Plant and Soil (2006) 281:247–254
DOI 10.1007/s11104-005-4293-x

Free atmospheric CO_2 enrichment (FACE) increased labile and total carbon in the mineral soil of a short rotation Poplar plantation

Marcel R. Hoosbeek[1,4], Yongtao Li[1,2] & Giuseppe E. Scarascia-Mugnozza[3]
[1]*Department of Environmental Sciences, Earth System Science group, Wageningen University, P.O. Box 37, 6700AA, Wageningen, The Netherlands.* [2]*Faculty of Natural Resources and Environment, South China Agricultural University, 510642, Guangzhou, P. R. China.* [3]*Department of Forest Environment and Resources, University of Tuscia, Via S. Camillo De Lellis, 01100 Viterbo, Italy.* [4]*Corresponding author**

Received 6 May 2005. Accepted in revised form 20 October 2005

Key words: carbon sequestration, elevated CO_2, FACE, labile carbon, nitrogen mineralization, soil carbon

Abstract

The global net terrestrial carbon sink was estimated to range between 0.5 and 0.7 Pg C y^{-1} for the early 1990s. FACE (free atmospheric CO_2 enrichment) studies conducted at the whole-tree and community scale indicate that there is a marked increase of primary production, mainly allocated into below-ground biomass. The enhanced carbon transfer to the root system may result in enhanced rhizodeposition and subsequent transfer to soil C pools. During the first rotation of the POP/EuroFACE experiment in a short-rotation Poplar plantation, total soil C content increased more under ambient CO_2 treatment than under FACE, while under FACE more new C was incorporated than under ambient CO_2. These unexpected and opposite effects may have been caused by a priming effect, where priming effect is defined as the stimulation of SOM decomposition caused by the addition of labile substrates. In order to gain insight into these processes affecting SOM decomposition, we obtained the labile, refractory and stable pools of soil C and N by chemical fractionation (acid hydrolysis) and measured rates of N-mineralization. Results of the first 2 years of the second rotation show a larger increase of total soil C% under FACE than under ambient CO_2. In contrast to the first rotation, total C% is now increasing faster under FACE than under ambient CO_2. Based on these observations we infer that the priming effect ceased during the second rotation. FACE treatment increased the labile C fraction at 0–10 cm depth, which is in agreement with the larger input of plant litter and root exudates under FACE. N-mineralization rates were not affected by FACE. We infer that the system switched from a state where extra labile C and sufficient N-availability (due to the former agricultural use of the soil) caused a priming effect (first rotation), to a state where extra C input is accumulating due to limited N-availability (second rotation). Our results on N-mineralization (second rotation) are in agreement with observations made at three forest FACE sites (Duke Forest, Oak Ridge, and Rhinelander), but our finding of increasing mineral soil C content contrasted with results at the Duke Forest where no significant increase in C content of the mineral soil occurred. However, the FACE induced increase in total C content occurred within the fraction with the shortest turnover time, i.e. the labile fraction. The refractory and stable fractions were not affected. The question remains whether the currently observed larger increase of total soil C and the increase of labile C under FACE will eventually result in long-term C storage in refractory and stable organic matter fractions.

---

* FAX No: +31-317-482419.
E-mail: marcel.hoosbeek@wur.nl

Introduction

The net exchange of carbon (C) between terrestrial ecosystems and the atmosphere is difficult to determine. This is in part due to the relatively high background levels of about 550 Pg C in vegetation, 1500–2000 Pg C in soils, and 780 Pg C in the atmosphere, as compared to net exchange rates of several Pg C per year (Houghton, 2003; Prentice et al., 2001). Two recent estimates of the global net terrestrial sink yielded respectively between 0.5 Pg C y^{-1} for the early 1990s (Houghton, 2003; Joos et al., 1999) and 0.7 Pg C y^{-1} during the 1990s (Plattner et al., 2002). These and other estimates led to many research efforts aimed at gaining insight into the mechanisms responsible for uptake of carbon by terrestrial ecosystems. Since forest vegetation and soils together hold almost half of the carbon of the Earth's terrestrial ecosystems, the effects of predicted future atmospheric CO_2 concentrations and nitrogen deposition rates are being investigated at several forest FACE (Free atmospheric CO_2 enrichment) experiments (Hoosbeek et al., 2004; Houghton, 2003; Norby et al., 2002; Schlesinger and Lichter, 2001). FACE technology has the great merit of not altering the microclimate of the test area and allows to perform research on global change impacts at the ecosystem level (Hendrey et al., 1999). Studies conducted at the whole-tree and community scale under elevated CO_2 indicate that there is a marked increase of primary production of forest vegetation (Calfapietra et al., 2001, 2003; Hamilton et al., 2002; Norby et al., 2002; Prentice et al., 2001). A critical point concerns the implications of belowground carbon allocation for long-term carbon storage (Lichter et al., 2005; Norby et al., 2002; Schlesinger and Lichter, 2001). Enhanced carbon transfer to the root system may result mainly in enhanced root respiration or, otherwise, in an increase of root dry matter, mycorrhizal activity and subsequent transfer of carbon to soil C pools.

Hoosbeek et al. (2004) assessed the effect of FACE treatment on the total soil C content and the incorporation of litter derived new C into soil organic matter (SOM) in a fast growing short rotation Poplar plantation in Italy. New soil C was estimated with the C3/C4 stable isotope method. Total soil C content increased under control and FACE respectively by 12% and 3%, i.e. 484 and 107 gC/m^2, while 704 and 926 gC/m^2 of new C was incorporated under control and FACE during the 3-year experiment. Although more new C was incorporated under FACE, the increase of total C was suppressed. Hoosbeek et al. (2004) hypothesized that these opposite effects may have been caused by a priming effect of the newly incorporated litter, where priming effect is defined as the stimulation of SOM decomposition caused by the addition of labile substrates. They calculated that during the experiment under ambient CO_2 and FACE treatment respectively 220 and 819 g/m2 of old C (from before the experiment) was lost from the soil.

DeLucia et al. (1997), Fox and Comerford (1990) and Jones et al. (1998) reported increased exudation of low-molecular-weight C compounds by roots of plants grown under elevated atmospheric CO_2 concentrations. Also, under CO_2 enrichment around natural CO_2 springs in central Italy, leaves of deciduous *Quercus pubscens* contained twice as much non-structural carbohydrates (NSC) inside as compared to outside of the vent area (Körner, 2003; Körner and Miglietta, 1994). And at the small-pot scale it has been demonstrated that, compared to ambient CO_2 concentrations, elevated CO_2 increases the amount of C allocated to the rhizosphere by enhancing root deposition (Cheng and Johnson, 1998; Hungate et al., 1997; Ineson et al., 1996). Based on these studies, Cheng (1999) concluded that total carbon input to the rhizosphere is significantly increased when plants are grown in elevated CO_2.

The fate and function of this extra labile C input into the soil in response to elevated CO_2 are not clear (Cheng, 1999). Depending on plant and soil conditions, the increased C input may result into an increase, a decrease, or no effect on SOM decomposition and nutrient mineralization. The additional C may be utilized by microorganisms and partly converted into SOM, thereby increasing soil C content. Or, the extra C may either stimulate SOM decomposition due to a priming effect, or suppress SOM decomposition resulting from microbial immobilization. Zak et al. (2000) argued that soil-N availability determines the fate of increased production entering the soil. Because microbial growth in soil is limited by substrate inputs from plant production, they reasoned that changes in the amount and chemistry of these organic substrates could affect the composition of soil microbial communities and the cycling of N in soil. In order to gain insight into these processes affecting SOM decomposition, we collected soil samples at the EuroFACE short-rotation Poplar plantation in central Italy. We obtained the labile, refractory and stable pools of soil C and N by chemical fractionation (acid hydrolysis) and measured rates of N-mineralization.

Methods

The POPFACE experiment was established early 1999 on former agricultural fields near Viterbo (42°37′04″ N, 11°80′87″ E, alt 150 m), Italy. The plantation and adjacent fields had been under forest until about 1950. Since then a variety of agricultural crops has been grown on these former forest soils until the inception of the POPFACE plantation. The annual precipitation is on average 700 mm with dry summers (Xeric moisture regime). During November of 1998 an initial soil survey took place (Table 1). The loamy soils classified as Pachic Xerumbrepts and were described in detail by Hoosbeek et al. (2004).

Nine ha were planted with *Populus x euramericana* hardwood cuttings at a density of 0.5 trees per m^2. Within this plantation three FACE and three control plots (30×30 m) were randomly assigned under the condition of minimum CO_2 enrichment pollution. The plots were divided into two parts by a physical resin-glass barrier (1 m deep in the soil) for nitrogen differential treatments in the two halves of each plot. However, because of the high inorganic N content of the soil (Table 1), no fertilization treatment was applied during the first 3-year rotation of the experiment. Each half plot was divided into three sectors, where each sector was planted at a density of 1 tree per m^2 using three different genotypes: *P. x euramericana* Dode (Guinier) (=*P. deltoides* Bart. ex Marsh.×*P. nigra* L.) genotype I-214, a genotype of *P. nigra* L. (Jean Pourtet) and a local selection of *P. alba* L. (genotype 2AS11). Carbon enrichment was achieved by injection of pure CO_2 through laser-drilled holes in tubing mounted on six masts (Miglietta et al., 2001). The FACE rings (octagons) within the FACE plots had a diameter of about 22 m. The elevated CO_2 concentrations, measured at 1-min intervals, were within 20% deviation from the pre-set target concentration (560 μmol mol^{-1}) for 91% of the time to 72.2% of the time, respectively, at the beginning and at the end of each rotation cycle of the plantation. The plantation was drip irrigated at a rate of 6–10 mm per day during the growing seasons.

The trees were coppiced after the first three growing seasons (1999–2001). The experiment continued with a second rotation under the name EuroFACE (2002–2004). A fertilization treatment was added to one half of each experimental plot because soil analyses showed the occurrence of limiting conditions of nitrogen availability in the soil (Scarascia-Mugnozza et al., *in press*). The total amount of nitrogen supplied was 212 kg ha^{-1} y^{-1} in 2002 and 290 kg ha^{-1} y^{-1} during 2003 and 2004.

Soil samples were collected from each sector within the 3 control and 3 FACE plots in June of 2004 and were transported to the laboratory in a mobile refrigerator. The soil samples were crushed by hand and live roots were removed. Carbon and nitrogen were determined by flash combustion in an elemental analyzer (EA 1108) (Van Lagen, 1996). Total soil organic C and N

Table 1. Initial soil properties at the inception of the POPFACE experiment (November 1998)

CO_2 treatment	N treatment	C_{total} %		N_{total} %		NO_3–N (μg N/g soil)		NH_4–N (μg N/g soil)		pH	
		Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
Ambient	Unfertilized	1.08	0.02	0.13	0.00	9.73	1.08	0.73	0.08	5.18	0.11
	N-fertilization	1.13	0.04	0.13	0.00	8.72	1.13	0.62	0.06	5.08	0.16
FACE	Unfertilized	0.91	0.12	0.12	0.01	7.17	0.66	0.59	0.12	4.89	0.03
	N-fertilization	1.06	0.08	0.13	0.01	7.64	1.19	0.62	0.12	4.98	0.06

content are expressed as weight percentage (g C or N per gram soil×100%). The change of soil C was calculated as: $(C\%_{2003}-C\%_{2001})/C\%_{2003}\times 100\%$. Soil N change was calculated similarly.

The labile, refractory and stable pools of soil C and N were obtained by acid hydrolyses according to a method largely based on a method described by Rovira and Vallejo (2002). Field moist soil (7.00 g) was placed in a dry pre-weighed sealable Pyrex (250 ml) tube. A sub-sample was used for determination of soil moisture. Next, 210 ml of 2.5 *M* H_2SO_4 (moist soil:acid = 1 g:30 ml) was added to the moist soil and thoroughly mixed. After the tubes were closed, they were placed in a water bath at 100 °C for 30 min. After cooling, the tubes were centrifuged at 3500 r/min for 3 min. Clear solution (10 ml) was drawn from the tube and kept in a glass bottle at 4 °C until analysis of C and N with TOC/TN analyzer (Shimadzu TOC-Vcsh, TNM-1). This hydrolysate was defined as the *labile* pool (mg C or N/g soil).

The remaining solution was drawn out of the Pyrex tube and the residue was washed with 200 ml of deionized water twice. The tube, containing the unhydrolyzed residue, was dried at 60 °C. Next, 16 ml of 13 M H_2SO_4 was added and the tubes were placed in an end-over-end shaker, overnight. The next morning, 184 ml of deionized water was added. The closed tubes were placed in a water bath at 100 °C for 3 h and shaken occasionally. After centrifugation at 3500 r/min for 3 min, 10 ml of clear hydrolysate was defined as the *refractory* pool (mg C or N/g soil). The unhydrolyzed residue was washed twice and dried at 60 °C. Carbon and nitrogen in this *stable* pool (mg C or N/g soil) were determined with an elemental analyzer (EA 1108).

At the beginning of the POPFACE experiment, the initial C and N content of the A horizons in plots 1–6 ranged from 6.5 to 11.8 mg C/g soil and from 1.1 to 1.3 mg N/g soil (Hoosbeek et al., 2004). In order to account for this spatial variability, the labile, refractory and stable pools are expressed as fractions:

$$\text{Fraction}_x = \text{Pool}_x/\text{Total soil C}$$

where x stands for labile, refractory or stable.

Field moist soil samples for N-mineralization were thoroughly mixed and sub-divided for determination of the initial moisture, $NO_3^-$and NH_4^+ content and for incubation. Incubation took place at 20 °C in a dark temperature controlled chamber for 28 days. After extraction with 1 *M* KCl solution, NO_3^- and NH_4^+ were measured colometrically with an autoanalyzer (Technicon II)(Van Lagen, 1996). The results are expressed as the change of elemental N in NO_3^- and NH_4^+ divided by the total N content of the sample ((mg N/g N)/ 28 days).

The SPSS (v 11.5) General Linear Model was used to calculate univariate analysis of variance and to evaluate CO_2 and N-fertilization treatment and species effects. Differences between means were considered significant when the *P*-value of the UNIANOVA F-test was < 0.05.

Results

Soil C and N% increased under all treatments and Poplar species at both depths during the first 2 years of the second rotation (Table 2). Moreover, soil C% of the upper 10 cm of the mineral soil increased significantly more under FACE than under ambient CO_2. The increase of N% (0–10 cm) under FACE was larger as well, but not significantly. There was no FACE effect on increases of C and N% between 10 and 20 cm depth. Nitrogen treatment and species had no effect on increases of soil C and N% at both depths.

FACE treatment significantly increased the labile C fraction at depth 0–10 cm (Table 3). This trend was not significant at depth 10–20 cm. Nitrogen fertilization decreased the labile C fraction at depth 0–10 cm, but had no effect at 10–20 cm depth. The labile N fraction was not affected by either FACE or N fertilization treatment. There was no Poplar species effect on labile C and N fractions. The refractory and stable C and N fractions were not affected by either CO_2 or N-fertilization treatment or species effects at both depths.

N-mineralization rates tended to be higher under N-fertilization and *P. euramericana*, although not significantly (Table 4). Mineralization rates were largely determined by the release of NO_3^- during the 28-day incubation period (data not shown). No significant CO_2 or N treatment or species effects were observed at either depth.

Table 2. Soil C and N% and the change (%) of C and N during the first 2 years of the second rotation (2001–2003)

Soil depth (cm)	Treatment			C% 2001		C% 2003		C change (%) 2001–2003		N% 2001		N% 2003		N change (%) 2001–2003	
			n	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
0–10	CO_2	Ambient	18	1.08	0.03	1.22	0.03	14.6[a]	3.5	0.11	0.00	0.12	0.00	10.3	3.8
		FACE	18	0.90	0.06	1.11	0.04	30.0[a]	6.5	0.09	0.01	0.11	0.01	22.9	5.9
	N	Unfertilized	18	0.94	0.04	1.18	0.04	28.6	5.2	0.10	0.00	0.11	0.01	19.6	4.6
		Fertilized	18	1.03	0.06	1.15	0.03	16.0	5.5	0.10	0.00	0.11	0.01	13.6	5.6
	Species	*alba*	12	1.05	0.07	1.15	0.04	14.2	7.0	0.10	0.01	0.11	0.01	10.0	4.7
		nigra	12	0.97	0.05	1.15	0.05	20.5	5.2	0.10	0.00	0.11	0.01	14.7	4.1
		euramericana	12	0.94	0.06	1.19	0.05	32.2	7.3	0.10	0.01	0.12	0.01	25.1	8.6
10–20	CO_2	Ambient	18	0.93	0.01	1.04	0.01	11.0	1.0	0.10	0.00	0.10	0.00	7.2	4.9
		FACE	18	0.83	0.04	0.90	0.04	8.8	2.4	0.08	0.00	0.09	0.01	9.1	5.8
	N	Unfertilized	18	0.87	0.04	0.97	0.04	12.6[b]	1.5	0.09	0.00	0.10	0.01	8.5	5.4
		Fertilized	18	0.90	0.03	0.96	0.04	7.3[b]	2.0	0.09	0.00	0.10	0.01	7.8	5.3
	Species	*alba*	12	0.90	0.04	0.96	0.04	6.3[c]	2.2	0.09	0.00	0.10	0.01	2.2	5.9
		nigra	12	0.89	0.04	0.98	0.05	9.5[c]	2.0	0.09	0.00	0.10	0.01	10.0	7.2
		euramericana	12	0.85	0.05	0.97	0.05	14.0[c]	2.3	0.09	0.00	0.10	0.01	12.1	6.5

[a] Significant FACE effect ($P = 0.044$) on soil C change at depth 0–10 cm.
[b] Significant N-fertilization effect ($P = 0.020$) on soil C change at depth 10–20 cm.
[c] Significant species effect ($P = 0.022$) on soil C change at depth 10–20 cm.

Table 3. Soil carbon and nitrogen fractions obtained by acid hydrolysis

Soil depth (cm)	Treatment		*n*	Labile				Refractory				Stable			
				C fraction		N fraction		C fraction		N fraction		C fraction		N fraction	
				Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
0–10	CO_2	Ambient	18	0.10[a]	0.01	0.05	0.00	0.18	0.00	0.09	0.00	0.57	0.04	0.74	0.04
		FACE	18	0.13[a]	0.01	0.06	0.01	0.18	0.01	0.09	0.01	0.63	0.02	0.60	0.05
	N	Unfertilized	18	0.12[b]	0.01	0.06	0.01	0.18	0.00	0.09	0.01	0.59	0.03	0.66	0.04
		N-fertilized	18	0.10[b]	0.00	0.06	0.00	0.18	0.01	0.09	0.01	0.61	0.03	0.68	0.05
	Species	*alba*	12	0.11	0.01	0.05	0.01	0.18	0.01	0.09	0.01	0.57	0.05	0.67	0.06
		nigra	12	0.12	0.01	0.06	0.01	0.17	0.01	0.09	0.01	0.58	0.03	0.63	0.06
		euramericana	12	0.11	0.01	0.06	0.01	0.19	0.01	0.09	0.01	0.64	0.03	0.70	0.06
10–20	CO_2	Ambient	18	0.10	0.00	0.05	0.00	0.18	0.00	0.09	0.01	0.64	0.04	0.72	0.06
		FACE	18	0.11	0.01	0.05	0.01	0.18	0.01	0.08	0.01	0.58	0.03	0.60	0.05
	N	Unfertilized	18	0.11	0.01	0.05	0.01	0.17	0.00	0.08	0.01	0.60	0.03	0.63	0.06
		N-fertilized	18	0.11	0.00	0.05	0.00	0.18	0.00	0.09	0.01	0.63	0.04	0.69	0.06
	Species	*alba*	12	0.11	0.01	0.06	0.01	0.18	0.01	0.09	0.01	0.65	0.03	0.72	0.06
		nigra	12	0.10	0.01	0.05	0.00	0.17	0.01	0.08	0.00	0.61	0.04	0.69	0.07
		euramericana	12	0.10	0.01	0.05	0.01	0.18	0.01	0.09	0.01	0.58	0.05	0.58	0.08

[a] Significant FACE effect ($P = 0.009$) on labile C pool at depth 0–10 cm.
[b] Significant N-fertilization effect ($P = 0.039$) on labile C pool at depth 0–10 cm.

Table 4. Nitrogen mineralization during 28-day incubation

Soil depth (cm)	Treatment		*n*	N-mineralization (mg N_{min}/g N_{sample})	
				Mean	s.e.
0–10	CO_2	Ambient	18	15.4	2.3
		FACE	18	14.2	2.9
	N	Ambient	18	12.3	2.5
		Fertilized	18	17.3	2.6
	Species	*alba*	12	13.4	2.2
		nigra	12	12.7	3.0
		euramericana	12	18.4	4.0
10–20	CO_2	Ambient	18	8.5	1.4
		FACE	18	8.2	1.4
	N	Ambient	18	8.3	1.4
		Fertilized	18	8.4	1.4
	Species	*alba*	12	8.3	1.4
		nigra	12	9.0	2.0
		euramericana	12	7.8	1.7

Discussion

Since the beginning of the experiment, soil C and N% have been increasing in all plots, i.e. under all treatments and Poplar species. This increase in SOM is largely due to the afforestation of agricultural land. During the first rotation Hoosbeek et al. (2004) observed that total soil C content increased more under ambient CO_2 treatment than under FACE, while under FACE more new C was incorporated than under ambient CO_2. They hypothesized that these opposite effects may have been caused by a priming effect of the newly incorporated litter. This extra input of labile C under FACE probably increased microbial activity with as side effect the increased decomposition of older SOM. This may also have induced an increase of N-mineralization.

Results of the first 2 years of the second rotation, however, show a larger increase of total C% under FACE than under ambient CO_2. Also total N% increased more under FACE, although not significantly. In contrast to the first rotation, SOM is now accumulating faster under FACE than under ambient CO_2. Based on these observations we infer that the priming effect ceased during the second rotation.

We applied chemical fractionation and measured N-mineralization rates in order to verify the priming effect hypothesis. The fractionation results confirm the presence of more labile C under FACE (0–10 cm), which would have been in agreement with the priming effect hypothesis. The presence of more labile C under FACE would have been a pre-requisite for a possible priming effect, but it does not necessarily induce a priming effect. The observed FACE effect on labile C does therefore not oppose the larger increase of total C% under FACE during the second rotation. Moreover, the presence of more labile C under FACE is in agreement with the larger input of plant litter and root exudates under FACE. This fraction is considered to consist largely of carbohydrates, which may have been excreted by roots or may have been released by turnover of root litter (Cheng, 1999). Increased root turnover under FACE was observed during the first rotation (Lukac et al., 2003).

We expected an increase of mineralization rates under FACE due to the increased decomposition of older SOM as was observed during the first rotation. Instead, FACE had no effect on N-mineralization rates. This is, however, in agreement with the observation that the priming effect ceased during the second rotation. The observed N-fertilization effect on labile C in the topsoil suggests that N has become limited for microbial decomposition during the second rotation in unfertilized plots. N-fertilization decreased the labile C fraction (0–10 cm) and also reduced the increase in C% at both depths, of which at 10–20 cm significantly. We infer that the system switched from a state where extra labile C and sufficient N-availability (due to the former agricultural use of the soil) caused a priming effect (first rotation), to a state where extra C input is accumulating due to limited N-availability (second rotation).

The absence of a FACE effect on N-mineralization is in agreement with observations on soil N cycling at three other forest FACE experiments. Finzi and Schlesinger (2003) found no significant change in the rate of N-mineralization and N-immobilization under elevated CO_2 at the Duke Forest FACE experiment. They postulated that the quantity and chemistry of the litter inputs may explain the limited response of microbially mediated soil N cycling. Zak et al. (2003) investigated and compared soil nitrogen cycling at three forest FACE experiments (Duke Forest, Oak Ridge, and Rhinelander). In these experiments, FACE increased the input of above- and below-ground litter production, which fuels heterotrophic metabolism in soil. Nonetheless, they found no FACE effect on any microbial N pool or process, indicating that greater litter production had not altered the microbial supply of N for plant growth. Zak et al. (2003) postulated that given the substantial amount of SOM already present in the soil, increases in above- and below-ground litter production under FACE were likely to be insufficient to influence microbial N demand over the duration of the experiments. They concluded that understanding the time scale over which greater plant production alters microbial N demand is important to be able to predict long-term changes in N availability.

Although the Duke Forest plantation was established 13 years prior to the start of the FACE experiment in 1996, C and N content of the mineral soil still increased under both FACE and ambient CO_2 due to recovery from agricultural land use prior to establishment of the plantation (Lichter et al., 2005). After 6 years of fumigation, FACE had no effect on the increase of total C and N content of the mineral soils at either depth. Density and physical fractionation techniques were applied to obtain the free light, coarse and fine intra-aggregate particulate, and the mineral-associated organic matter fractions. Lichter et al. (2005) found no significant FACE effect on either organic matter fraction. However, fractionation results of the upper mineral soil suggest that the increase in C content occurred entirely within the free light fraction.

After 5 years of CO_2 fumigation at the EuroFACE site, we observed that the FACE induced increase in total C content occurred within the fraction with the shortest turnover time, i.e. the labile fraction. The refractory and stable fractions were not affected by FACE. Although physical and chemical fractionation results may not be compared directly, in both studies FACE did not affect the more stable and/or protected fractions with relatively longer turnover times. The question remains whether the currently observed larger increase of total soil C and the increase of labile C under FACE will eventually result in long-term C sequestration in refractory and stable organic matter fractions.

Acknowledgements

Dr. Pere Rovira (Dept de Biologia Vegetal, Facultat de Biologia, Universitat de Barcelona, Spain) is gratefully acknowledged for providing detailed information on the method for acid hydrolysis. We thank Judith Vos, Eef Velthorst and Marcel Meinders for their help in the field and laboratory. We also thank two anonymous reviewers and section editor Dr. Chris Neill for providing useful comments. Funding was provided by the European Commission Fifth Framework Program, Environment and Climate RTD Program, research contract EVR1–CT- 2002–40027 (EUROFACE) and by the Centre of Excellence "Forest and Climate" of the Italian Ministry of University and Research (MIUR).

References

Calfapietra C, Gielen B, Galema A N J, Lukac M, De Angelis P, Moscatelli M C, Ceulemans R and Scarascia-Mugnozza G 2003 Free-air CO_2 enrichment (FACE) enhances biomass production in a short-rotation Poplar plantation. Tree Physiol. 23, 805–814.

Calfapietra C, Gielen B, Sabatti M, De Angelis P, Scarascia-Mugnozza G and Ceulemans R 2001 Growth performance of Populus exposed to "Free-Air Carbon dioxide Enrichment" during the first growing season in the POPFACE experiment. Ann. For. Sci. 58, 819–828.

Cheng W 1999 Rhizosphere feedbacks in elevated CO_2. Tree Physiol. 19, 313–320.

Cheng W and Johnson D W 1998 Elevated CO_2, rhizosphere processes, and soil organic matter decomposition. Plant and Soil 202, 167–174.

DeLucia E H, Callaway R M, Thomas E M and Schlesinger W H 1997 Mechanisms of phosphorus acquisition for ponderosa pine under high CO_2 and temperature. Ann. Bot. 79, 111–120.

Finzi A C and Schlesinger W H 2003 Soil–nitrogen cycling in a Pine forest exposed to 5 years of elevated carbon dioxide. Ecosystems 6, 444–456.

Fox T R and Comerford N B 1990 Low-molecular-weight organic acids in selected forest soils of the southeastern USA. Soil Sci. Soc. Am. J. 54, 1139–1144.

Hamilton J G, DeLucia E H, George K, Naidu S L, Finzi A C and Schlesinger W H 2002 Forest carbon balance under elevated CO_2. Oecologia 131, 250–260.

Hendrey G H, Ellsworth D S, Lewin K F and Nagy J 1999 A free-air enrichment system for exposing tall forest vegetation to elevated atmospheric CO_2. Glob. Change Biol. 5, 293–309.

Hoosbeek M R, Lukac M, Van Dam D, Godbold D L, Velthorst E J, Biondi F A, Peressotti A, Cotrufo M F, De Angelis P and Scarascia-Mugnozza G, 2004 More new carbon in the mineral soil of a Poplar plantation under Free

Air Carbon Enrichment (POPFACE): Cause of increased priming effect? Global Biogeochem. cycles 18, GB1040.

Houghton R A 2003 The contemporary carbon cycle. *In* Biogeochemistry. Ed W H Schlesinger. pp 473–513. Elsevier.

Hungate B A, Holland E A, Jackson R B, Chapin III F S, Mooney H A and Field C B 1997 The fate of carbon in grasslands under carbon dioxide enrichment. Nature 388, 576–579.

Ineson P, Cotrufo M F, Bol R, Harkness D D and Blum H 1996 Quantification of soil carbon inputs under elevated CO_2: C_3 plants in a C_4 soil. Plant and Soil 187, 345–350.

Jones T H, Thompson L J, Lawton J H, Bezemer T M, Bardgett R D, Blackburn T M, Bruce K D, Cannon P F, Hall G S, Hartley S E, Howson G, Jones C G, Kampichler C, Kandeler E and Ritchie D A 1998 Impacts of Rising Atmospheric carbon dioxide on model terrestrial ecosystems. Science 280, 441–443.

Joos F, Meyer R, Bruno M and Leuenberger M 1999 The variability in the carbon sinks as reconstructed for the last 1000 years. Geophys. Res. Lett. 26, 1437–1440.

Körner C 2003 Carbon limitation in trees. J. Ecol. 91, 4–17.

Körner C and Miglietta F 1994 Long term effects of naturally elevated CO_2 on Mediterranean grassland and forest trees. Oecologia 99, 343–351.

Lichter J, Barron S H, Bevacqua C E, Finzi A C, Irving K F, Stemmler E A and Schlesinger W H 2005 Soil carbon sequestration and turnover in a pine forest after six years of atmospheric CO_2 enrichment. Ecology 86, 1835–1847.

Lukac M, Calfapietra C and Godbold D L 2003 Production, turnover and mycorrhizal colonization of root systems of three *Populus* species grown under elevated CO_2 (POPFACE). Glob. Change Biol. 9, 838–848.

Miglietta F, Peressotti A and Vaccari F P 2001 Free-air CO2 enrichment (FACE) of a Poplar plantation: The POPFACE fumigation system. New Phytol. 150, 465–476.

Norby R J, Hanson P J, O'Neill E G, Tschaplinski T J, Weltzin J F, Hansen R A, Cheng W, Wullschleger S D, Gunderson C A, Edwards N T and Johnson D W 2002 Net primary productivity of a CO_2–enriched deciduous forest and the implications for carbon storage. Ecol. Appl. 12, 1261–1266.

Plattner G-K, Joos F and Stocker T F 2002 Revision of the global carbon budget due to changing air–sea oxygen fluxes. Global Biogeochem. cycles 16–1096, doi:1010.1029/2001GB001746.

Prentice I C, Farquhar G D, Fasham M J R, Goulden M L, Heimann M, Jaramillo V J, Kheshgi H S, Le Quere C, Scholes R J and Wallace D W R 2001 The carbon cycle and atmospheric carbon dioxide. *In* Climate Change 2001: The scientific basis. Ed. IPCC. pp 183–237. Cambridge University Press, Cambridge and New York.

Rovira P and Vallejo V R 2002 Labile and recalcitrant pools of carbon and nitrogen in organic matter decomposing at different depths in soil: an acid hydrolysis approach. Geoderma 107, 109–141.

Scarascia-Mugnozza G E, Calfapietra C, Ceulemans R, Gielen B, Cotrufo M F, De Angelis P, Godbold D L, Hoosbeek M R, Kull O, Lukac M, Marek M, Miglietta F, A. P, Raines C, Sabatti M, Anselmi N and Taylor G *in press* Responses to elevated CO_2 of a short rotation, multispecies Poplar plantation. In Short- and long-term effects of elevated atmospheric CO_2 on managed ecosystems. Springer Verlag, Berlin.

Schlesinger W H and Lichter J 2001 Limited carbon storage in soil and litter of experimental forest plots under increased atmospheric CO_2. Nature 411, 466–469.

Van Lagen B 1996 Soil Analyses. *In* Manual for Soil and Water Analyses. Eds P Buurman, B Van Lagen and E J Velthorst. pp. 1–120. Backhuys Publishers, Leiden, The Netherlands.

Zak D R, Holmes W E, Finzi A C, Norby R J and Schlesinger W H 2003 Soil nitrogen cycling under elevated CO_2: A synthesis of forest FACE experiments. Ecol. Appl. 13, 1508–1514.

Zak D R, Pregitzer K S, Curtis P S and Holmes W E 2000 Atmospheric CO_2 and the composition and function of soil microbial communities. Ecol. Appl. 10, 47–59.

Section editor: C. Neill