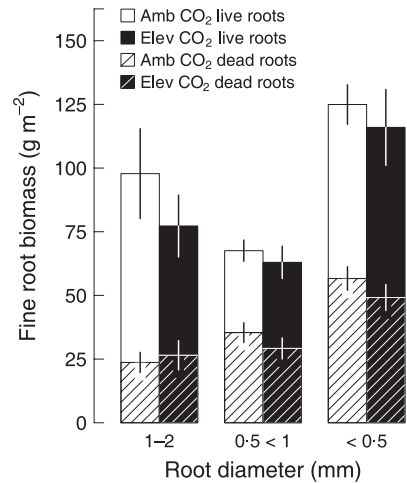
**Table 1.** Linear mixed effects model results for fine root biomass in soil cores and ingrowth cores and <sup>13</sup>C signatures of fine roots (soil cores 2007) under ambient and elevated CO<sub>2</sub>

Factor	Df	F-value	P
<b>Soil cores (2005)</b>			
CO <sub>2</sub>	1, 24	5.27	0.031*
Diameter	1, 74	38.95	< 0.001***
Living status	1, 74	133.33	< 0.001***
CO <sub>2</sub> × diameter	1, 74	4.78	0.032*
Diameter × living status	1, 74	17.18	< 0.001***
<b>Ingrowth cores (2005–2006)</b>			
CO <sub>2</sub>	1, 24	3.58	0.071(*)
Diameter	1, 74	92.40	< 0.001***
Living status	1, 74	100.54	< 0.001***
CO <sub>2</sub> × living status	1, 74	3.62	0.061(*)
Diameter × living status	1, 74	82.62	< 0.001***
<b>Soil cores (2007)</b>			
CO <sub>2</sub>	1, 14	1.02	0.33
Species	2, 14	0.84	0.45
Diameter	2, 83	30.21	< 0.001***
Living status	1, 83	8.30	0.005**
Species × living status	2, 83	16.75	< 0.001***
Diameter × living status	2, 83	8.25	< 0.001***
<b>δ<sup>13</sup>C (soil cores 2007)</b>			
CO <sub>2</sub>	1, 15	9.71	0.007**
Diameter	2, 86	45.09	< 0.001***
Species	2, 15	0.31	0.740
CO <sub>2</sub> × diameter	2, 86	6.89	0.002**
Diameter × species	4, 86	2.59	0.042*

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

was about 30% lower under elevated compared to ambient CO<sub>2</sub> (Fig. 2). However, this reduction was only marginally significant (Table 1). Although both methods revealed similar differences in fine root biomass between ambient and elevated CO<sub>2</sub>, the fine root biomass in soil cores had no significant influence on newly produced fine root biomass in ingrowth cores (ANCOVA,  $P > 0.05$ ). Thus, ingrowth cores installed at the same locations where soil cores showed high fine root biomass did not necessarily yield high fine root biomass. Live fine roots in soil cores contributed 92% and 83% to total fine root biomass (TFRB = live + dead fine root biomass across all diameters  $\leq 2$  mm) under elevated and ambient CO<sub>2</sub>, respectively. Irrespective of CO<sub>2</sub> treatment, live fine roots in ingrowth cores made up around 95% of TFRB. In 2007, fine root biomass at peak season did not differ significantly between CO<sub>2</sub>-treatments regardless of root diameter and living status of fine roots (Fig. 3, TFRB:  $290 \pm 23$  g m<sup>-2</sup> under ambient vs.  $256 \pm 36$  g m<sup>-2</sup> under elevated CO<sub>2</sub>, mean  $\pm$  SE). Also, fractions of root size classes were similar across CO<sub>2</sub> treatments with root diameters  $< 0.5$  mm contributing 43–45% while  $0.5 < 1$  mm diameters contributed 23–25% and  $1 \leq 2$  mm diameters added 30–34% to TFRB. Irrespective of the CO<sub>2</sub> treatment, live fine roots made up *c.* 60% of TFRB.

TFRB in 2007 was 54% and 42% higher in ambient and elevated CO<sub>2</sub>, respectively, compared to TFRB in 2005, which is probably due to the interannual variability and the different sampling dates in the respective years (peak season vs. spring).

**Fig. 3.** Fine root biomass of three dominant deciduous forest trees (*F. sylvatica*, *Q. petraea*, *C. betulus*) growing under elevated and ambient CO<sub>2</sub> based on a peak season harvest in July 2007. Means  $\pm$  SE,  $n = 9$ .**Table 2.** Carbon and nitrogen concentrations in live fine roots ( $< 1$  mm, ingrowth cores) formed within two growing periods (2005–2006) under ambient ( $n = 14$ ) and elevated CO<sub>2</sub> ( $n = 12$ )

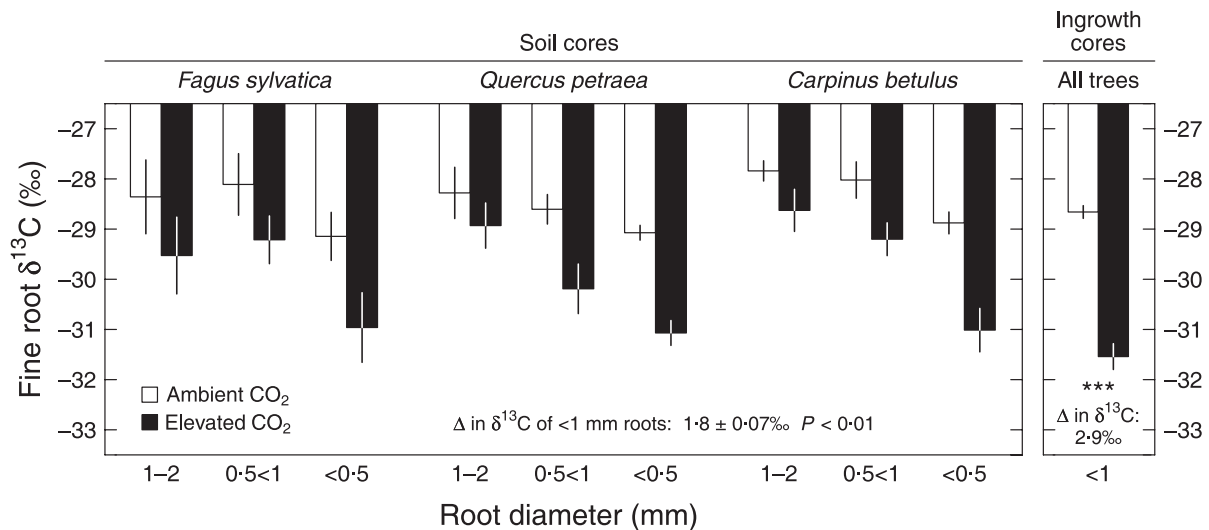
	Ambient CO <sub>2</sub>	Elevated CO <sub>2</sub>	P
	Mean $\pm$ SE	Mean $\pm$ SE	
Fine root C concentration (mg g <sup>-1</sup> )	421.7 $\pm$ 9.5	422.0 $\pm$ 9.3	0.98
Fine root N concentration (mg g <sup>-1</sup> )	15.7 $\pm$ 0.5	16.5 $\pm$ 0.6	0.33
Fine root C : N	27.0 $\pm$ 0.8	25.9 $\pm$ 0.8	0.32

#### C AND N CONCENTRATIONS IN FINE ROOTS

Elevated CO<sub>2</sub> had no effect on C and N concentration and C : N ratio in live fine roots ( $< 1$  mm) of deciduous forest trees harvested from ingrowth cores that were installed for 20 months, including two full growing seasons during years 5 and 6 of CO<sub>2</sub> enrichment (Table 2).

#### FINE ROOT <sup>13</sup>C SIGNALS

Growth in elevated CO<sub>2</sub> and root diameter had significant effects on the isotopic signal in fine roots (soil cores 2007), while living status and species identity did not influence fine root  $\delta^{13}$ C (Fig. 4, Table 1). The isotopic label in fine roots sampled from CO<sub>2</sub>-enriched trees was strongest in the finest diameters ( $< 0.5$  mm) that presumably represented the most recent fraction of the fine root system (significant CO<sub>2</sub> × root diameter interaction, Table 1, Fig. 4). In fine roots ( $< 1$  mm) exclusively formed during years 5 and 6 of CO<sub>2</sub> enrichment (ingrowth cores), 51% of the carbon carried the isotopic signature of the fossil CO<sub>2</sub> released in the tree canopies.



**Fig. 4.** Left panel,  $\delta^{13}\text{C}$  of fine roots from the three dominant forest trees (*F. sylvatica*, *Q. Petraea*, *C. betulus*) exposed to ambient or elevated  $^{13}\text{C}$ -depleted  $\text{CO}_2$  in year 7 of the FACE experiment (soil cores). Means  $\pm$  SE,  $n = 9$ . Right panel,  $\delta^{13}\text{C}$  of fine roots (all species pooled) exclusively formed during years 5 and 6 of the  $\text{CO}_2$  enrichment (ingrowth cores). Means  $\pm$  SE,  $n = 14$  in ambient and  $n = 12$  in elevated  $\text{CO}_2$ . Differences in  $\delta^{13}\text{C} \pm$  SE between fine roots  $< 1$  mm collected in the control and  $\text{CO}_2$ -enriched areas are shown by numbers in graph. \*\*\* $P < 0.001$ .

## Discussion

### TREE FINE ROOT BIOMASS

The Swiss web-FACE experiment is the only study worldwide where mature deciduous trees growing in a near-natural forest have been exposed to elevated  $\text{CO}_2$ . After 7 years of  $\text{CO}_2$  enrichment our data suggest unaltered (soil cores 2007, Fig. 3) or even reduced fine root compartments (soil cores 2005, Fig. 2) in these trees which comes as a surprise, given we had expected a  $\text{CO}_2$ -induced increase rather than no change or a reduction in fine root biomass. We also anticipated elevated  $\text{CO}_2$  to foster fine root growth but the declining trend observed in ingrowth cores rather suggests a  $\text{CO}_2$ -driven reduction in fine root growth of  $\text{CO}_2$ -enriched trees (Fig. 2).

Earlier stable isotope data and soil air  $\text{CO}_2$  concentration together with the lack of above-ground growth stimulation while leaf-level photosynthesis was enhanced implied increases in the flow of carbon to below-ground sinks under elevated  $\text{CO}_2$  (Steinmann *et al.* 2004; Körner *et al.* 2005; Zott *et al.* 2005; Asshoff, Zott & Körner 2006; Keel *et al.* 2006). Soil N limitation may offset potential  $\text{CO}_2$  effects on fine root growth (Pregitzer *et al.* 1995, 2000; Oren *et al.* 2001; Spinnler *et al.* 2002) but can be ruled out as an explanation for the negative or lacking fine root responses because the calcareous forest soils in this region are nutrient-rich (Walther *et al.* 2004) and there is substantial wet nitrogen deposition in this area of 20–25 kg N ha<sup>-1</sup> a<sup>-1</sup>, feeding plenty of N to trees and soils (Swiss Federal Office for the Environment 2000). Consequently, nitrate concentrations in the soil solution are rather higher-than-average, often exceeding the threshold value of 25 mg L<sup>-1</sup> given in the Swiss Water Protection Ordinance (Gschv 1998; Bucher-Wallin *et al.* 2003; P. Schleppei, personal communication). Given that mycorrhizal colonization in most ecosystems increases substantially under elevated  $\text{CO}_2$  (+47%, Treseder 2004), the

mycorrhizal network may offer an avenue for dissipation of excess carbon. Mycorrhization has not been studied on the mature trees of our site but very rapid transfer of new carbon from these trees to mycorrhizal fungi had been shown previously (Steinmann *et al.* 2004; Keel *et al.* 2006). At the SCC site we measured consistently enhanced soil moisture resulting from reduced tree water consumption under elevated  $\text{CO}_2$  (Cech *et al.* 2003; Leuzinger & Körner 2007, Fig. 1). These soil water savings offer a more likely explanation for decreases in fine root biomass as soil water supply *per se* and thus, nutrient availability is improved. This  $\text{CO}_2$ -induced facilitation in water and nutrient uptake might diminish the need for extensive fine root systems.

Contrary to our findings, below-ground biomass of both broad-leaved and coniferous trees had often been reported to increase under elevated  $\text{CO}_2$  (Rogers *et al.* 1994; Curtis & Wang 1998; Tingey, Phillips & Johnson 2000; Zak *et al.* 2000; Nowak, Ellsworth & Smith 2004). However, as emphasized in these reviews, plant developmental stage, study duration and environmental factors, particularly soil conditions may have strongly biased the observed  $\text{CO}_2$ -responses. Most of the studies were of short duration ( $\leq 3$  years) and tested young trees in ‘decoupled’ or expanding systems (‘coupling’ refers to the linkage between the carbon and the nutrient cycle, Körner 2006), the responses of which may not be extrapolated to mature trees (Loehle 1995; Körner 2006).

Data from multi-year FACE experiments conducted in ‘coupled’ systems with constant leaf area index and (near to) steady state nutrient cycle are still scarce. Among the few steady state systems the well-documented Oak Ridge FACE site is exceptional insofar as the pronounced initial above-ground growth stimulation of sweetgum (*Liquidambar styraciflua*) rapidly ceased while fine root production remained enhanced during 9 years of  $\text{CO}_2$  enrichment (Norby *et al.* 2004; Iversen, Ledford & Norby 2008). Both fine root

production and mortality were roughly doubled resulting in significantly greater peak season fine root biomass, but declining turnover rates (Norby *et al.* 2004; Iversen *et al.* 2008). Surprisingly, the largest increase in fine root production in this system occurred below 30 cm soil depth (Iversen *et al.* 2008). As the continuous positive fine root response was attributed to previously unexplored soil volume, it will be interesting to observe how long fine root proliferation will persist in this system, once soil exploration by roots has reached a new steady state.

At the Duke FACE site, loblolly pine (*Pinus taeda*) initially showed substantial increases in fine root increment under elevated CO<sub>2</sub>, but the effects on fine root biomass were rather modest (Matamala & Schlesinger 2000). Subsequent minirhizotron observations yielded a mean annual (insignificant) increase of 23% in fine root biomass during 6 years of CO<sub>2</sub>-exposure matching the 13–27% stimulation in annual tree basal area increment (Moore *et al.* 2006; Pritchard *et al.* 2001, 2008). Elevated CO<sub>2</sub> progressively increased soil moisture during the early years of enrichment. Unlike the soil water savings observed at our site, enhanced soil moisture was largely attributed to enhanced needle litter accumulation, which restricted evaporation from the soil (Schäfer *et al.* 2002).

After 7 years of CO<sub>2</sub> enrichment in a scrub oak system in Florida, the initial stimulation of fine root growth and mortality had completely ceased and, unexpectedly, like in our case, the biomass of surface roots < 0.25 mm diameter was even significantly reduced by 32% (Dilustro *et al.* 2002; Day *et al.* 2006; Brown *et al.* 2007). Similar to what we propose for our deciduous forest trees, the reduction in finest roots had been ascribed to improved soil moisture and/or enhanced nutrient availability under high CO<sub>2</sub> (Hungate *et al.* 2002; Johnson *et al.* 2003).

Consistent with the current study, 4 years of CO<sub>2</sub>-exposure failed to stimulate fine root growth in a tree line ecosystem in the Swiss Central Alps, dominated by 30-year-old European larch (*Larix decidua*) and mountain pine (*Pinus uncinata*; Handa, Hagedorn & Hättenschwiler 2008). Similarly, at the Nevada desert FACE facility, elevated CO<sub>2</sub> did not affect fine root dynamics of shrub communities, except for community transects where fine root biomass and turnover were even significantly lower under high CO<sub>2</sub> (Phillips *et al.* 2006). Unlike our site, soil moisture was not measurably increased under elevated CO<sub>2</sub> at the desert site, suggesting that improved water-use efficiency compensates for smaller fine root systems.

#### C AND N CONCENTRATION OF TREE FINE ROOTS

Tree fine root C and N concentrations remained unaffected under elevated CO<sub>2</sub>. This illustrates that not even relative increase in C allocation to the fine roots of our mature trees occurred under elevated CO<sub>2</sub>. Plant tissues developed under elevated CO<sub>2</sub> frequently show lower nitrogen and protein concentrations and therefore increases in the C:N-ratio (Cotrufo *et al.* 1998; Norby *et al.* 1999). However, irrespective of life form, fine roots seem to be more variable in their nutrient

response to elevated CO<sub>2</sub> as increases as well as decreases but mostly no changes were observed.

In line with our results, other steady state systems also reported no effects of elevated CO<sub>2</sub> on fine root C or N concentrations, including unaltered C:N-ratios in fine roots of *Pinus taeda* at the Duke FACE site, unchanged root nitrogen concentrations in *Larix decidua* and *Pinus uncinata* at the Swiss tree line FACE (Handa *et al.* 2008), and unaltered fine root N concentration in sweetgum at the Oak Ridge FACE site (Iversen *et al.* 2008). Even in the longest CO<sub>2</sub> experiment worldwide, where sour orange trees grew 17 years under elevated CO<sub>2</sub> with orchard-like irrigation and nutrient supply, the elemental composition, including C and N, remained largely unaffected (Kimball *et al.* 2007). In the Florida scrub-oak ecosystem, C and N concentration of surface roots < 0.25 mm decreased significantly after 7 years of CO<sub>2</sub> enrichment, but no differences in deeper soil or larger root diameters were found (Brown *et al.* 2007). Soil N availability can have a greater impact on fine root N than elevated CO<sub>2</sub> (Pregitzer *et al.* 2000; King *et al.* 2005) and in CO<sub>2</sub>-enriched spruce and beech trees growing on N-rich soils even declines in fine root N have been observed (Hagedorn *et al.* 2002).

Given the prolonged CO<sub>2</sub> enrichment in some of the studies (≥ 5 years), it is unlikely that initially lacking CO<sub>2</sub> effects on fine root C and N concentration will emerge in the long-term.

#### ROOT <sup>13</sup>C SIGNALS

At the SCC the isotopic signature of the fossil CO<sub>2</sub> used for canopy enrichment, was rapidly traceable within the study trees and also within soil compartments and mycorrhizal fungi, providing evidence for effective CO<sub>2</sub> enrichment and enabling us to estimate the fraction of labelled C (new C) in plant organs, including fine roots (Steinmann *et al.* 2004; Keel *et al.* 2006). After 6 years of CO<sub>2</sub>-exposure, half of the carbon in newly produced fine roots (< 1 mm, ingrowth cores) of CO<sub>2</sub>-enriched trees was isotopically labelled, suggesting a C-pool turnover of *c.* 12 years which is likely to reflect a slow root turnover as well. This is in line with the findings of Keel *et al.* (2006) who found 38% new C in fine roots (< 1 mm) of the same CO<sub>2</sub>-enriched trees after four growing seasons of enrichment implying a C turnover > 10 years. However, we do not believe that such data permit fully reliable root turnover estimates. In a pulse labelling experiment, an intense mixing of new and existing C was shown at branch-level for *F. sylvatica* and *Q. petraea* (Keel *et al.* 2007), and the slow replacement of old by new C in recent fine roots (ingrowth cores) after 6 years of continuous labelling provides strong evidence for slowly diluting C pools on a whole-tree level in these species. Root <sup>13</sup>C signals, thus reflect a combination of a near to endless dilution process of old by new mobile C-pools and new tissue formation.

Soil core sampling was likely to include fine roots that originated from periods prior to the start of the FACE experiment, which would further dilute the isotopic signal. In fact, the magnitude of the isotopic label (difference in

$\delta^{13}\text{C}$  between ambient and elevated  $\text{CO}_2$ ) was substantially lower in soil core samples compared to samples derived from ingrowth cores in which all roots were exclusively formed during  $\text{CO}_2$  enrichment in 2005–2006 (Fig. 4). Therefore, fractions of new C in soil core samples were not estimated.

Similarly long C turnover times were observed at the Swiss tree line FACE experiment with *Pinus* and *Larix*. After 5 years of  $\text{CO}_2$ -exposure, fine roots (pooled across all species including understorey vegetation) consisted of 25% (< 0.1 mm diameter) and 15% (0.1–2 mm) new C (Handa *et al.* 2008).

## Conclusions

Our findings and those from several other  $\text{CO}_2$  enrichment experiments studying woody plants suggest that unaltered or even reduced fine root biomass may meet plant resource demands under elevated  $\text{CO}_2$ . Improved water and thus, probably nutrient supply, through reduced transpiration and resultant soil water savings might cause trees to reduce their fine root investments in this late successional temperate forest in a future  $\text{CO}_2$ -enriched atmosphere. Whether such soil water savings will persist will depend on atmospheric feedback and future climatic trends. To the degree that soil humus formation (carbon sequestration) depends on enhanced fine root mass or accelerated turnover, our data do not provide any evidence that greater carbon deposits will be formed under such types of tree stands in the future.

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