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C and N in soil organic matter density fractions under elevated atmospheric CO_2: Turnover vs. stabilization

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ABSTRACT

Turnover of C and N in an arable soil under Free Air Carbon Dioxide (FACE) experiment was studied by the use of ^{13}C natural abundance and ^{15}N-labeled fertilizers. Wheat was kept four growing seasons under ambient and elevated CO_2 concentrations and fertilized for three growing seasons. Density fractionation of soil organic matter (SOM) allowed to track ^{13}C and ^{15}N in free particulate organic matter (fPOM; <1.6 g cm^{-3}), particulate organic matter occluded within aggregates with two densities (oPOM 1.6, oPOM 1.6–2.0 g cm^{-3}), and in mineral-associated organic matter (>2.0 g cm^{-3}) fractions. Elevated CO_2 and N fertilization did not significantly affect C and N contents in the bulk soil. Calculated mean residence time (MRT) of C and N revealed the qualitative differences of SOM density fractions: (i) the shortest MRT_C and MRT_N in fPOM confirmed high availability of this fraction to decomposition. Larger C/N ratio of fPOM under elevated vs. ambient CO_2 indicated an increasing recalcitrance of FACE-derived plant residues. (ii) There was no difference in MRT of C and N between lighter and heavier oPOMs probably due to short turnover time of soil aggregates which led to oPOM mixing. The increase of MRT_C and MRT_N in both oPOMs during the experiment confirmed the progressive degradation of organic material within aggregates. (iii) Constant turnover rates of C in the mineral fraction neither confirmed nor rejected the assumed stabilization of SOM to take place in the mineral fraction. Moreover, a trend of decreasing of C and N amounts in the Min fraction throughout the experiment was especially pronounced for C under elevated CO_2. Hence, along with the progressive increase of C_{FACE} in the Min fraction the overall losses of C under elevated CO_2 may occur at the expense of older "pre-FACE" C.

1. Introduction

During the past two centuries, the CO_2 concentration in the atmosphere increased by 35%, mainly because of fossil fuel combustion and land-use changes (IPCC, 2007). Studies exploring ecosystem responses to elevated CO_2 have gained widespread attention in the last few decades (reviewed in: Ainsworth and Long, 2005; De Graaff et al., 2006; Jastrow et al., 2005). Most investigations have been focused on forest (Hoosbeek and Scarascia-Mugnozza, 2009; Lichter et al., 2008; Miglietta et al., 2001) and grassland ecosystems (De Graaff et al., 2008; Niklaus et al., 2001; van Kessel et al., 2006). Agricultural ecosystems (Giesemann and Weigel, 2008; Leavitt et al., 1996) are scarcely investigated, although crop-based agriculture (i.e. excluding grazing land) occupies around 1.7 billion hectares globally (Paustian et al., 2000) and has higher potential for sink of atmospheric C because of lower soil organic C content when compared to e.g. grassland ecosystems (Marhan et al., 2010; Niklaus et al., 2001).

Regardless of ecosystem type the most efficient technique to study the pathways of carbon (C) and nitrogen (N) incorporation to soil organic matter (SOM) under elevated atmospheric CO_2 is the Free Air CO_2 Enrichment (FACE) approach (Ainsworth and Long, 2005; Miglietta et al., 2001). Since most FACE experiments use CO_2 deriving from fossil fuel, which is depleted in ^{13}C when compared to atmospheric CO_2, this stable C isotope composition can be used as a tracer and allows to study the fate of C in soils under elevated CO_2 (Dorodnikov et al., 2007, 2008; van Kessel et al., 2006; Wiesenberg et al., 2008). Additionally, the application of N fertilizers labeled with ^{15}N in FACE experiments provides an opportunity to study N turnover under elevated atmospheric CO_2 (De Graaff et al., 2008; van Kessel et al., 2006). The mini-FACE experiment in Hohenheim, Stuttgart, Germany was initiated in 2002 to investigate the effect of elevated atmospheric CO_2 on an agricultural ecosystem of summer wheat. The use of stable C

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isotope tracers under CO_2 enrichment and labeled ^{15}N-fertilizers allows to track C and N in soil organic matter (SOM). This enables assessment whether newly assimilated FACE-derived C (C_{FACE}) and fertilizer-derived N ($N_{fertil.}$) enter inert and recalcitrant SOM pools hereby increasing the potential for SOM sequestration. In turn, C sequestration in soil has been considered an important issue, since this process may partly counterbalance anthropogenic CO_2 emissions and decrease the rise of CO_2 in the atmosphere (van Kessel et al., 2006; Hoosbeek and Scarascia-Mugnozza, 2009; IPCC, 2007; Lichter et al., 2008).

One of the prerequisites for SOM sequestration under elevated atmospheric CO_2 is the stabilization of C_{FACE} and $N_{fertil.}$ in inert or protected SOM pools. To reveal the fate of the new C and N in SOM different fractionation techniques based on physical (reviewed in von Lützow et al., 2007), chemical (Ellerbrock and Kaiser, 2005), thermal (Dorodnikov et al., 2007, 2008; Kuzyakov et al., 2006) or biological properties of SOM, as well as combinations of methods (Balesdent, 1996; Flessa et al., 2008; Marschner et al., 2008) have been widely used. Among others, physical fractionation techniques such as particle size, aggregate and density fractionations are considered less destructive than chemical fractionation procedures, and their results are assumed to be more directly related to the structure and function of SOM *in situ* (Balesdent, 1996; Christensen, 2001). These techniques have been applied to determine the association of SOM with primary particles and to quantify the amount of particulate organic matter (POM) between and within soil aggregates (Golchin et al., 1997; John et al., 2005; von Lützow et al., 2007). Golchin et al. (1997) proposed a conceptual model linking POM decomposition and association with soil mineral particles. Following their hierarchical concept these authors assumed that the protection of organic matter increases with increasing density of SOM, i.e. with increasing association to soil mineral particles. Thus, the level of association between the free light POM (fPOM; density <1.6 g cm^{-3}) and the soil mineral matrix is low and fPOM corresponding to fresh plant litter is the most available for decomposition. In turn, occluded POM (oPOM) separated by densities of 1.6 and 2.0 g cm^{-3} derives from microaggregates. The decomposability of organic matter in microaggregates decreases with the increasing density of oPOM (from 1.6 to 2.0 g cm^{-3}). However, the oPOM 2.0 fraction also partly derives from macroaggregates where it acts as a binding agent between microaggregates. Organic matter in the residual mineral fraction (Min; >2.0 g cm^{-3}) has the largest association with mineral particles, i.e. the highest protection against decomposition and so, the strongest stabilization (Golchin et al., 1997). Accordingly, the turnover time of SOM increases with the increase in density of SOM fractions, because of decreasing availability for soil microbial biomass (Flessa et al., 2008; John et al., 2005; Marschner et al., 2008). The concept of SOM turnover in density fractions was applied in studies investigating the effects of elevated atmospheric CO_2 on forest (Hoosbeek and Scarascia-Mugnozza, 2009; Lichter et al., 2005) or grassland ecosystems (Niklaus et al., 2001; Six et al., 2001), but has never been used, to our knowledge, for agricultural ecosystems. In the current study we tracked the C_{FACE} and $N_{fertil.}$ in free, occluded within aggregates and mineral-associated SOM fractions and we calculated turnover rates of C and N in density fractions to test whether an intensively tilled agroecosystem with wheat could sequester extra C and N under elevated atmospheric CO_2. We hypothesized that (i) the contribution of C_{FACE} and $N_{fertil.}$ will decrease with the increasing density of SOM fractions showing the highest portion of C_{FACE} and $N_{fertil.}$ in fPOM and the lowest in mineral-associated OM; (ii) accordingly, the turnover time of C and N should be shorter in light and longer in heavy fractions of SOM demonstrating the increasing stabilization of the latter with the increase of association to soil mineral particles.

2. Materials and methods

2.1. Experimental set-up and soil sampling

The set-up of the mini-FACE experiment "Heidfeldhof", Hohenheim, Stuttgart, Germany, is described in details elsewhere (Erbs and Fangmeier, 2006; Högy et al., 2009; Marhan et al., 2008, 2010). In brief, the mini-FACE experiment was started in spring 2002 and included plots (diameter 2 m) with elevated atmospheric CO_2 level (540 ppm, $\delta^{13}C$ −19‰, because of using CO_2 at −48‰ for fumigation), ambient plots (380 ppm, $\delta^{13}C$ −8‰), and control plots (ambient CO_2 level, no frames). Each treatment was replicated five times. Beginning in 2003, inorganic NPK fertilizers including KNO_3 labeled with ^{15}N ($\delta^{15}N$ of the fertilizer was 333.75‰) were applied in equal amounts of 140 kg N ha^{-1}, 60 kg K ha^{-1}, 13 kg P ha^{-1} to each plot under ambient and elevated CO_2 treatments. No organic fertilizers were applied. All the plots have been manually tilled twice per year: before planting in February–March and after the harvest in September–October to the depth of ca. 25 cm. The aboveground plant biomass (summer wheat *Triticum aestivum* cv. Triso) was harvested after each growing season (except the first one in 2002, when only weeds have been grown on the site). The wheat stubble and root residues remained in the soil after harvest.

The soil is a semi-stagnic Luvisol (9% sand, 69% silt, 22% clay) without carbonates, pH 6.8 and mean bulk density of 1.2 g cm^{-3} (Marhan et al., 2010). Soil samples were taken annually two times before tillage from the depth of 0–10 cm. The samples were air-dried at room temperature and sieved through 2 mm mesh. All visible roots and plant remains were carefully removed with tweezers. Five replications of bulk soil each of ambient and elevated CO_2 treatments were available for analysis. To obtain sufficient soil amount for density fractionation and subsequent analytical procedures, the soil samples from October (Oct.) 2002–March (Mar.) 2006 from replicate plots were combined according to date and CO_2 treatment. Even then, two analytical replications were available for soil under ambient and elevated CO_2 treatment for Oct. 2002, and Mar. 2004, whereas for Oct. 2004, Mar.–Oct. 2005 and Mar. 2006 only one replication was available. No soil was available for Mar.–Oct. 2003. Samples of plant residues (straw of summer wheat *T. aestivum* L. cv. Triso) were available from two rings each of ambient and elevated CO_2 treatments taken in 2004 and 2006.

2.2. Separation by density fractionation

The isolation of soil density fractions was carried out according to John et al. (2005) and was only slightly modified. Eight grams of air-dried soil (sieved through 2 mm) were placed in a centrifugation tube and 35 ml of sodium polytungstate solution (Sometu, Berlin, Germany) of a density of 1.6 g cm^{-3} was added. The tube was gently turned upside down by hand about five times and the solution was centrifuged at 4000 rpm for 1 h in Eppendorf Centrifuge 5810R at room temperature. The supernatant with floating particles was filtered (cellulose acetate, 0.45 μm; Sartorius) using a stainless steel pressurized filtration device (Sartorius) and washed with distilled water to gain the free particulate organic matter <1.6 g cm^{-3} (fPOM; Fig. 1a). Then, the pellet was dispersed with 35 ml of sodium polytungstate solution (1.6 g cm^{-3}) and 5 glass beads with a diameter of 5 mm were added. The solution was shaken for 16 h with a frequency of 60 movements per minute to crack the soil aggregates (John et al., 2005). After disaggregation, the soil suspension was centrifuged for 1 h at 4000 rpm. The supernatant with floating particles (light occluded particulate organic matter with a density <1.6 g cm^{-3}, oPOM 1.6; Fig. 1b) was filtered (0.45 μm) using the pressurized filtration cylinder (Sartorius) and washed with distilled water. Again, the pellet was dispersed but using sodium

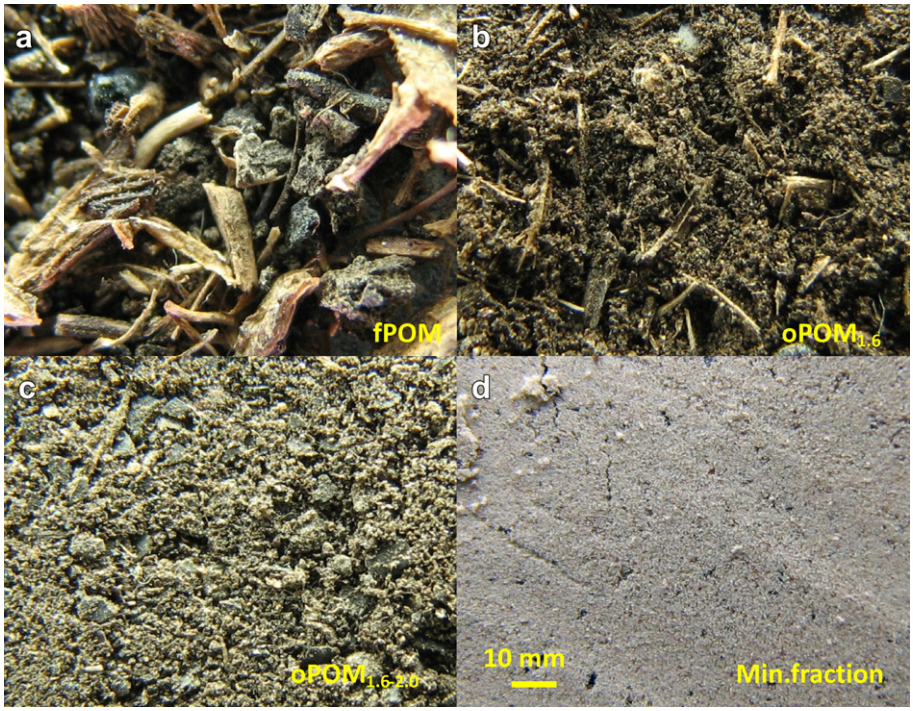

Fig. 1. (a) Free particulate organic matter (fPOM, density < 1.6 g cm^{-3}), (b) occluded particulate organic matter ($oPOM_{1.6}$, <1.6 g cm^{-3}), (c) occluded particulate organic matter ($oPOM_{1.6-2.0}$, 1.6–2.0 g cm^{-3}) and (d) mineral fraction (Min, >2.0 g cm^{-3}).

polytungstate solution of a density 2.0 g cm^{-3}. The supernatant with floating particles (dense occluded particulate organic matter with a density of 1.6–2.0 g cm^{-3}, oPOM 2.0; Fig. 1c) was filtered as described above and washed. To remove the salt, the pellet containing the mineral fraction (>2.0 g cm^{-3}, Min fraction; Fig. 1d) was washed four times with distilled water, each time centrifuging and discarding the supernatant. We did not isolate fine silt and clay fraction in this study. The dissolved organic matter was discarded with supernatant hence not measured. Bulk unfractionated soil and all fractions were dried at 40 °C, weighed and ground to powder by means of a ball mill (MM2, Fa Retsch) for 15 s.

2.3. Elemental (C, N) and stable isotope ($\delta^{13}C$, $\delta^{15}N$) analyses

Ten to forty milligrams of milled bulk soil samples and isolated density fractions were weighed in tin capsules to determine stable carbon ($\delta^{13}C$) and stable nitrogen ($\delta^{15}N$) isotope composition, as well as C and N contents. The samples were measured at the Bayreuth Center of Ecology and Environmental Research (BayCEER), University of Bayreuth, using an elemental analyzer in a dual-element analysis mode (Carlo Erba 1108, Milano, Italy) for Dumas combustion. This was followed by gas chromatographic separation of the gaseous combustion products, which were then fed into a gas-isotope ratio mass spectrometer (delta S Finnigan MAT, Bremen, Germany) via a ConFlo III open-split interface (Finnigan MAT). Standard gases were calibrated with respect to international standards (N_2 in the air and CO_2 in PeeDee belemnite) by use of the reference substances N1 and N2 for nitrogen isotope ratios and Australian National University (ANU) sucrose and NBS 19 for the carbon isotopes. Relative isotope abundances are denoted as δ-values, which were calculated according to the following equation: $\delta^{15}N$ or $\delta^{13}C = (R_{sample}/R_{standard} - 1) \times 1000‰$, where R_{sample} and $R_{standard}$ are the ratios of heavy isotope to light isotope of the samples and the respective standards. Samples of bulk soil were measured in one replication, and samples of plant residues, as well as isolated SOM density fractions were measured in two replications.

2.4. Calculations and statistics

Calculations of new C portions (% new C) in soil due to the incorporation of fresh plant-derived deposits under elevated and ^{13}C-depleted CO_2 can be calculated as follows:

$$\text{new C} = (\delta_{FACEsoil} - \delta_{AMBIENTsoil})/(\delta_{FACEplant} - \delta_{AMBIENTplant}) \times 100[\%] \quad (1)$$

where $\delta_{FACEplant}$ (−38.03 ± 0.69‰) and $\delta_{AMBIENTplant}$ (−27.61 ± 0.54‰) are the average isotope signatures of plants grown under FACE or ambient atmospheric conditions, respectively; $\delta_{FACEsoil}$ and $\delta_{AMBIENTsoil}$ are the isotope signatures of the soil under FACE and under a reference site kept with ambient conditions, respectively (Wiesenberg et al., 2008). Average $\delta^{13}C$ of plants were calculated from respective isotope signatures of plants grown under FACE or ambient atmospheric conditions for the period of the experiment (2002–2006) (Table 1).

A similar equation was used to calculate the portion of N (new N) deriving from labeled fertilizers:

$$\text{new N} = (\delta^{15}N_{soil\ actual} - \delta^{15}N_{soil\ initial})/(\delta^{15}N_{fertil.} - \delta^{15}N_{soil\ initial}) \times 100\ [\%] \quad (2)$$

where $\delta^{15}N_{soil\ actual}$ was the $\delta^{15}N$ value of the bulk SOM and density fractions after fertilization, $\delta^{15}N_{soil\ initial}$ was the $\delta^{15}N$ value of the bulk SOM and density fractions before fertilization with labeled N, and $\delta^{15}N_{fertil.}$ was the $\delta^{15}N$ signature of ^{15}N labeled fertilizers.

To calculate the contribution of new C and N from fertilizers in SOM for a particular period of the FACE experiment and annual turnover rates (k) of SOM, a simple exponential approach was selected (Balesdent and Mariotti, 1996):

$$k = -\ln(1 - M/100)/t\ [\text{year}^{-1}] \quad (3)$$

Table 1
Amount of C, N, and $\delta^{13}C$, $\delta^{15}N$ values in plant tissues* under ambient and elevated CO_2 concentrations within four years (2002–2006) of the FACE experiment. Errors are standard errors of measurement ($n = 4$). Treatments followed by the same letters are not significantly different between elevated and ambient CO_2 of one sampling date (uppercase letters) and between sampling dates of each CO_2 treatments (lowercase letters) at $p \leq 0.05$ (according to two-way-ANOVA and Fischer LSD test).

Date/treatment	C (mg g^{-1} dry weight)	N (mg g^{-1} dry weight)	$\delta^{13}C$ (‰ PDB)	$\delta^{15}N$ (‰ air N_2)	C/N
2002**					
ambient	n.a.	n.a.	-29.85 ± 0.19^{Ad}	not applicable	n.a.
elevated	n.a.	n.a.	-40.38 ± 0.28^{Bc}	not applicable	n.a.
2003**					
ambient	n.a.	n.a.	-28.26 ± 0.26^{Ac}	108.81 ± 9.16^{Aa}	n.a.
elevated	n.a.	n.a.	-36.20 ± 0.54^{Ba}	104.98 ± 5.70^{Aa}	n.a.
2004					
ambient	444.8 ± 1.8^{Aa}	3.39 ± 0.04^{Bb}	-25.34 ± 0.15^{Aa}	223.75 ± 6.09^{Ad}	131.1 ± 1.1^{Aa}
elevated	446.4 ± 0.4^{Aa}	2.66 ± 0.14^{Aa}	-36.53 ± 0.23^{Ba}	235.27 ± 12.19^{Ad}	168.0 ± 8.6^{Ba}
2005**					
ambient	n.a.	n.a.	-26.58 ± 0.21^{Ab}	206.59 ± 3.99^{Ac}	n.a.
elevated	n.a.	n.a.	-38.10 ± 0.15^{Bb}	197.34 ± 8.14^{Ac}	n.a.
2006					
ambient	447.2 ± 2.8^{Aa}	2.77 ± 0.18^{Ba}	-28.01 ± 0.26^{Ac}	160.22 ± 0.08^{Bb}	161.6 ± 3.7^{Ab}
elevated	446.9 ± 3.5^{Aa}	2.18 ± 0.23^{Aa}	-38.90 ± 0.85^{Bb}	140.76 ± 0.54^{Ab}	205.3 ± 10.2^{Bb}

*Straw of summer wheat *Triticum aestivum* L. cv. Triso.
**Data provided by M. Erbs (personal communications) included only isotopic composition of plants ($n = 5$).

where M was the % of new C or N (Eqs. (1) and (2)) in bulk SOM and SOM density fractions, and t was the time of soil exposure to elevated CO_2 concentration and the period of N fertilization.

The mean residence time (MRT) of C and N in bulk soil and in SOM fractions was calculated as a reciprocal to the turnover rates (Gregorich et al., 1995):

$$\mathrm{MRT} = 1/k \ [\mathrm{years}] \quad (4)$$

The C isotope signatures of bulk SOM, density fractions and plants growing under ambient and elevated CO_2 conditions, the duration of CO_2 enrichment and duration of N fertilization used for calculations are presented in Tables 1–5. The C and N contents in unfractionated soil and isolated density fractions were calculated for 1 g of bulk soil.

The determination of statistically significant differences (where possible) between values for bulk soil and isolated SOM density fractions under ambient and elevated CO_2 treatments within one sampling period as well as between sampling periods from Oct. 2002 to Mar. 2006 were performed via two-way-ANOVA and Fischer LSD test ($p \leq 0.05$) using STATISTICA 7.0 software (StatSoft, USA).

3. Results

3.1. Elemental (C, N) amounts and stable isotope ($\delta^{13}C$, $\delta^{15}N$) composition of bulk SOM

The amount of C and N in bulk soil did not differ significantly between ambient and elevated CO_2 treatments for any of the sampling dates (Table 2). The highest amount of C was observed for soil sampled in Oct. 2002 ($p = 0.0011$) as compared to the subsequent dates of sampling. The amount of N did not differ significantly ($p = 0.148$) between the sampling dates except of the significantly higher N in Oct. 2002 vs. Mar. 2004 ($p = 0.032$) (Table 2). The C-to-N ratio (C/N) of the bulk soil showed slightly

Table 2
Amount of C, N, and $\delta^{13}C$, $\delta^{15}N$ values in bulk soil under ambient and elevated atmospheric CO_2 within four years (2002–2006) of the FACE experiment. Errors are standard errors of measurement ($n = 5$). Treatments followed by the same letters are not significantly different between elevated and ambient CO_2 of one sampling date (uppercase letters) and between sampling dates of each CO_2 treatment (lowercase letters) at $p \leq 0.05$ (according to two-way-ANOVA and Fischer LSD test).

Date/treatment	C (mg g^{-1} soil)	N (mg g^{-1} soil)	$\delta^{13}C$ (‰ PDB)	$\delta^{15}N$ (‰ air N_2)	C/N
Oct. 2002					
ambient	18.3 ± 0.5^{Ab}	1.81 ± 0.03^{Ab}	-25.72 ± 0.07^{Aa}	6.91 ± n.a.*	10.1 ± 0.3^{Ab}
elevated	18.5 ± 0.2^{Ab}	1.80 ± 0.02^{Ab}	-26.46 ± 0.13^{Ba}	6.83 ± n.a.	10.3 ± 0.1^{Ab}
Mar. 2004					
ambient	15.9 ± 0.3^{Aa}	1.68 ± 0.05^{Aa}	-25.64 ± 0.04^{Aa}	12.32 ± n.a.	9.5 ± 0.1^{Aa}
elevated	16.5 ± 0.6^{Aa}	1.70 ± 0.06^{Aa}	-26.47 ± 0.15^{Ba}	12.87 ± n.a.	9.7 ± 0.2^{Aa}
Oct. 2004					
ambient	16.0 ± 0.8^{Aa}	1.74 ± 0.07^{Aab}	-25.72 ± 0.08^{Aa}	16.82 ± n.a.	9.2 ± 0.2^{Aa}
elevated	16.5 ± 0.2^{Aa}	1.73 ± 0.05^{Aab}	-26.62 ± 0.07^{Ba}	16.43 ± n.a.	9.5 ± 0.3^{Aa}
Mar. 2005					
ambient	16.0 ± 0.2^{Aa}	1.72 ± 0.03^{Aab}	-25.67 ± 0.07^{Aa}	15.40 ± 0.62^{Aa}	9.3 ± 0.1^{Aa}
elevated	16.3 ± 0.2^{Aa}	1.74 ± 0.04^{Aab}	-26.55 ± 0.08^{Ba}	16.98 ± 1.51^{Aa}	9.4 ± 0.1^{Aa}
Oct. 2005					
ambient	16.6 ± 0.5^{Aa}	1.81 ± 0.05^{Aab}	-26.81 ± 0.06^{Ac}	19.60 ± 0.49^{Ab}	9.2 ± 0.2^{Aa}
elevated	16.4 ± 0.2^{Aa}	1.76 ± 0.04^{Aab}	-27.70 ± 0.09^{Bc}	19.40 ± 0.35^{Aa}	9.3 ± 0.1^{Aa}
Mar. 2006					
ambient	15.8 ± 0.6^{Aa}	1.76 ± 0.07^{Aab}	-26.46 ± 0.06^{Ab}	20.04 ± n.a.	9.0 ± 0.2^{Aa}
elevated	16.2 ± 0.7^{Aa}	1.79 ± 0.07^{Aab}	-27.31 ± 0.08^{Bb}	19.19 ± n.a.	9.1 ± 0.2^{Aa}

*One replication available.

Table 3
Amount of C, N, and $\delta^{13}C$, $\delta^{15}N$ values in free particulate organic matter (fPOM), occluded in aggregates density fractions (oPOM 1.6 and oPOM 2.0) of SOM and soil mineral residue (Min) under ambient and elevated CO_2 treatments during four years (2002–2006) of the FACE experiment. Errors are standard errors of measurement ($n = 4$: 2 "real" × 2 analytical replications). Treatments followed by the same letters are not significantly different between elevated and ambient CO_2 (uppercase letters) and SOM density fractions of one sampling date (lowercase letters) at $p \leq 0.05$ (according to two-way-ANOVA and Fischer LSD test). Stars show the significance of differences ($p \leq 0.05$) between SOM density fractions under each of the CO_2 treatments of two sampling dates Oct. 2002 and Mar. 2004, where replications were available (according to *t*-test).

Date/SOM fraction	CO_2 treatment	C (mg g^{-1} soil)	N (mg g^{-1} soil)	$\delta^{13}C$ (‰ PDB)	$\delta^{15}N$ (‰ air N_2)	C/N
Oct. 2002						
fPOM	ambient	2.3 ± n.a.[#]	0.09 ± n.a.	−25.99 ± n.a.	8.19 ± n.a.	25.6 ± n.a.
	elevated	$2.4 \pm 0.0^{b*}$	$0.08 \pm 0.00^{b*}$	$-29.73 \pm 0.27^{c*}$	$8.12 \pm 0.31^{b*}$	$30.0 \pm 0.6^{d*}$
oPOM 1.6	ambient	0.9 ± 0.1^{Aa}	$0.04 \pm 0.00^{Aa*}$	$-26.08 \pm 0.06^{Ab*}$	$6.68 \pm 0.16^{Aa*}$	$22.5 \pm 0.4^{Ac*}$
	elevated	$1.2 \pm 0.1^{Ba*}$	$0.05 \pm 0.00^{Ba*}$	$-26.89 \pm 0.07^{Bb*}$	$6.52 \pm 0.14^{Aa*}$	$24.0 \pm 0.2^{Bc*}$
oPOM 2.0	ambient	2.6 ± 0.1^{Ab}	$0.17 \pm 0.01^{Ab*}$	$-26.39 \pm 0.08^{Ac*}$	$7.54 \pm 0.39^{Ab*}$	$15.3 \pm 0.2^{Ab*}$
	elevated	3.8 ± 0.2^{Bc}	0.24 ± 0.02^{Bc}	$-27.16 \pm 0.09^{Bb*}$	$6.58 \pm 0.28^{Aa*}$	15.8 ± 0.5^{Ab}
Min	ambient	10.4 ± 0.5^{Ac}	1.34 ± 0.08^{Ac}	$-25.73 \pm 0.02^{Aa*}$	$7.65 \pm 0.17^{Ab*}$	7.8 ± 0.9^{Aa}
	elevated	$9.6 \pm 0.2^{Ad*}$	$1.27 \pm 0.05^{Ad*}$	-25.88 ± 0.01^{Ba}	$7.52 \pm 0.12^{Ab*}$	7.6 ± 0.1^{Aa}
Mar. 2004						
fPOM	ambient	1.2 ± 0.1^{Ba}	0.05 ± 0.00^{Ba}	-26.61 ± 0.12^{Ab}	36.97 ± 0.59^{Ac}	24.0 ± 0.4^{Ad}
	elevated	0.7 ± 0.1^{Aa}	0.03 ± 0.00^{Aa}	-31.21 ± 0.08^{Bd}	39.33 ± 1.94^{Ac}	23.3 ± 0.2^{Ac}
oPOM 1.6	ambient	1.1 ± 0.1^{Ba}	0.06 ± 0.01^{Ba}	-26.69 ± 0.01^{Ab}	14.45 ± 1.56^{Ab}	18.3 ± 0.4^{Bc}
	elevated	0.6 ± 0.0^{Aa}	0.04 ± 0.00^{Aa}	-27.43 ± 0.08^{Bb}	12.24 ± 0.30^{Aa}	15.0 ± 0.3^{Ab}
oPOM 2.0	ambient	2.9 ± 0.1^{Ab}	0.23 ± 0.01^{Ab}	-27.03 ± 0.03^{Ac}	13.51 ± 0.20^{Bb}	12.6 ± 0.1^{Ab}
	elevated	3.3 ± 0.2^{Ab}	0.24 ± 0.01^{Ab}	-27.88 ± 0.00^{Bc}	12.68 ± 0.32^{Aa}	13.8 ± 0.2^{Bb}
Min	ambient	9.8 ± 0.2^{Bc}	1.28 ± 0.05^{Bc}	-25.39 ± 0.00^{Aa}	12.74 ± 0.30^{Aa}	7.7 ± 0.1^{Aa}
	elevated	7.3 ± 0.2^{Ac}	0.84 ± 0.01^{Ac}	-25.80 ± 0.07^{Ba}	14.85 ± 0.48^{Bb}	8.7 ± 0.1^{Ba}
Oct. 2004						
fPOM	ambient	0.4 ± n.a.	0.03 ± n.a.	−26.87 ± n.a.	66.46 ± n.a.	13.3 ± n.a.
	elevated	0.6 ± n.a.	0.03 ± n.a.	−31.68 ± n.a.	111.72 ± n.a.	20.0 ± n.a.
oPOM 1.6	ambient	0.7 ± n.a.	0.05 ± n.a.	−26.91 ± n.a.	24.09 ± n.a.	14.0 ± n.a.
	elevated	1.1 ± n.a.	0.06 ± n.a.	−27.72 ± n.a.	25.78 ± n.a.	18.3 ± n.a.
oPOM 2.0	ambient	3.0 ± n.a.	0.23 ± n.a.	−27.03 ± n.a.	19.42 ± n.a.	13.0 ± n.a.
	elevated	2.2 ± n.a.	0.19 ± n.a.	−27.81 ± n.a.	20.32 ± n.a.	11.6 ± n.a.
Min	ambient	9.0 ± n.a.	1.07 ± n.a.	−25.50 ± n.a.	18.09 ± n.a.	8.4 ± n.a.
	elevated	7.4 ± n.a.	0.87 ± n.a.	−26.15 ± n.a.	16.93 ± n.a.	8.5 ± n.a.
Mar. 2005						
fPOM	ambient	0.7 ± n.a.	0.04 ± n.a.	−27.27 ± n.a.	71.01 ± n.a.	17.5 ± n.a.
	elevated	0.8 ± n.a.	0.04 ± n.a.	−32.27 ± n.a.	95.87 ± n.a.	20.0 ± n.a.
oPOM 1.6	ambient	0.5 ± n.a.	0.03 ± n.a.	−26.89 ± n.a.	15.48 ± n.a.	16.7 ± n.a.
	elevated	0.5 ± n.a.	0.03 ± n.a.	−27.79 ± n.a.	20.13 ± n.a.	16.7 ± n.a.
oPOM 2.0	ambient	3.1 ± n.a.	0.25 ± n.a.	−27.19 ± n.a.	17.02 ± n.a.	12.4 ± n.a.
	elevated	3.2 ± n.a.	0.25 ± n.a.	−28.07 ± n.a.	20.98 ± n.a.	12.8 ± n.a.
Min	ambient	10.0 ± n.a.	1.27 ± n.a.	−25.55 ± n.a.	14.64 ± n.a.	7.9 ± n.a.
	elevated	8.4 ± n.a.	1.03 ± n.a.	−26.27 ± n.a.	17.24 ± n.a.	8.2 ± n.a.
Oct. 2005						
fPOM	ambient	0.5 ± n.a.	0.03 ± n.a.	−26.94 ± n.a.	64.40 ± n.a.	16.7 ± n.a.
	elevated	0.6 ± n.a.	0.03 ± n.a.	−33.07 ± n.a.	112.34 ± n.a.	20.0 ± n.a.
oPOM 1.6	ambient	0.6 ± n.a.	0.03 ± n.a.	−26.55 ± n.a.	21.51 ± n.a.	20.0 ± n.a.
	elevated	0.4 ± n.a.	0.02 ± n.a.	−27.84 ± n.a.	23.14 ± n.a.	20.0 ± n.a.
oPOM 2.0	ambient	2.9 ± n.a.	0.23 ± n.a.	−27.14 ± n.a.	21.25 ± n.a.	12.6 ± n.a.
	elevated	3.0 ± n.a.	0.24 ± n.a.	−28.04 ± n.a.	21.73 ± n.a.	12.5 ± n.a.
Min	ambient	8.4 ± n.a.	1.20 ± n.a.	−25.41 ± n.a.	23.99 ± n.a.	7.0 ± n.a.
	elevated	8.0 ± n.a.	1.00 ± n.a.	−26.27 ± n.a.	22.35 ± n.a.	8.0 ± n.a.
Mar. 2006						
fPOM	ambient	0.3 ± n.a.	0.02 ± n.a.	−27.48 ± n.a.	81.29 ± n.a.	15.0 ± n.a.
	elevated	0.5 ± n.a.	0.03 ± n.a.	−32.01 ± n.a.	91.15 ± n.a.	16.7 ± n.a.
oPOM 1.6	ambient	0.7 ± n.a.	0.04 ± n.a.	−26.71 ± n.a.	20.52 ± n.a.	17.5 ± n.a.
	elevated	0.7 ± n.a.	0.04 ± n.a.	−27.26 ± n.a.	21.45 ± n.a.	17.5 ± n.a.
oPOM 2.0	ambient	3.3 ± n.a.	0.26 ± n.a.	−26.78 ± n.a.	20.62 ± n.a.	12.7 ± n.a.
	elevated	3.1 ± n.a.	0.26 ± n.a.	−28.27 ± n.a.	22.75 ± n.a.	11.9 ± n.a.
Min	ambient	10.3 ± n.a.	1.20 ± n.a.	−25.44 ± n.a.	20.13 ± n.a.	8.6 ± n.a.
	elevated	6.9 ± n.a.	1.09 ± n.a.	−26.31 ± n.a.	20.24 ± n.a.	6.3 ± n.a.

[#] One replication available.

higher values under elevated vs. ambient CO_2 and decreased under both CO_2 treatments throughout the experiment (Table 2).

Application of ^{13}C-depleted CO_2 significantly ($p = <0.01$) shifted $\delta^{13}C$ in plants growing under elevated CO_2 by about −10.5‰ (Table 1) and by −0.7‰ in bulk SOM (Table 2) after the first growing period already. In the following seasons the average shift in $\delta^{13}C$ under elevated vs. ambient CO_2 treatments was about −10.4‰ for plants (Table 1) and −085‰ for SOM (Table 2). The $\delta^{15}N$ of SOM substantially increased after the start of fertilization in 2003 from initial +6.8 and +6.9 to +19.0 and +20.0‰ under elevated and ambient CO_2, respectively (Table 2).

3.2. Elemental (C, N) amounts and stable isotope ($\delta^{13}C$, $\delta^{15}N$) composition of SOM density fractions

Density fractionation allowed isolating free, occluded and mineral-associated organic matter with different amounts of C and N. The Min fraction dominated by weight (90–96% from the initial

Table 4
Portion of replaced C in total C_{org} (new C) and turnover rates of C (TR) in bulk soil and in free particulate organic matter (fPOM), occluded in aggregates density fractions (oPOM 1.6 and oPOM 2.0) of SOM and in soil mineral residue (Min). Errors are standard errors of measurement (bulk soil: $n = 5$; density fractions: $n = 4$: 2 "real" × 2 analytical replications). Treatments followed by the same letters are not significantly different between bulk soil and SOM density fractions of one sampling date (uppercase letters) and between sampling dates for each bulk soil and SOM density fractions (where possible) at $p \leq 0.05$ (according to two-way-ANOVA and Fischer LSD test).

Date/SOM fraction	New C# (%)	Duration of CO_2 enrichment (years)	TR* (year^{-1})
Oct. 2002			
FPOM	36.0 ± 2.6^{Ca}	0.7	0.671 ± 0.060^{Cb}
OPOM 1.6	7.9 ± 0.0^{Bb}	0.7	$0.122 \pm 0.001^{Bb*}$
OPOM 2.0	7.4 ± 0.7^{Ba}	0.7	$0.115 \pm 0.011^{Bb*}$
Min	1.4 ± 0.1^{Aa}	0.7	0.021 ± 0.002^{Aa}
Bulk soil	7.1 ± 0.4^{Ba}	0.7	$0.111 \pm 0.06^{Bd*}$
Mar. 2004			
FPOM	44.3 ± 0.8^{Cb}	2	0.292 ± 0.007^{Ca}
OPOM 1.6	7.1 ± 0.4^{Ba}	2	0.037 ± 0.002^{Ba}
OPOM 2.0	7.1 ± 0.2^{Ba}	2	0.037 ± 0.001^{Ba}
Min	3.9 ± 0.4^{Ab}	2	0.020 ± 0.002^{Aa}
Bulk soil	7.8 ± 0.6^{Ba}	2	0.041 ± 0.003^{Bc}
Oct. 2004			
FPOM	46.2 ± n.a.**	2.7	0.233 ± n.a.
OPOM 1.6	7.8 ± n.a.	2.7	0.030 ± n.a.
OPOM 2.0	7.5 ± n.a.	2.7	0.029 ± n.a.
Min	6.2 ± n.a.	2.7	0.024 ± n.a.
Bulk soil	8.8 ± 1.1^{a}	2.7	0.035 ± 0.004^{bc}
Mar.2005			
FPOM	48.1 ± n.a.	3	0.218 ± n.a.
OPOM 1.6	8.6 ± n.a.	3	0.030 ± n.a.
OPOM 2.0	8.4 ± n.a.	3	0.029 ± n.a.
Min	6.9 ± n.a.	3	0.024 ± n.a.
Bulk soil	8.5 ± 1.1^{a}	3	0.030 ± 0.004^{b}
Oct. 2005			
FPOM	58.9 ± n.a.	3.7	0.242 ± n.a.
OPOM 1.6	12.4 ± n.a.	3.7	0.036 ± n.a.
OPOM 2.0	8.6 ± n.a.	3.7	0.025 ± n.a.
Min	8.3 ± n.a.	3.7	0.024 ± n.a.
Bulk soil	8.6 ± 0.9^{a}	3.7	0.024 ± 0.003^{ab}
Mar. 2006			
FPOM	43.5 ± n.a.	4	0.143 ± n.a.
OPOM 1.6	5.3 ± n.a.	4	0.014 ± n.a.
OPOM 2.0	14.4 ± n.a.	4	0.039 ± n.a.
Min	8.3 ± n.a.	4	0.022 ± n.a.
Bulk soil	8.2 ± 0.6^{a}	4	0.021 ± 0.001^{a}

New C was calculated based on the Eq. (1).
*TR's were calculated based on the Eq. (3).
**One replication available.

soil) and contained the largest C pool, ranging from 6.85 to 10.42 mg g^{-1} soil and N (0.84–1.34 mg g^{-1} soil) under both ambient and elevated CO_2 treatments (Table 3). Carbon and N amounts in POM decreased with decreasing density: oPOM 2.0 > oPOM 1.6 ≥ fPOM, except for Oct. 2002, when fPOM was substantially larger than oPOM 1.6 under both CO_2 treatments (Table 3).

There was no pronounced effect of elevated CO_2 on the amount of C and N in POM density fractions. In contrast to POM fractions, Min fraction showed higher C and N contents under ambient vs. elevated CO_2 for all sampling dates (Table 3).

The C-to-N ratios of isolated fractions generally decreased with the increasing density. The highest values corresponded to fPOM (up to 30) and the lowest to the Min fraction (6.3–8.7) (Table 3). The fPOM had higher values of C/N under elevated vs. ambient CO_2 (except for Mar. 2004), coinciding with the trend of C/N in plant residues (Table 1). After initiation of N fertilization,

Table 5
Portion of fertilizer-derived N (new N) in total N_{min} and turnover rates of N (TR) in bulk soil and in free particulate organic matter (fPOM), occluded in aggregates density fractions (oPOM 1.6 and oPOM 2.0) of SOM and in soil mineral residue (Min). Errors are standard errors of measurement (bulk soil: $n = 5$; density fractions: $n = 4$: 2 "real" × 2 analytical replications). Treatments followed by the same letters are not significantly different between ambient and elevated CO_2 in bulk soil and SOM density fractions of one sampling date (uppercase letters) and between sampling dates for each bulk soil under both CO_2 treatments (lowercase letters) of March and October 2005 at $p \leq 0.05$ (according to t-test).

Date/SOM fraction	CO_2 treatment	New N# (%)	Duration of N fertilization (years)	TR* (year^{-1})
Mar. 2004				
FPOM	ambient	8.84 ± 0.18^{A}	1	0.093 ± 0.002^{A}
	elevated	9.59 ± 1.85^{A}	1	0.101 ± 0.020^{A}
OPOM 1.6	ambient	2.38 ± 0.48^{A}	1	0.024 ± 0.005^{A}
	elevated	1.75 ± 0.09^{A}	1	0.018 ± 0.001^{A}
OPOM 2.0	ambient	1.83 ± 0.01^{A}	1	0.018 ± 0.000^{A}
	elevated	1.87 ± 0.10^{A}	1	0.019 ± 0.001^{A}
Min	ambient	1.56 ± 0.09^{A}	1	0.017 ± 0.000^{A}
	elevated	2.25 ± 0.15^{B}	1	0.023 ± 0.003^{B}
Bulk soil	ambient	1.66 ± n.a.	1	0.017 ± n.a.
	elevated	1.85 ± n.a.	1	0.019 ± n.a.
Oct. 2004				
FPOM	ambient	17.90 ± n.a.**	1.7	0.118 ± n.a.
	elevated	31.82 ± n.a.	1.7	0.230 ± n.a.
OPOM 1.6	ambient	5.32 ± n.a.	1.7	0.033 ± n.a.
	elevated	5.88 ± n.a.	1.7	0.036 ± n.a.
OPOM 2.0	ambient	3.64 ± n.a.	1.7	0.022 ± n.a.
	elevated	4.20 ± n.a.	1.7	0.026 ± n.a.
Min	ambient	3.20 ± n.a.	1.7	0.020 ± n.a.
	elevated	2.88 ± n.a.	1.7	0.018 ± n.a.
Bulk soil	ambient	3.03 ± n.a.	1.7	0.019 ± n.a.
	elevated	2.94 ± n.a.	1.7	0.018 ± n.a.
Mar. 2005				
FPOM	ambient	19.30 ± n.a.	2	0.107 ± n.a.
	elevated	26.95 ± n.a.	2	0.157 ± n.a.
OPOM 1.6	ambient	2.69 ± n.a.	2	0.014 ± n.a.
	elevated	4.16 ± n.a.	2	0.021 ± n.a.
OPOM 2.0	ambient	2.91 ± n.a.	2	0.015 ± n.a.
	elevated	4.40 ± n.a.	2	0.023 ± n.a.
Min	ambient	2.16 ± n.a.	2	0.011 ± n.a.
	elevated	2.98 ± n.a.	2	0.015 ± n.a.
Bulk soil	ambient	2.60 ± 0.19^{Aa}	2	0.013 ± 0.001^{Aa}
	elevated	3.11 ± 0.46^{Aa}	2	0.016 ± 0.002^{Aa}
Oct. 2005				
FPOM	ambient	18.80 ± n.a.	2.7	0.078 ± n.a.
	elevated	32.00 ± n.a.	2.7	0.145 ± n.a.
OPOM 1.6	ambient	4.53 ± n.a.	2.7	0.017 ± n.a.
	elevated	5.08 ± n.a.	2.7	0.020 ± n.a.
OPOM 2.0	ambient	4.20 ± n.a.	2.7	0.016 ± n.a.
	elevated	4.63 ± n.a.	2.7	0.018 ± n.a.
Min	ambient	5.01 ± n.a.	2.7	0.019 ± n.a.
	elevated	4.54 ± n.a.	2.7	0.017 ± n.a.
Bulk soil	ambient	3.88 ± 0.15^{Ab}	2.7	0.015 ± 0.001^{Aa}
	elevated	3.84 ± 0.11^{Aa}	2.7	0.015 ± 0.000^{Aa}
Mar. 2006				
FPOM	ambient	22.45 ± n.a.	3	0.085 ± n.a.
	elevated	25.50 ± n.a.	3	0.098 ± n.a.
OPOM 1.6	ambient	4.23 ± n.a.	3	0.014 ± n.a.
	elevated	4.56 ± n.a.	3	0.016 ± n.a.
OPOM 2.0	ambient	4.01 ± n.a.	3	0.014 ± n.a.
	elevated	4.94 ± n.a.	3	0.017 ± n.a.
Min	ambient	3.83 ± n.a.	3	0.013 ± n.a.
	elevated	3.90 ± n.a.	3	0.013 ± n.a.
Bulk soil	ambient	4.02 ± n.a.	3	0.014 ± n.a.
	elevated	3.78 ± n.a.	3	0.013 ± n.a.

New N was calculated based on the Eq. (2).
*TR's were calculated based on the Eq. (x).
**One replication available.

the C/N ratios decreased in POM fractions, but not in the mineral fraction, where C/N did not vary substantially throughout the experiment (Table 3).

Corresponding to the bulk soil, $\delta^{13}C$ of all density fractions under elevated CO_2 decreased starting from the first growing season. The largest shift in $\delta^{13}C$ of −3.7 to −6.1‰ under elevated vs. ambient CO_2 treatment was observed for the fPOM fraction and the smallest shift (−0.1 to −0.9‰) was detected in the Min fraction. The depletion in ^{13}C under elevated CO_2 with time occurred in all fractions except fPOM and oPOM 1.6 for the last sampling date (Mar. 2006) (Table 3). The application of labeled ^{15}N-fertilizers that started in 2003 significantly ($p = <0.0001$) increased $\delta^{15}N$ of all SOM density fractions from initial +6.5 and +8.2‰ (Oct. 2002) to +9.6 and +112.3‰ for subsequent sampling dates. The largest increase in $\delta^{15}N$ was observed in fPOM under elevated CO_2 (Table 3).

3.3. Amount of replaced C and fertilizers-derived N in bulk soil and SOM density fractions

The portion of new C reached 7% in bulk soil after the first growing season (Oct. 2002) and increased up to 8.6% after three growing seasons (Oct. 2005) (Table 4). The distribution of new C in SOM density fractions followed, as hypothesized, the pattern of density increase: the highest % of new C was observed in fPOM and the lowest in the Min fraction with intermediate portions in oPOM 1.6 and oPOM 2.0. However, this trend changed, when the portion of new C in SOM fractions was recalculated into mass units based on weight of the density fractions (C_{FACE}) (Fig. 2). Here, the highest amount of C_{FACE} in fPOM occurred only in the very first growing season, whereas its amount decreased in subsequent periods of the experiment. A similar trend was observed also for the oPOM 1.6 fraction (Fig. 2). In contrast to fPOM and oPOM 1.6, oPOM 2.0 showed no change and Min fractions showed a progressive increase in C_{FACE} during the experiment (Fig. 2). Starting from Oct. 2004 the largest pool of C_{FACE} was found in the Min fraction.

The portion of fertilizer-derived N (new N) increased in bulk soil from the initial 1.7% in Mar. 2004 to 4% in Mar. 2006 under ambient CO_2 and from 1.8 to 3.8% within the same period under elevated CO_2, respectively (Table 5). The highest fraction of new N was observed in fPOM, and this was especially pronounced under elevated vs. ambient CO_2 treatment. The total amount of new N ($N_{fertil.}$) progressively increased in bulk soil mainly due to the increase in the Min fraction (Fig. 3). The increase in $N_{fertil.}$ in the Min fraction during the growing seasons 2004 and especially 2005 was more pronounced under ambient than under elevated CO_2 (Fig. 3). However, the significance of the observed trend could not be determined. No difference in the pattern of $N_{fertil.}$ distribution among POM fractions was observed between ambient and elevated CO_2 treatments (Fig. 3).

3.4. Mean residence time of C and N in bulk soil and SOM density fractions

The mean residence time of C (MRT_C) assessed for elevated CO_2 treatment had increased in bulk soil from 10 to 50 years during 4 years of the experiment (Fig. 4). Such an increase in bulk MRT_C was determined for the occluded POM (oPOM 1.6 and oPOM 2.0). Mean RT_C of fPOM was the shortest (1.5–7 years) and MRT_C of Min fraction was the longest (41–52 years) of all fractions (Fig. 4). Mean RT_C of fPOM demonstrated a slight increase with time (Fig. 4).

Mean residence times of N (MRT_N) in bulk soil and density fractions were calculated for both CO_2 treatments, because ^{15}N-labeled fertilizer was applied equally under ambient and elevated CO_2. Mean

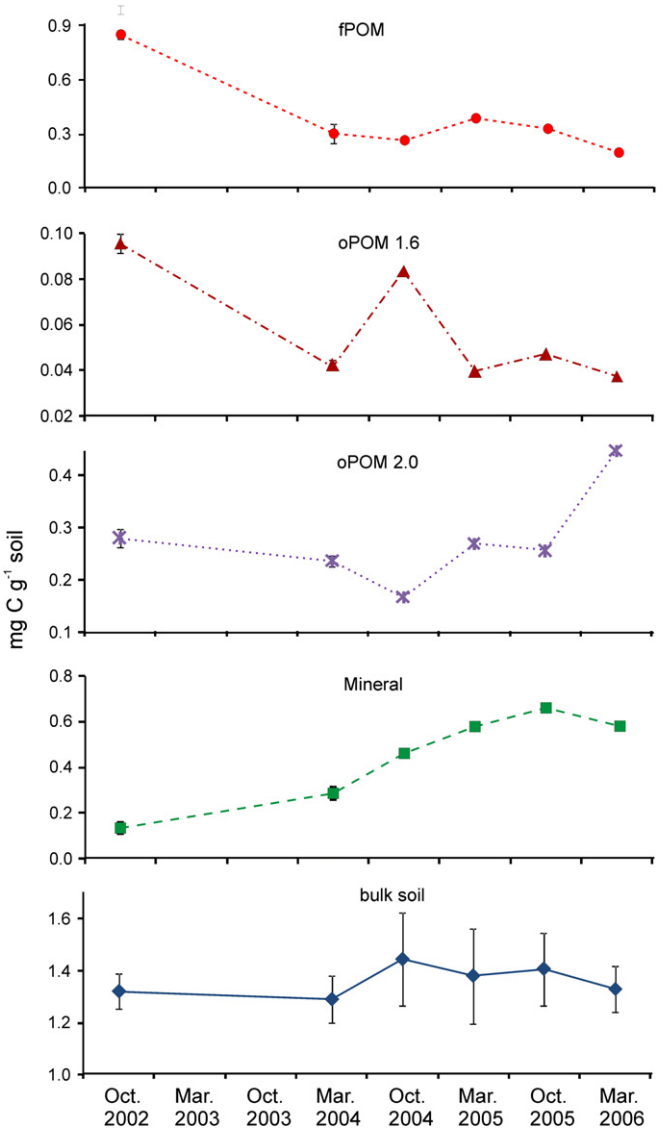

Fig. 2. Amount of replaced C in bulk soil (± SE, $n = 5$) and in isolated free particulate organic matter (fPOM), occluded particulate OM with density up to 1.6 g cm^{-3} (oPOM 1.6), occluded particulate OM with density up to 2.0 g cm^{-3} (oPOM 2.0) and mineral fraction (± SE, $n = 4$: 2 "real" × 2 analytical replications) during 4.5 years of Free Air CO_2 Enrichment.

RT_N of the bulk soil under ambient CO_2 increased from 60 to 73 years and from 54 to 78 years under elevated CO_2 during 3 years (Mar. 2003–Mar. 2006). The pronounced effect of elevated CO_2 on MRT_N was observed in fPOM: MRT_N was 45–48% shorter under elevated vs. ambient CO_2 in Oct. 2004 and Oct. 2005 and 5–30% shorter in Mar. 2004–2006 as compared to respective values under ambient CO_2 treatment (Fig. 4b,c).

4. Discussion

4.1. C and N dynamics in bulk soil under ambient and elevated atmospheric CO_2: "FACE-derived" vs. "pre-FACE" SOM

Atmospheric CO_2 enrichment during four growing seasons as well as NPK fertilization during three growing seasons did not significantly affect total C and N of bulk soil of an agricultural site

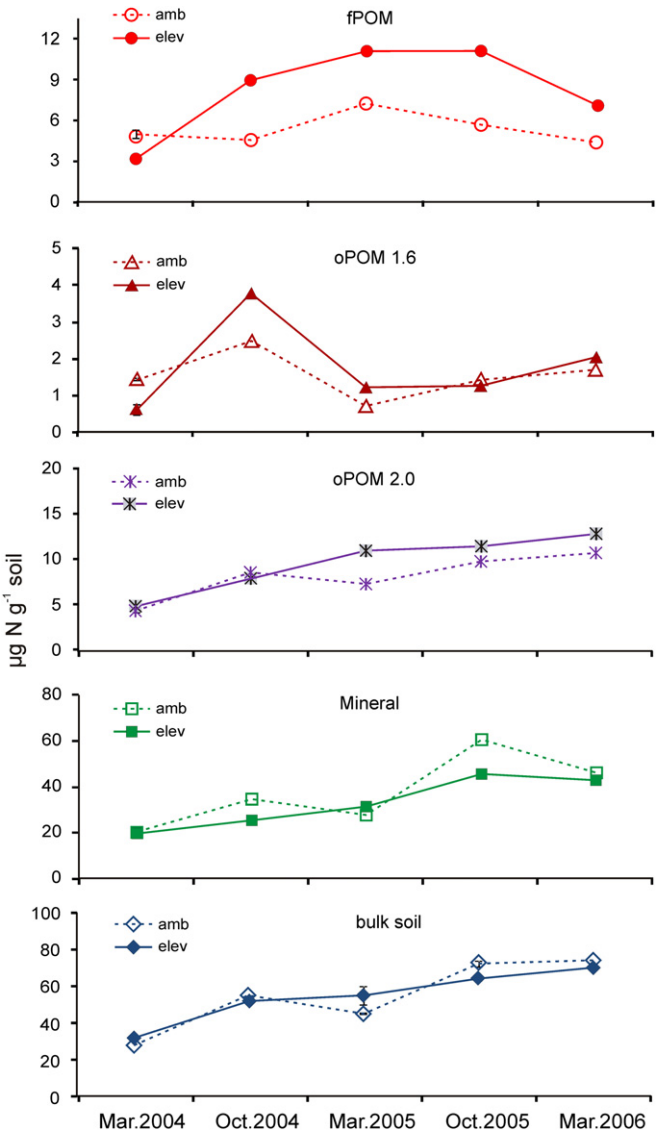

Fig. 3. Amount of replaced N (dots) in bulk soil (± SE, $n = 5$) and in isolated free particulate organic matter (fPOM), occluded particulate OM with density up to 1.6 g cm^{-3} (oPOM 1.6), occluded particulate OM with density up to 2.0 g cm^{-3} (oPOM 2.0) and mineral fraction under ambient (top) and elevated (bottom) CO_2 treatments (± SE, $n = 4$: 2 "real" × 2 analytical replications) during 3 years of fertilization.

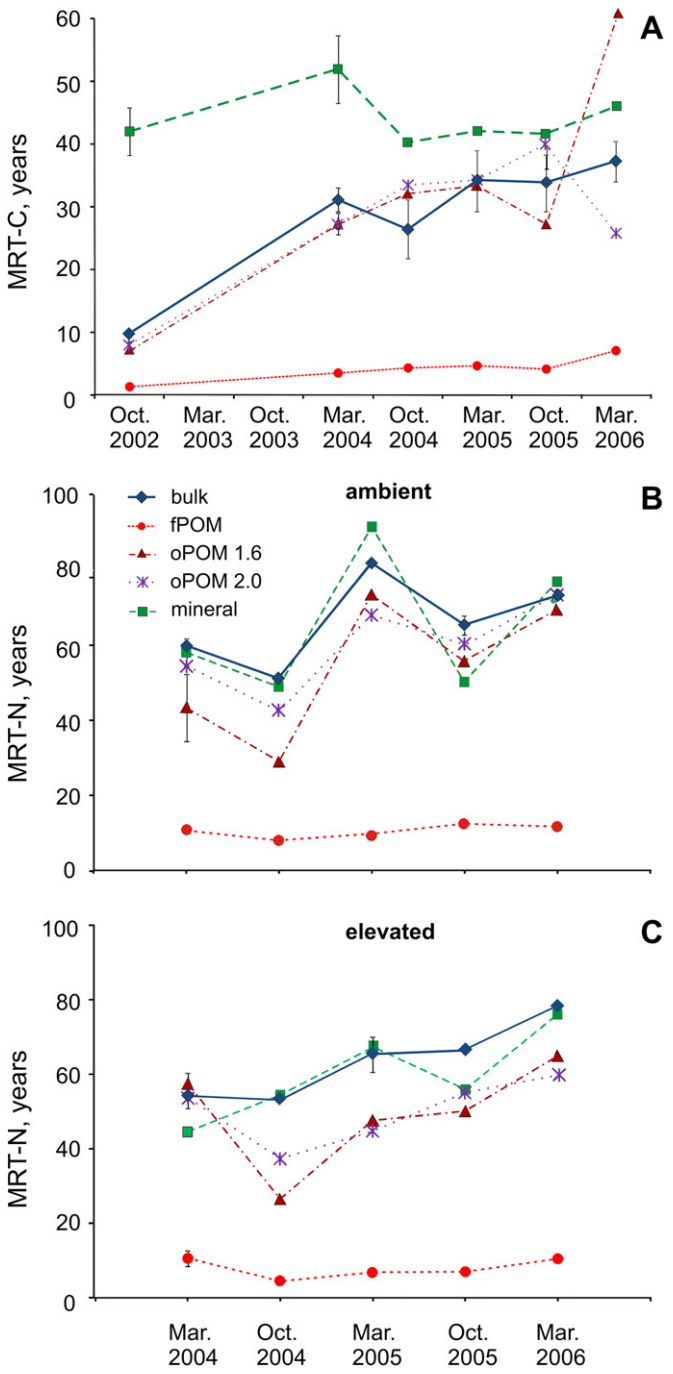

Fig. 4. Mean residence time (MRT) of (A) C turnover rates, (B) N turnover rates under ambient CO_2 and (C) under elevated CO_2 in bulk soil (± SE, $n = 5$) and in isolated free particulate organic matter (fPOM), occluded particulate OM with density up to 1.6 g cm^{-3} (oPOM 1.6), occluded particulate OM with density up to 2.0 g cm^{-3} (oPOM 2.0) and mineral fraction (± SE, $n = 4$: 2 "real" × 2 analytical replications) during 4.5 years of Free Air CO_2 Enrichment and 3 years of fertilization.

cropped with summer wheat (Table 1). This is in agreement with the results of many FACE studies including the current experimental site (Marhan et al., 2008, 2010) and other agricultural field experiments (Dorodnikov et al., 2008; Giesemann and Weigel, 2008; Leavitt et al., 1996), as well as grassland (De Graaff et al., 2008; Niklaus et al., 2001; van Kessel et al., 2006) and forest experiments (Lichter et al., 2005; Norby et al., 2002). The lack of total C and N change under elevated CO_2 could be explained, on the one hand, by the short magnitude (and/or low amount) of CO_2-stimulated C inputs relative to the duration of the experiment (Jastrow et al., 2005). On the other hand, the process of SOM sequestration could be counterbalanced by the increasing decomposition of SOM by soil microbial biomass under elevated atmospheric CO_2 (Blagodatskaya et al., 2010; Heath et al., 2005; Lagomarsino et al., 2009). The increasing activity of soil microorganisms under elevated vs. ambient CO_2 was previously reported for the current FACE experiment (Blagodatskaya et al., 2010; Dorodnikov et al., 2009). Using the approach of the assessment of new C turnover rates (van Kessel et al., 2006) we estimated the MRT of new C under elevated CO_2 to be about 2 months (0.15 years, data not shown) in the current study. Such high turnover rates are apparently driven by the activity of soil microorganisms (Blagodatskaya et al., 2011). However, the recalcitrance of FACE-derived plant residues has been simultaneously increasing in the experiment as shown by increase in C/N ratio of plant tissues (Table 1) and by earlier findings (Marhan et al., 2008). Moreover, the amount of C_{FACE} in bulk soil showed an increasing trend in the course of the experiment (Fig. 2, bottom). Although, the increase of C_{FACE}

along with the lack of total C changes cannot directly indicate the effect of elevated CO_2 on decomposition rates, since newly synthesized C under ambient CO_2 could potentially follow the same pattern, which is not possible to detect without a label. Still, along with the increasing C/N ratio in plant residues (Table 1) and decrease of old SOM pool predicted by modeling for the current experimental site (Marhan et al., 2010), the reported increasing activity of soil microorganisms under elevated CO_2 (Blagodatskaya et al., 2010; Dorodnikov et al., 2009) could accelerate decomposition of older "pre-FACE" C vs. FACE-derived fresh plant residues. This process will lead to the gradual replacement of the unprotected and/or less recalcitrant soil C pool by C_{FACE} (Marhan et al., 2010). However, the mentioned mechanism should be tested for the current experimental site by labeling of ambient CO_2 treatment and/or by long-term incubation study, which could provide information about SOM decomposition under both ambient and elevated CO_2.

In contrast to the assessment of C turnover possible just for elevated CO_2 treatment, the N turnover in bulk soil was assessed for both elevated and ambient CO_2. The general trend in the replacement of total N by $N_{fertil.}$ was similar for both CO_2 treatments (Fig. 3). Significantly lower amount of $N_{fertil.}$ was observed only after vegetation season 2005 in bulk soil under elevated vs. ambient CO_2 (Fig. 3, bulk soil values). This may occur due to higher N uptake by plants because of increased demand for N to support plant growth stimulated by elevated CO_2 (Ainsworth and Long, 2005; De Graaff et al., 2008; Luo et al., 2004). However, neither current results of N content in plant tissues (N content was significantly lower under elevated vs. ambient CO_2) (Table 1) nor statistical evidence (just one significant event during four years of the experiment) do support this hypothesis. Furthermore, the MRT_N during 3 years showed no significant difference in bulk soil under elevated and under ambient CO_2 (Fig. 4b,c). Nevertheless, bulk soil values very often fail to provide sufficient information about the processes involved into SOM turnover (Neff et al., 2002). Thus, to reveal the effect of fertilization onto C and N dynamics under both elevated and ambient CO_2 the fractionation of total SOM pool into subpools of different stability is an adequate and effective tool (Neff et al., 2002).

4.2. C and N dynamics in SOM density fractions under ambient and elevated atmospheric CO_2: turnover vs. stabilization

4.2.1. fPOM

Free particulate organic matter represents coarse and poorly decomposed plant residues mostly of roots and stubbles origin (Fig. 1a; John et al., 2005). Predomination of plant-derived material in fPOM fraction was also confirmed by lipids distribution patterns (Wiesenberg et al., 2010). Carbon and N content of fPOM was the highest after the first growing season of 2002 under both CO_2 treatments as compared to the subsequent sampling dates (Table 3). This was due to the experimental set-up, when all growing plants on the site were not harvested since the FACE system worked in a testing regime. Thus, the aboveground plant biomass was incorporated into the soil hereby increasing its fPOM (and total C) content. The fPOM decrease in subsequent seasons was, correspondingly, due to fewer inputs of plant residues into the soil because of the removal of aboveground plant biomass during harvest. Notably higher C content (except of Mar. 2004), nearly the same N content and, hence, broader C/N ratio in fPOM (Table 3) were detected under elevated vs. ambient CO_2 indicating the changing quality of plant tissues under atmospheric CO_2 enrichment (Ainsworth and Long, 2005; Cotrufo et al., 2005; Wiesenberg et al., 2008).

The highest portions of new C and N (Tables 4, 5) along with the shortest MRT_C and MRT_N of fPOM fraction (<13 years; Fig. 4) as compared to other fractions supported the hypothesized high availability of the former to decomposition. This is in agreement with the hierarchical concept of SOM turnover in density fractions (Bol et al., 2003; Flessa et al., 2008; Golchin et al., 1997; John et al., 2005; Marschner et al., 2008) and the effect of fertilization onto SOM turnover in density fractions (Neff et al., 2002). Although gradual decrease of MRT_C in fPOM (Fig. 4) cannot alone indicate changing quality of plant tissues under elevated atmospheric CO_2 because, in theory, a slowing down of MRT_C over time could also occur under ambient CO_2. The MRT_C along with broader C/N ratio in fPOM under elevated vs. ambient CO_2 supports previous findings of increasing recalcitrance of plant-derived inputs in FACE studies (Cotrufo et al., 2005; Marhan et al., 2008; Wiesenberg et al., 2008). However, as it was reported (Bol et al., 2009; Flessa et al., 2008; Marschner et al., 2008), the chemical recalcitrance of plant-derived inputs cannot solely provide the potential of SOM stabilization within a long-term period. Thus, other factors, such as association of organic compounds with mineral particles should be considered for the assessment of SOM turnover under elevated atmospheric CO_2.

4.2.2. oPOM

Particulate organic matter occluded in soil aggregates represented visually more decomposed organic materials with stronger association to soil mineral particles as compared to fPOM (Fig. 1b, c; John et al., 2005). Analysis of lipids distribution patterns within SOM density fractions showed the increased contribution of microbial-derived compounds in oPOM vs. fPOM and mineral fractions (Wiesenberg et al., 2010).

Despite the amount of C and N (Table 3) as well as C_{FACE} and $N_{fertil.}$ (Figs. 2 and 3) were at least 2-folds higher in oPOM 2.0 vs. oPOM 1.6 fraction, the MRT_C (Fig. 4a) and particularly MRT_N (Fig. 4b,c) did not reveal a functional difference between lighter oPOM 1.6 and heavier oPOM 2.0. This contradicts the conceptual model of SOM turnover in density fractions proposed by Golchin et al. (1997). Authors assumed a functional difference between lighter and heavier oPOMs – oPOM 1.6 and 2.0 derives from microaggregates. However, the fraction oPOM 2.0 also partly derives from macroaggregates where it acts as a binding agent between microaggregates. The lack of the difference in MRT of C and N between lighter and heavier oPOMs could be caused by (i) weak association of lighter and heavier oPOM fractions to aggregates of different sizes and/or (ii) short turnover time of aggregates. Thus, regular intensive tillage of soil on experimental site accelerated the brakedown of macroaggregates into microaggregates with the simultaneous rearrangement of the latter into "new" macroaggregates, etc. So, POM occluded in macro- and microaggregates has been mixing hereby showing no difference in turnover rates between lighter and heavier oPOM fractions.

Both MRT_C and MRT_N of oPOM fractions have substantially increased throughout the experiment (Fig. 4a). Moreover, Fig. 4 shows that the increase in MRT_C of bulk soil over time was largely driven by the response of the oPOM fractions. Shorter turnover time of C in the beginning and its subsequent slowing down during an experiment was reported for studies utilizing C_3/C_4 vegetation change to assess the turnover time of C (Klumpp et al., 2007; Marschner et al., 2008) and for those under FACE (van Kessel et al., 2006). This is consistent with the intensive incorporation of new FACE-derived C into the soil within a relatively short time interval (in our case, about 7% of total C was replaced after the first growing season already, Table 4). Thereafter, the rate of the replacement decreases and after some time the replacement of C is very slow. This means that the C of the "labile" and "intermediate-stable" C pools is mostly replaced after this time (Marschner et al., 2008). For the current site, not only progressive degradation of organic material, which was incorporated in the initial year of the experiment (Wiesenberg et al., 2010), but also substantial decrease of the amount of plant residues (aboveground plant biomass has

been harvested after 2002) resulted in 4-fold increase of MRT_C during subsequent 3.5 years. Finally, identical trends of MRT_C increase in oPOM and bulk soil support the concept of the critical role of aggregation on stabilization of organic matter in entire soil (Golchin et al., 1997; Jastrow et al., 2005; Six et al., 2001).

4.2.3. Mineral-associated OM

The residual fraction during aggregate separation, the mineral fraction, left after the removal of POM comprised 89–96% of an initial soil sample by weight (Fig. 1d). This fraction contained the largest pool of C and N (Table 3). There is strong evidence in the literature that the Min fraction (also referred as silt and clay fraction) is responsible for SOM storage and stabilization. This was shown by chemical composition of SOM (Min fraction mostly contains derivates of plant material decomposition) and increasing turnover times or MRTs of SOM (Bol et al., 2003, 2009; Flessa et al., 2008; Golchin et al., 1997; von Lützow et al., 2007; Wiesenberg et al., 2010). Our results similarly showed that SOM in the Min fraction had the longest MRT_C and MRT_N of all fractions, hereby supporting the hypothesized highest protection of SOM provided by association to soil mineral particles. However, the dynamics of MRT_C and MRT_N in Min fraction over 3–4 years cannot strongly suggest SOM to stabilize in this fraction. From the one hand, the MRT_N has been decreasing during the experiment under both ambient and elevated CO_2 (Fig. 4 b, c) indicating the gradual process of total N replacement by $N_{fertil.}$ in the form of low availability for microbial decomposition and/or plant uptake (i.e. stabilization). From the other hand, the MRT_C of the Min fraction assessed for elevated CO_2 was constant (about 45 years) during the period of measurements (4 years) (Fig. 4). Generally, the lack of the difference in MRT_C of the Min fraction over 4 years could mostly relate to the fact that MRT_C of the fraction is much longer than the duration of the experiment. Hence, MRT_C will remain nearly constant in the short-term time interval. However, another mechanism may exist: stabilization of new C derived from coarse plant residues under elevated CO_2 (discussed for bulk soil and fPOM fraction), which theoretically should result in an increase of MRT_C, is counterbalanced by increasing decomposition of older "pre-FACE" C. Some evidence to this provides a trend of decreasing of C and N amounts in the Min fraction throughout the experiment, especially pronounced for C under elevated CO_2 (Table 3). Hence, along with the progressive increase of C_{FACE} in the Min fraction (Fig. 2) the overall losses of C under elevated CO_2 may occur at the expense of older "pre-FACE" C. Still, regarding the above discussion for the bulk soil, either labeling of ambient CO_2 treatment and/or long-term incubation of isolated fractions under both ambient and elevated CO_2 could support/reject the proposed hypothesis.

5. Conclusions

Atmospheric CO_2 enrichment of an agricultural field plot cropped with wheat did not affect C and N contents in bulk soil during four growing seasons. However, fractionation of SOM by density and application of isotope analysis provided information about the quantity and intensity of C_{FACE} and $N_{fertil.}$ incorporation into: the inter-aggregate OM (fPOM), POM occluded within soil aggregates (oPOM 1.6, oPOM 2.0) and mineral-associated OM during the experiment. Calculated turnover rates and mean residence times of C and N revealed the qualitative differences of SOM density fractions:

(i) The shortest MRT_C and MRT_N in fPOM fraction as compared to other fractions confirmed high availability of this fraction to decomposition by soil microbial biomass. At the same time, larger C/N ratio and gradual increase in MRT_C of fPOM may result from the increasing recalcitrance of plant-derived inputs under elevated CO_2.

(ii) The results of the study did not reveal an assumed difference between lighter oPOM 1.6 and heavier oPOM 2.0. The lack of the difference in MRT of C and N between lighter and heavier oPOM fractions could be caused by short turnover time of soil aggregates. At the same time, the gradual increase of MRT_C in both oPOM fractions during the experiment confirmed the progressive degradation of organic material within aggregates of different sizes and supported the concept of the critical role of aggregation on stabilization of organic matter in entire soil.

(iii) The longest MRT_C and MRT_N values were found for the Min fraction supporting the hypothesis that the highest protection of SOM provided by association to soil mineral particles. However, constant turnover rates of C in mineral fraction during the experiment neither confirm nor neglect the assumed stabilization of SOM to take place in the Min fraction. Moreover, a trend of decreasing of C and N amounts in the Min fraction throughout the experiment was especially pronounced for C under elevated CO_2. Hence, along with the progressive increase of C_{FACE} in the Min fraction the overall losses of C under elevated CO_2 may occur at the expense of older "pre-FACE" C.

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