

## Plant and soil lipid modification under elevated atmospheric CO<sub>2</sub> conditions: II. Stable carbon isotopic values ( $\delta^{13}\text{C}$ ) and turnover

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### Abstract

Future enrichment of atmospheric CO<sub>2</sub> and its effect on ecosystems were studied using grassland free air CO<sub>2</sub> enrichment (FACE) experiments. Plant waxes have been shown to be directly modified under elevated CO<sub>2</sub> concentration. Lipids, as major components of plant waxes, are important constituents of plant surfaces and their position at the plant/atmosphere interface makes them susceptible to environmental change. The main focus of this study was to improve knowledge about modifications to stable carbon isotopic ( $\delta^{13}\text{C}$ ) values of individual lipids within plant biomass and soils as a result of the increased atmospheric CO<sub>2</sub> concentration, implying an addition of <sup>13</sup>C labelled CO<sub>2</sub>. The isotopically labelled biomass facilitates turnover time determination of lipids in soils due to the direct comparison of identical plants grown under ambient and <sup>13</sup>C-depleted atmospheric conditions. We demonstrate which lipids were influenced by modified CO<sub>2</sub> concentration and how the lipid isotopic values of plant biomass and soil were influenced under elevated vs. ambient CO<sub>2</sub> conditions. Most plant carboxylic acids and alkanes were uniformly depleted in <sup>13</sup>C by ca. 6‰ when compared to plant biomass bulk isotope values. In soil, short chain carboxylic acids (< C<sub>20</sub>), derived mainly from microbial sources, revealed a lower depletion in isotope value than plant-derived long chain acids (≥ C<sub>20</sub>). The isotopic differences between individual compounds in soil under ambient vs. elevated CO<sub>2</sub> conditions varied significantly between 2 and 6‰ for individual acids and 0–6‰ for individual alkanes. This argues against plant/soil turnover determinations for individual compounds. Preferably, weighted mean average isotopic values of the most abundant lipids provide reliable calculation of replaced carbon proportions and turnover times. Carboxylic acids were turned over fastest in grassland soil, followed by bulk carbon, whereas alkanes exhibited the slowest turnover times. This is in contrast to previous studies of arable soil, but confirms observations made on peaty soil indicating that alkanes may be part of the relatively stable carbon fraction in soils. The turnover of total organic carbon, carboxylic acids and long chain alkanes was observed to be significantly greater in soil under *Lolium perenne* (ryegrass) than in soil under the leguminose plant *Trifolium repens* (white clover).  
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## 1. Introduction

Increase in atmospheric CO<sub>2</sub> concentration has led to an intensive debate about climate change (IGBP, 1998; IPCC, 2007). To improve our knowledge about the influence of increasing CO<sub>2</sub> concentration on plants and ecosystems, numerous experiments have been carried out. These studies employ free air carbon dioxide enrichment (FACE) experiments, where plants are grown under otherwise natural conditions. In the experiments identical plants are usually exposed to ambient and elevated CO<sub>2</sub> conditions on neighbouring plots. As summarized previously (Wiesenberg et al., 2007), bioproductivity and degradability of plant biomass have been documented to be influenced variously under elevated CO<sub>2</sub> concentration for numerous plants. Commonly, bioproductivity was found to increase for numerous C<sub>3</sub> plants (Nowak et al., 2004; Poorter and Navas, 2003; Pendall et al., 2004; Wang et al., 2004) under elevated CO<sub>2</sub> concentration. Simultaneously, the degradability of plant biomass often increased (Norby and Luo, 2004; Pendall et al., 2004). For some plants, such as *Lolium perenne*, a decrease in productivity was described (Nijs et al., 1996).

Under elevated CO<sub>2</sub> concentration the translocation of plant-derived carbon into soil was observed to be similar to that under ambient conditions, resulting in similar carbon proportions in both situations (e.g. van Kessel et al., 2000a; Six et al., 2001). Also, microbial communities were not significantly changed under elevated CO<sub>2</sub> conditions (Glaser et al., 2006). Studies combining molecular approaches, including compositional changes and compound specific isotope ( $\delta^{13}\text{C}$ ) modifications of plant biomass and soil organic carbon under elevated CO<sub>2</sub> concentration are still scarce, except for some recent studies on lignin derivatives (Heim and Schmidt, 2006) and lipids (Wiesenberg et al., 2007).

As described in a complementary study (Wiesenberg et al., 2007), lipids contain several diagnostic markers for source apportionment of organic carbon in soils (e.g. Wiesenberg and Schwark, 2006) and facilitate turnover rate determinations for plant-derived lipids in soils (Lichtfouse et al., 1994, 1997; Cayet and Lichtfouse, 2001; Wiesenberg et al., 2004b). Commonly, agricultural or forest soils have been used for input and turnover time determinations of lipids (e.g. Wiesenberg et al., 2004b; Qu  n  a et al., 2006). While

lipid distribution patterns in arable soil (e.g. Lichtfouse et al., 1994; Wiesenberg et al., 2004b; Qu  n  a et al., 2006; Jandl et al., 2007), forest soil (e.g. Ambl  s et al., 1994; Nierop, 1998; Qu  n  a et al., 2006) or peat (e.g. Ficken et al., 1998; Yi and Lanhua, 2001) have been frequently studied, knowledge of the behaviour of lipids in grassland soils is limited (van Bergen et al., 1997; Bull et al., 2000). Plant lipid distribution patterns and isotopic signatures were found to be directly correlated with several environmental and growth factors, including exposition or plant part (Collister et al., 1994; Lockheart et al., 1997; Wiesenberg et al., 2004b), plant growth or degradation stage (e.g. Nguyen Tu et al., 2001, 2004; Conte et al., 2003; Chikaraishi and Naraoka, 2006). The influence of several growth parameters such as precipitation on plant lipid isotope values has been discussed controversially in terms of effect (e.g. Conte et al., 2003; Bi et al., 2005). However, only a few studies have investigated the influence of modified atmospheric conditions on plant lipids. Variables addressed in these studies include elevated temperature (e.g. Larkindale and Huang, 2004) or elevated atmospheric CO<sub>2</sub> concentration (e.g. Hussain et al., 2001; Pe  uelas et al., 2002; Wiesenberg et al., 2007). These studies on plant lipids under ambient vs. elevated CO<sub>2</sub> concentration document diverse influences on lipid composition depending on plant species and plant part analysed. Modification of lipid proportions was documented for tree seeds (Hussain et al., 2001), while lipids of selected shrubs remained unchanged (Pe  uelas et al., 2002). The modification of lipid concentration and distribution patterns of plants and soils under FACE discussed in this paper has been described in a complementary study (Wiesenberg et al., 2007).

Stable carbon isotope analysis has been used in combination with lipid analysis, first in terms of bulk isotopic measurement of total soil organic carbon (SOC), and then as compound specific isotopic analysis of individual lipids (Lichtfouse and Budzinski, 1995). The combination of structural and isotopic analyses allows differentiation of compound sources, assessment of how fast they are incorporated into soil and calculation of their residence times in the pedosphere. Except for a study of lignin derivatives (Heim and Schmidt, 2006) no publications employing compound specific isotope analysis for turnover calculations of pasture plant-derived compounds like lipids in soil under elevated CO<sub>2</sub>

conditions were available. Compound specific isotope values facilitate determination of turnover times for plant-derived components in soil on a molecular level, as demonstrated for lignin monomers (Heim and Schmidt, 2006) and for lipids for C<sub>3</sub>/C<sub>4</sub> plant crop changes (Wiesenberg et al., 2004b).

Here, we discuss lipid isotopic modification under elevated CO<sub>2</sub> conditions for several pasture plants and how this signal is transferred from plant biomass to soil lipids by determining turnover times for short and long chain carboxylic acids, as well as for aliphatic hydrocarbons in pasture soil.

## 2. Materials and methods

### 2.1. Sampling site

Soil and plant samples were taken from the free air carbon dioxide enrichment (FACE) experiment of the Swiss Federal Institute of Technology (ETH) experimental trial near Eschikon, 20 km NE of Zurich. The influence of elevated atmospheric CO<sub>2</sub> on a grassland ecosystem was the main objective of the trial. The added atmospheric CO<sub>2</sub> was characterized by significantly lower δ<sup>13</sup>C values than the ambient air (van Kessel et al., 2000b). While ambient air contained ca. 350 ppm CO<sub>2</sub> with a δ<sup>13</sup>C value of ca. −8‰, fumigated plots had received for 10 years an elevated CO<sub>2</sub> concentration of ca. 600 ppm with a δ<sup>13</sup>C value of ca. −18‰, when the soil was sampled in 2002. The soil is classified as Eutric Cambisol (FAO-UNESCO, 1994) and experimental details have been described elsewhere (Zanetti et al., 1997; Hebeisen et al., 1997). Clover [*Trifolium repens* (L.)] and ryegrass [*Lolium perenne* (L.)] were grown for ten years on neighbouring plots receiving continuous ambient and elevated atmospheric CO<sub>2</sub> concentrations.

### 2.2. Samples

Soil and plant samples were derived from plots without nitrogen fertilization. Plants were harvested in autumn 1998. Soil samples (0–10 cm depth) were taken in summer 2002 after the termination of the FACE experiment. Aliquots of the samples from each plot were combined with corresponding samples from analogous replicate experiments and homogenized. Air dried samples were finely ground using a ball mill.

### 2.3. Bulk analysis

Samples were analysed for their stable carbon isotopic composition using a continuous flow Heraeus CHN-O-Rapid elemental analyser coupled to a Finnigan MAT Delta-S mass spectrometer. Carbon isotopic values were expressed in permil relative to the Vienna Pee Dee Belemnite (V-PDB) standard:

$$\delta^{13}\text{C} = \left[ \left( \frac{{}^{13}\text{C}/{}^{12}\text{C}_{\text{sample}}}{{}^{13}\text{C}/{}^{12}\text{C}_{\text{std}}} \right) - 1 \right] \times 10^3, \quad (1)$$

where  ${}^{13}\text{C}/{}^{12}\text{C}_{\text{std}} = 0.0112372$ .

### 2.4. Lipid extraction and separation

Lipids were extracted using accelerated solvent extraction (Dionex ASE 200) with CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>OH (93/7; v/v). Stainless steel extraction vessels contained 5 g of plant or 30 g of soil material, respectively. Samples were sequentially extracted for 20 min in two steps at 5 × 10<sup>6</sup> Pa and 75 °C and 140 °C, respectively. Extracts were combined thereafter. The detailed extraction scheme has been described previously (Wiesenberg et al., 2004a). Lipids were sequentially separated into eight fractions of different polarity (Wiesenberg et al., 2004a) using heterocompound medium pressure liquid chromatography (H-MPLC) described by Willsch et al. (1997), followed by medium pressure liquid chromatography (MPLC) separation of the neutral fraction as described by Radke et al. (1980). This gave eight lipid fractions, of which the aliphatic hydrocarbon and carboxylic acid fractions were studied in detail. The other fractions were not amenable to compound specific isotope analysis because of the low concentrations of lipids or, in some cases, the required derivatisation would result in problematic isotope fractionation of individual compounds. Volume reduction was performed via a turbo vapouriser (Zymark) or rotary evaporation.

### 2.5. Gas chromatography/mass spectrometry (GC/MS)

Defined amounts of deuteriated standards (d<sub>39</sub>-n-C<sub>20</sub> carboxylic acid, d<sub>50</sub>-n-C<sub>24</sub> alkane) were added to the carboxylic acid and aliphatic hydrocarbon fractions, respectively. Compound identification was performed with a HP 5890 Series II gas chromatograph coupled to a HP 5989A mass spectrometer. Carboxylic acids were methylated with CH<sub>2</sub>N<sub>2</sub>,

aliphatic hydrocarbons being directly amenable to GC.

### 2.6. Compound specific isotope analysis (CSIA)

CSIA of individual aliphatic hydrocarbons and carboxylic acids was carried out under continuous He flow using an Agilent 6890 gas chromatograph coupled to a Finnigan GC combustion unit and a Finnigan DeltaPlusXL mass spectrometer. Ion currents were monitored continuously ( $m/z$  44, 45 and 46). Acids were methylated with  $\text{BF}_3\text{-CH}_3\text{OH}$  (10%, w/w), correction being made for the introduced methyl group. All measurements were carried out at least in triplicate. The averages of the CSIA results used for the calculation of replaced carbon proportions represent the mean of the four (for alkanes) or six (for carboxylic acids) most abundant compounds ( $\delta_{\text{Malkanes}}$ ;  $\delta_{\text{Macids}}$ ) normalised to the proportion of each compound

$$\delta_{\text{Malkanes}} = (\text{A} \times \delta_{\text{A}}) + (\text{B} \times \delta_{\text{B}}) + (\text{C} \times \delta_{\text{C}}) + (\text{D} \times \delta_{\text{D}}) \quad (2)$$

$$\delta_{\text{Macids}} = (\text{A} \times \delta_{\text{A}}) + (\text{B} \times \delta_{\text{B}}) + (\text{C} \times \delta_{\text{C}}) + (\text{D} \times \delta_{\text{D}}) + (\text{E} \times \delta_{\text{E}}) + (\text{F} \times \delta_{\text{F}}), \quad (3)$$

with A, B, C, D, E, F as the relative proportions of the most abundant compounds and  $\delta_{\text{A}}$ ,  $\delta_{\text{B}}$ ,  $\delta_{\text{C}}$ ,  $\delta_{\text{D}}$ ,  $\delta_{\text{E}}$ ,  $\delta_{\text{F}}$  as their  $\delta^{13}\text{C}$  isotopic values.

### 2.7. Calculation of replaced carbon proportions and turnover times

The introduction of  $\text{CO}_2$  elevated in concentration and isotopically ( $^{13}\text{C}$ ) depleted (FACE) allowed calculation of new carbon proportions in soil originally developed under ambient  $\text{CO}_2$  concentration. Turnover time calculations were based on turnover rates under assumed steady state conditions, i.e. the carbon content (net balance of input and degradation) was constant in the soil (Balesdent and Mariotti, 1996). Steady state conditions could be assumed for the soil from the FACE experiment in Eschikon, because Schneider et al. (2004) had already demonstrated that the biomass productivity was virtually identical for the plants grown under the ambient and elevated  $\text{CO}_2$  concentration on the plots without nitrogen fertilization. Comparable to using a  $\text{C}_4/\text{C}_3$  crop change, after the introduction of FACE conditions, the admixture of the carbon fraction originating from the new vegetation grown

under these conditions ( $F_{\text{FACE}}$ ) can be calculated as follows (Balesdent et al., 1987):

$$F_{\text{FACE}} = \frac{(\delta_{\text{FACEsoil}} - \delta_{\text{AMBIENTsoil}})}{(\delta_{\text{FACEplant}} - \delta_{\text{AMBIENTplant}})}, \quad (4)$$

where  $\delta_{\text{FACEplant}}$  and  $\delta_{\text{AMBIENTplant}}$  are the isotopic signatures of plants grown under FACE or ambient atmospheric conditions, respectively;  $\delta_{\text{FACEsoil}}$  and  $\delta_{\text{AMBIENTsoil}}$  are the isotopic signatures of the soil under FACE and initial soil isotopic signatures at the beginning of the experiment, respectively. Alternatively,  $\delta_{\text{AMBIENTsoil}}$  is taken as equivalent to a reference site kept under ambient conditions (Balesdent and Mariotti, 1996). Due to the fact that plants were harvested each autumn and that the bioproductivity was similar over time (Schneider et al., 2004), it can be assumed that the isotopic labelling of lipids and bulk carbon of the plant biomass sampled after the vegetation period should have been virtually identical throughout the duration of the experiment. This justifies the applicability of the plant and soil samples from different years for the turnover time determinations. The residual fraction of carbon derived from plants grown under ambient conditions ( $F_{\text{AMBIENT}}$ ) in the soil under FACE conditions can be expressed as

$$F_{\text{AMBIENT}} = F_{\text{AMBIENT } t_0} - F_{\text{FACE}}, \quad (5)$$

with  $F_{\text{AMBIENT } t_0}$  as the fraction of initial carbon at the time of conversion ( $t_0$ ) to FACE conditions. Because the plots were managed for several decades with  $\text{C}_3$  crops under ambient atmospheric  $\text{CO}_2$  conditions prior to the FACE experiment, it can be assumed that soil organic carbon (SOC) was completely  $\text{C}_3$ -labelled, without any elevated contribution from  $\text{CO}_2$ -fertilized biomass, so  $F_{\text{AMBIENT } t_0} = 1$ .

Calculation of replaced carbon proportions based on natural stable isotope labelling in soil is widely used to calculate total soil carbon budgets (e.g. Balesdent et al., 1987; Collins et al., 1999; Cayet and Lichtfouse, 2001). Simple assumptions are based on bulk soil carbon turnover with only one carbon pool of uniform turnover rate (e.g. Balesdent et al., 1987). In contrast, models with several carbon pools of different turnover rates exist (e.g. Jenkinson and Rayner, 1977; Huggins et al., 1998; Paul et al., 2001). SOC decomposition in most models is assumed to follow first order kinetics at steady state conditions (Balesdent and Mariotti, 1996; Huggins et al., 1998). The decomposition of

SOC per unit time was introduced as turnover rate equivalent to the decay rate or decomposition rate ( $k$ ), and can be calculated (Huggins et al., 1998; Collins et al., 1999) as

$$k = \ln(F_{\text{AMBIENT}}/F_{\text{AMBIENT } t_0})/(t - t_0), \quad (6)$$

with the remaining  $F_{\text{AMBIENT}}$  in the soil at time  $t$ . Based on this calculation, the turnover time ( $T$ ), used synonymously with the mean residence time (MRT) of organic carbon in soils can be calculated (Huggins et al., 1998; Collins et al., 1999) as

$$T = 1/k. \quad (7)$$

To study the new carbon proportions and turnover times of bulk soil organic matter and individual lipid fractions we applied the above equations to bulk SOC, individual alkanes and individual carboxylic acids using stable isotope signatures ( $\delta^{13}\text{C}$ ) for each compartment.

### 3. Results and discussion

The bulk isotopic ( $\delta^{13}\text{C}$ ) values of plants and soils are presented first, followed by the compound specific isotopic signatures ( $\delta^{13}\text{C}$ ) of carboxylic acids and alkanes in the samples. Based on bulk and compound specific isotope signatures the proportions of replaced carbon are discussed for the soil kept under FACE conditions in comparison to that under ambient atmospheric compositions. The data are then used to calculate turnover times for bulk plant carbon and individual plant lipid fractions.

#### 3.1. Bulk isotopic composition

The  $\delta^{13}\text{C}$  values of *T. repens* and *L. perenne* grown under ambient atmospheric  $\text{CO}_2$  conditions were typical for  $\text{C}_3$  plants, at  $-27\text{‰}$  V-PDB and  $-28\text{‰}$  V-PDB, respectively (Fig. 1). As expected, the same plants grown under elevated  $\text{CO}_2$  concentration and isotopically-depleted conditions revealed significantly lighter isotopic values and were depleted by  $9.2\text{‰}$  and  $9.5\text{‰}$ , respectively (Figs. 1 and 2). The isotopic shift was mainly related to the supply of  $^{13}\text{C}$ -depleted  $\text{CO}_2$  under FACE conditions. Additionally, there might have been an increased isotopic fractionation in the plants as a result of the elevated atmospheric  $\text{CO}_2$  concentration, which was previously found to result in enhanced isotopic fractionation during biosynthesis (Zhao et al., 2001).

Soil derived from the ambient plots showed uniform isotopic signatures under both plants (Fig. 1). The contribution of more biomass from *L. perenne* plants resulted in a stronger isotopic modification of the soil carbon isotopic value in comparison to *T. repens*-cropped soil under FACE conditions (Figs. 1 and 2).

#### 3.2. Carboxylic acids

In comparison to the bulk isotopic values, compound specific values for individual acids of plants from ambient plots were depleted by ca.  $7\text{‰}$  (Fig. 1), in agreement with results reporting a similar depletion for wax lipids of numerous plants (Collister et al., 1994; Lockheart et al., 1997; Ballentine et al., 1998; Conte et al., 2003; Wiesenberg et al., 2004b; Rommerskirchen et al., 2006). In general, the isotopic signatures of individual acids revealed marginal variability, but with increasing chain length, lighter isotopic values were reported more often (Conte et al., 2003). Unsaturated  $\text{C}_{18}$  acids were characterized by heavier isotopic values than most other acids. This may be related to their origin as primary lipids in epicuticular wax, whereas other fatty acids biosynthesized via different metabolic pathways might exhibit deviating  $^{13}\text{C}$  fractionation. Plants grown under FACE conditions revealed the same minor variations in isotopic signature. When comparing short chain to long chain acids, the isotopic difference for plants grown under ambient vs. FACE conditions showed less variation for *L. perenne* than for *T. repens*. A different plant habit and variable proportions of plant internal vs. epicuticular wax may account for the difference. The isotopic shift for acids paralleled that of bulk values under FACE vs. ambient conditions. Short chain acids are major components of cuticular waxes and reveal a biosynthetically induced constant offset against bulk tissue (Hayes, 2001). In contrast to plant biomass, the soil isotopic values were different for long ( $> \text{C}_{19}$ ) and short chain ( $\leq \text{C}_{19}$ ) acids (Fig. 1). This can be attributed to the contribution of microbially-derived organic material to short chain acids in soil (e.g. Boeschker and Middelburg, 2002; Bossio et al., 1998; Lichtfouse et al., 1995), resulting in isotopic values different from those of long chain acids in soils, which are almost exclusively plant-derived (Wiesenberg et al., 2004b, 2006). Under FACE conditions, a similar separation in the isotopic values between long and short chain acids was observed for both soils. While

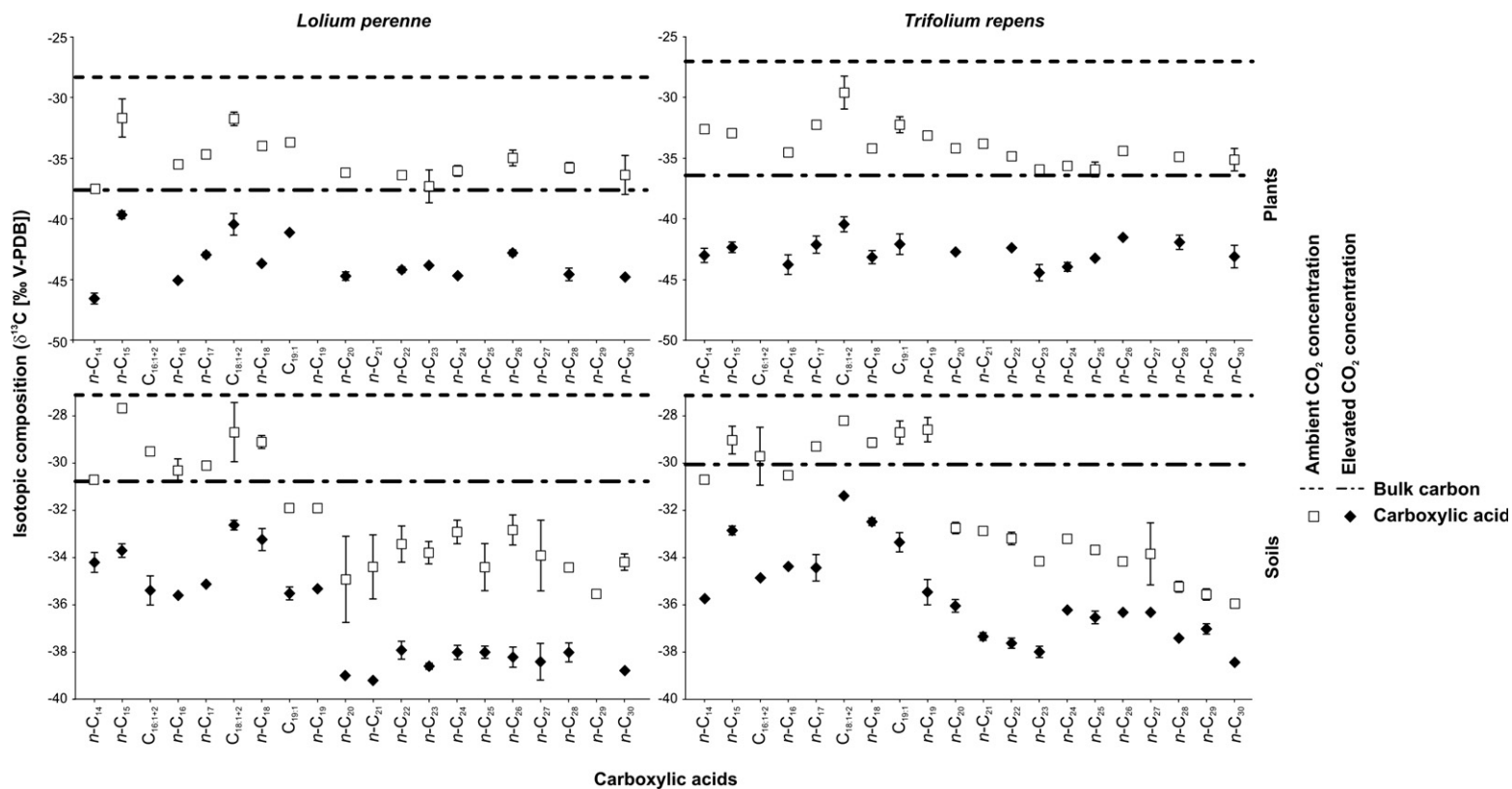


Fig. 1. Isotopic composition ( $\delta^{13}\text{C}$ ) of bulk samples and individual carboxylic acids for *L. perenne* and *T. repens* plants and soil under ambient and elevated  $\text{CO}_2$  conditions. Error bars indicate standard deviation for at least three compound specific isotope determinations of individual lipids.

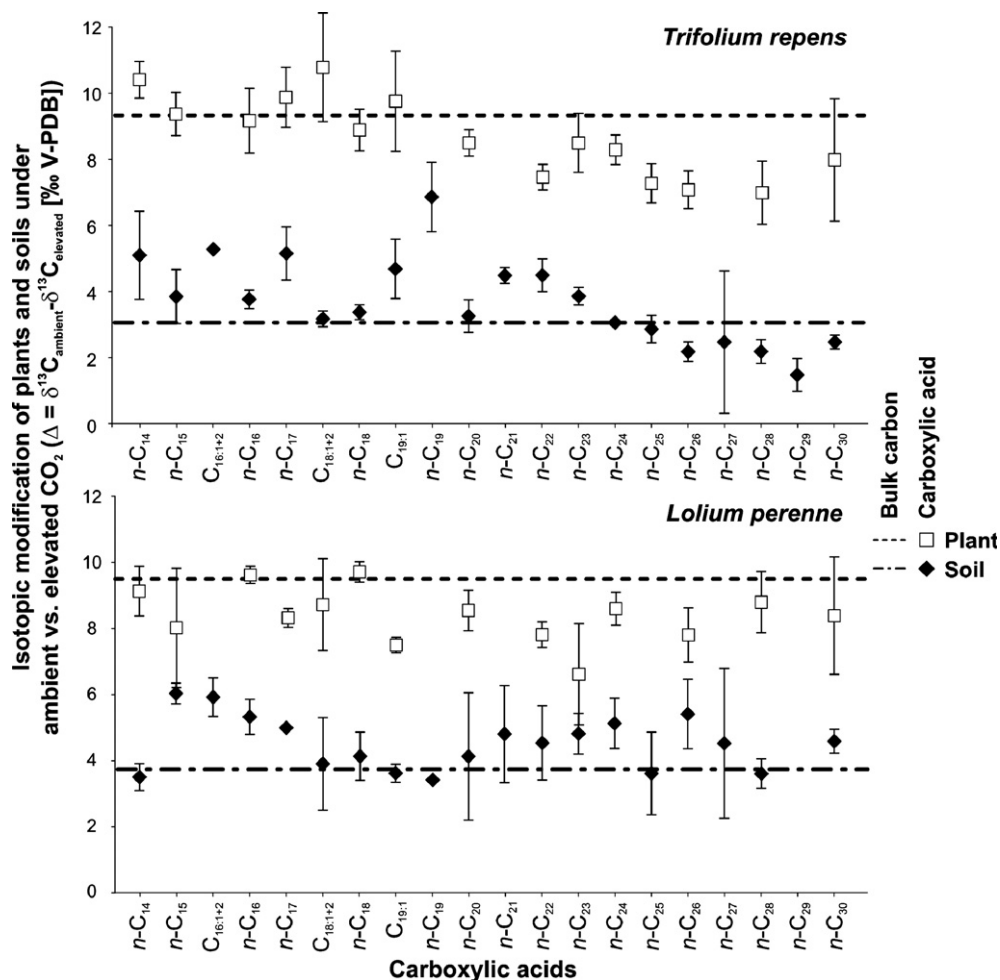


Fig. 2. Isotopic differences in plant and soil carboxylic acids between ambient and elevated  $\text{CO}_2$  conditions. Error bars indicate standard deviation for at least three compound specific isotope determinations of individual lipids.

for *L. perenne* soil a uniform isotopic depletion of acids under FACE conditions occurred, long chain acids in the soil under *T. repens* received a lower isotopic labelling (Fig. 2). This is attributed to a more effective turnover of long chain acids under grass than under clover.

### 3.3. Alkanes

In comparison with the acids, the isotopic values of the alkanes showed greater variation (Fig. 3). Short chain alkane homologues of *L. perenne* were less depleted in  $^{13}\text{C}$  than long chain homologues. This general feature of alkane isotopic values is in agreement with literature results (Conte et al., 2003; Bi et al., 2005) and consistent for plants grown under ambient and elevated  $\text{CO}_2$  conditions. It thus seems to be related to biosynthetic isotopic fraction-

ation processes being more prevalent in long chain homologues. For *L. perenne*, the differences between individual alkane and bulk carbon isotope values for plants grown under ambient vs. FACE conditions were similar to differences between individual acid and bulk carbon isotope values for plants grown under ambient vs. FACE conditions in the range of 8–9‰. The slightly heavier isotopic values for even numbered short chain alkanes in comparison to their odd numbered counterparts (Fig. 4) might be caused by the low abundance of the even numbered compounds and thus greater uncertainty in determining compound specific isotope values. For *T. repens*, the even numbered short chain alkanes showed slightly lighter isotopic values. Again, this might be attributed to the low concentration, as samples from the FACE experiment did not reveal any isotopic discrimination. As for

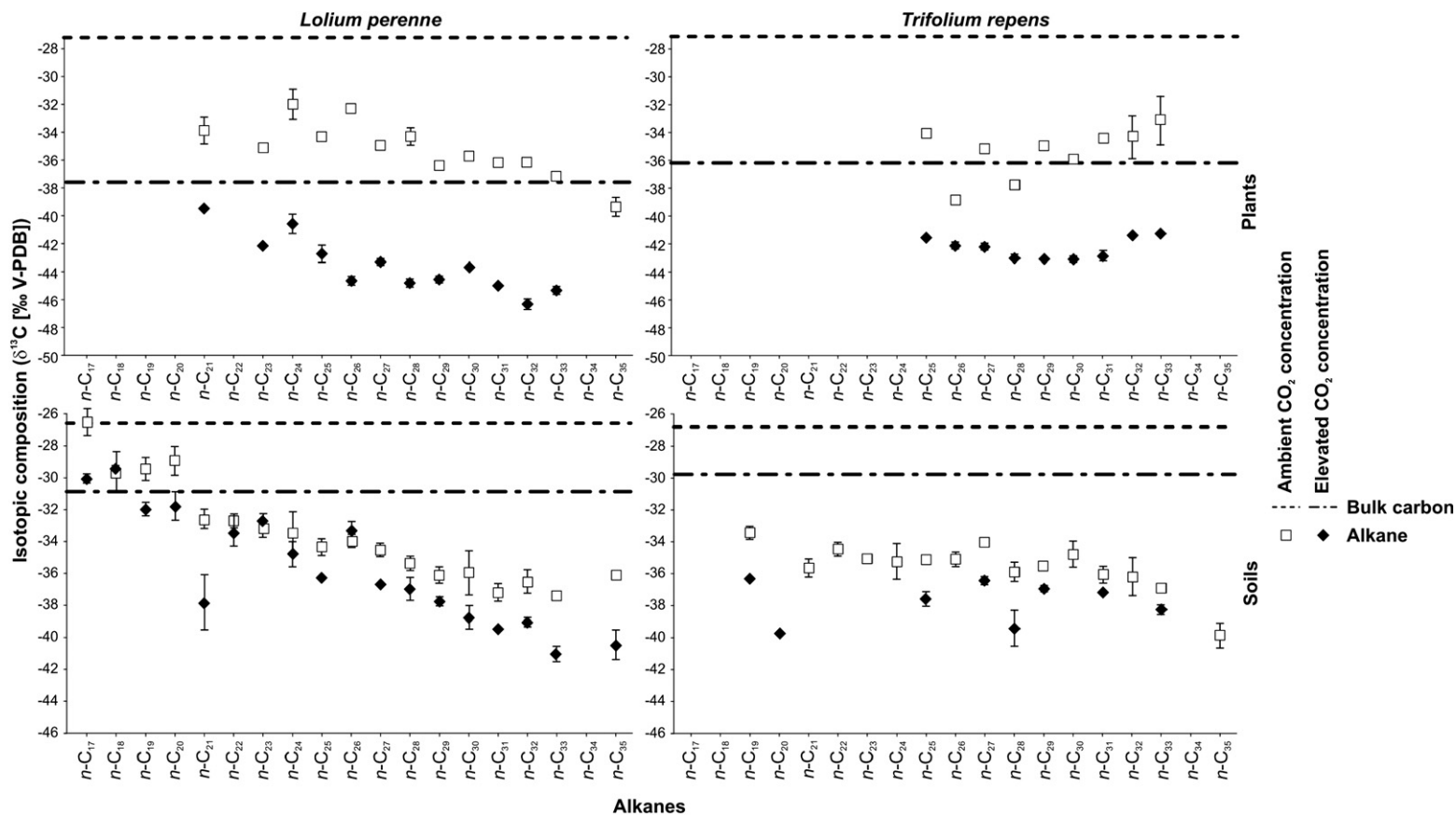


Fig. 3. Isotopic composition ( $\delta^{13}\text{C}$ ) of bulk samples and individual *n*-alkanes for *L. perenne* and *T. repens* plants and soil under ambient and elevated CO<sub>2</sub> conditions. Error bars indicate standard deviation for at least three compound specific isotope determinations of individual lipids.

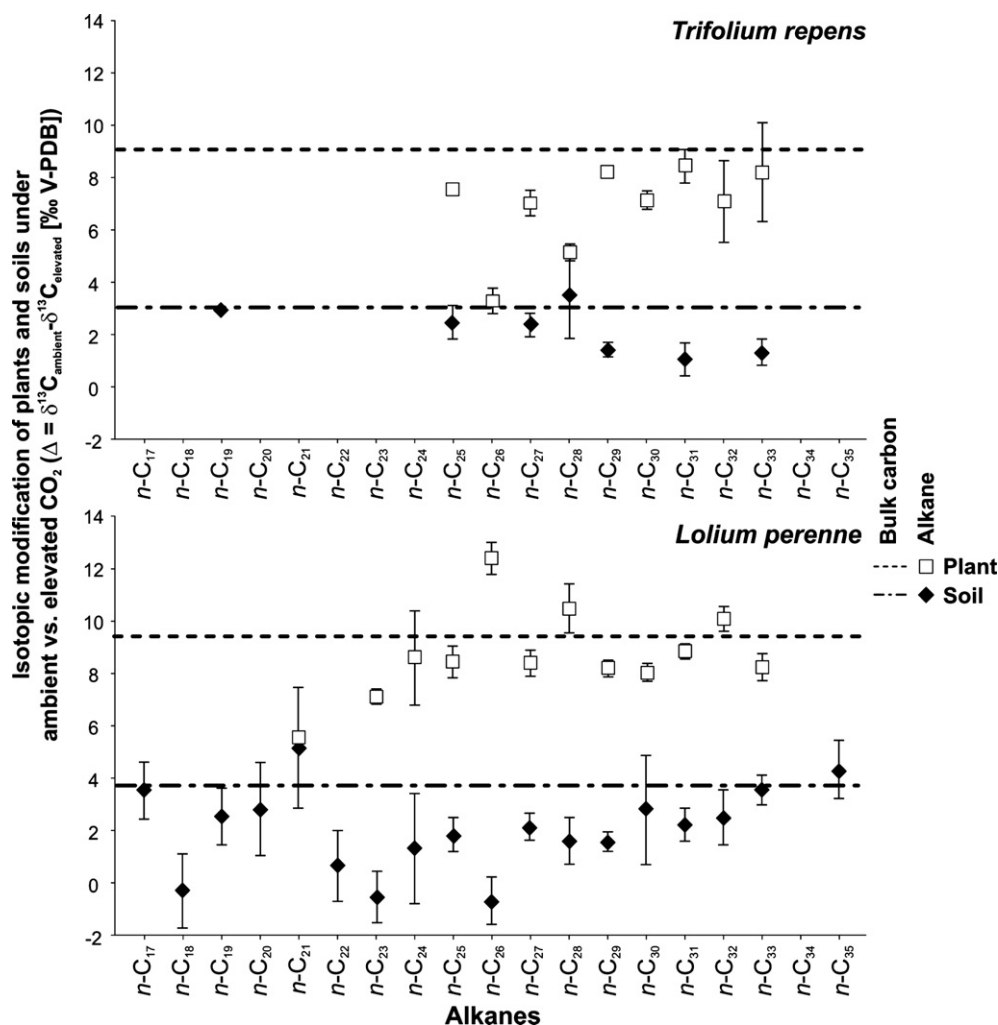


Fig. 4. Isotopic differences in *n*-alkanes from soils and plants grown under ambient and elevated CO<sub>2</sub> conditions. Error bars indicate standard deviation for at least three compound-specific isotope determinations of individual lipids.

*L. perenne*, no isotopic fractionation with increasing chain length was observed for *T. repens* alkanes. The isotopic difference for alkanes derived from plants grown under ambient vs. elevated CO<sub>2</sub> conditions was lower than the isotopic difference for bulk carbon (Fig. 4) and compound specific acid isotopic signatures, especially of short chain homologues, under ambient vs. elevated CO<sub>2</sub> concentrations.

For soil under *L. perenne* the correlation of increasing chain length and decreasing  $\delta^{13}\text{C}$  value was obvious (Fig. 3). The isotopic differences between soil alkanes under ambient vs. FACE conditions varied around 2‰, lower than the difference in the bulk isotopes (Fig. 4), suggesting a slower turnover of alkanes in comparison to that of bulk

carbon. Similar to signatures of the plant biomass, for soil alkanes under *T. repens* no correlation between chain length and isotopic composition could be observed. The isotopic differences for alkanes under ambient vs. elevated CO<sub>2</sub>-treated soil were almost in the range of the bulk carbon differences or slightly lower. Hence, for soil alkanes under *L. perenne* and *T. repens*, lower isotopic differences in comparison to bulk carbon indicated a slower replacement of these compounds in the soil. The small difference in isotopic value between soil alkanes under ambient vs. elevated CO<sub>2</sub> conditions can be attributed to: (i) a low contribution and thus slow replacement of alkanes derived from fresh plant biomass and (ii) the contribution of soil

alkanes from different sources, including deposition of atmospheric particles derived from incomplete combustion processes, as discussed as being relevant for different soils (Rethemeyer et al., 2004; Wiesenberg et al., 2004a,b) or aerosols (Conte et al., 2003).

### 3.4. Turnover of lipids

Weighted averages of the isotopic values of most abundant *n*-alkanes and most abundant long chain acids were calculated on the basis of formulae (2) and (3), using the mean isotopic values normalized to relative abundance (Table 1) and are shown in Fig. 5. Plants grown under ambient conditions were characterized by uniform isotopic values for *n*-alkanes and carboxylic acids, which argues for a similar biosynthetic pathway for both lipid fractions, i.e. decarboxylation of primary acids led to the formation of *n*-alkane wax lipids (Hayes, 2001). Under FACE conditions, *n*-alkane and carboxylic acid isotopic values are modified to the same extent, i.e. there is no biosynthetic isotope fractionation within the analysed lipid fractions as a result of elevated CO<sub>2</sub> concentration and depleted atmospheric δ<sup>13</sup>C values.

Soil alkanes revealed significantly lower isotopic values than (i) soil carboxylic acids and (ii) plant alkanes. This can be explained by a contribution from compounds derived from different sources to the soil *n*-alkanes. Such alternate sources may include the degradation of other precursor compounds, like e.g. alcohols or esters, carrying a different isotopic signature, to form soil *n*-alkanes, or the contribution of *n*-alkanes from fossil carbon sources. The higher δ<sup>13</sup>C values observed for carboxylic acids argue for a different source for these compounds. Under FACE conditions especially, carboxylic acids showed a significant contribution

of new plant-derived components, reflected in a large isotopic difference, whereas the isotopic differences between *n*-alkanes of ambient and FACE cropped soil were lower for both pasture plants. This indicates a fast incorporation of carboxylic acids into soil organic matter, whereas the replacement of *n*-alkanes seems to be slower.

Previous studies have shown the complexity of turnover of individual phospholipids (Kramer and Gleixner, 2006). However, for free lipids, similar studies under FACE conditions have not been published. Hence, we calculated proportions of replaced carbon for carboxylic acids and *n*-alkanes. This was based on the mean isotopic values of the isotopic signatures of plants and soil under FACE vs. ambient conditions using the fraction of FACE-derived carbon ( $F_{\text{FACE}}$ ) from formula (4) multiplied by 100 to obtain % values

$$\% \text{ replaced carbon} = F_{\text{FACE}} \times 100. \quad (8)$$

For carboxylic acids, between 30 and 75% of the individual components are replaced after ten years of FACE treatment, whereby the variation is less when regarding exclusively the replaced carbon proportions of carboxylic acids in *L. perenne* or *T. repens* cropped soils (Fig. 6). Greater proportions of carboxylic acids have been replaced in the soil under *L. perenne* (42–75%) than in the soil under *T. repens* (30–60%). In particular, short chain ( $\leq C_{16}$ ) and long chain ( $\geq C_{23}$ ) acids have been replaced to a greater degree, whereas the components of intermediate chain length were replaced to equal extents in soil under both pasture plants. This was surprising because of the fact that the carboxylic acids were more abundant in *T. repens*. Probably, larger amounts of biomass, including root biomass were incorporated into *L. perenne*-cropped soil, resulting in faster replacement of fatty acids in soil under

Table 1

Relative contribution (%) of individual long chain acids and alkanes to total *n*-carboxylic acids and *n*-alkanes, respectively (modified after Wiesenberg et al., 2007)

|                   |        |          | Fatty acids               |                           |                           |                           |                           |                           | Alkanes                   |                           |                           |                           |
|-------------------|--------|----------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
|                   |        |          | <i>n</i> -C <sub>20</sub> | <i>n</i> -C <sub>22</sub> | <i>n</i> -C <sub>24</sub> | <i>n</i> -C <sub>26</sub> | <i>n</i> -C <sub>28</sub> | <i>n</i> -C <sub>30</sub> | <i>n</i> -C <sub>27</sub> | <i>n</i> -C <sub>29</sub> | <i>n</i> -C <sub>31</sub> | <i>n</i> -C <sub>33</sub> |
| <i>L. perenne</i> | Plants | Ambient  | 9.9                       | 3.9                       | 3.0                       | 4.6                       | 3.7                       | 2.9                       | 7.0                       | 21.2                      | 35.4                      | 12.1                      |
|                   |        | Elevated | 10.0                      | 3.9                       | 3.1                       | 4.9                       | 3.9                       | 2.7                       | 7.0                       | 21.6                      | 36.5                      | 11.2                      |
|                   | Soil   | Ambient  | 9.2                       | 14.2                      | 14.4                      | 10.8                      | 7.5                       | 5.3                       | 6.9                       | 21.8                      | 32.1                      | 14.2                      |
|                   |        | Elevated | 9.5                       | 20.5                      | 26.3                      | 18.6                      | 10.6                      | 6.8                       | 7.3                       | 23.0                      | 32.1                      | 13.8                      |
| <i>T. repens</i>  | Plants | Ambient  | 7.4                       | 2.5                       | 3.2                       | 1.4                       | 2.4                       | 1.8                       | 9.6                       | 42.3                      | 33.8                      | 1.0                       |
|                   |        | Elevated | 7.7                       | 3.7                       | 5.3                       | 2.7                       | 5.5                       | 3.7                       | 9.9                       | 44.2                      | 32.1                      | 0.3                       |
|                   | Soil   | Ambient  | 6.7                       | 14.1                      | 13.0                      | 11.2                      | 8.4                       | 5.8                       | 8.8                       | 23.4                      | 29.5                      | 11.0                      |
|                   |        | Elevated | 9.2                       | 25.7                      | 26.6                      | 18.1                      | 11.7                      | 7.7                       | 8.0                       | 25.0                      | 30.1                      | 10.8                      |

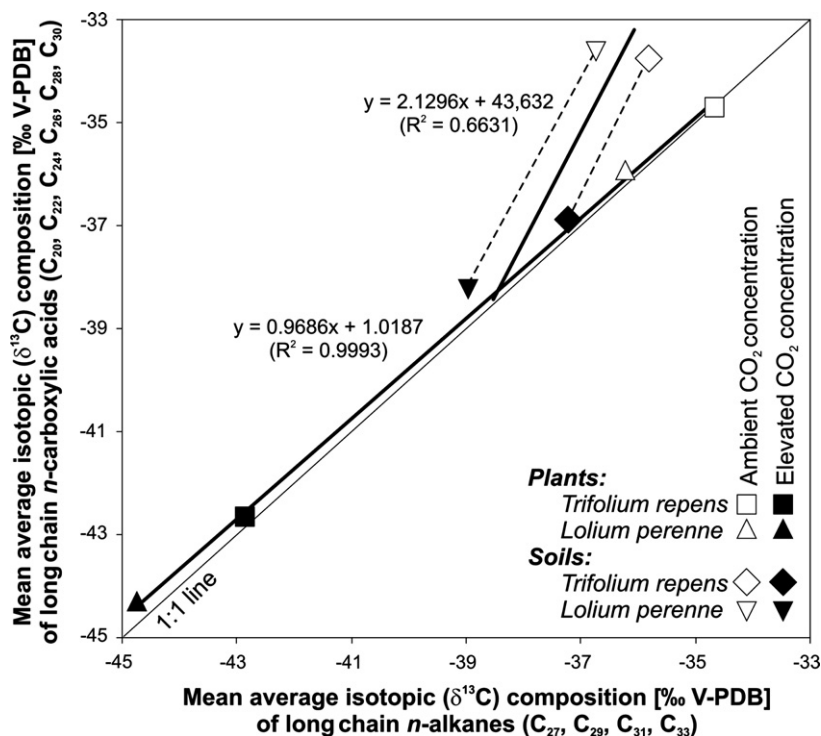


Fig. 5. Isotopic composition of most abundant long chain fatty acids vs. *n*-alkanes.

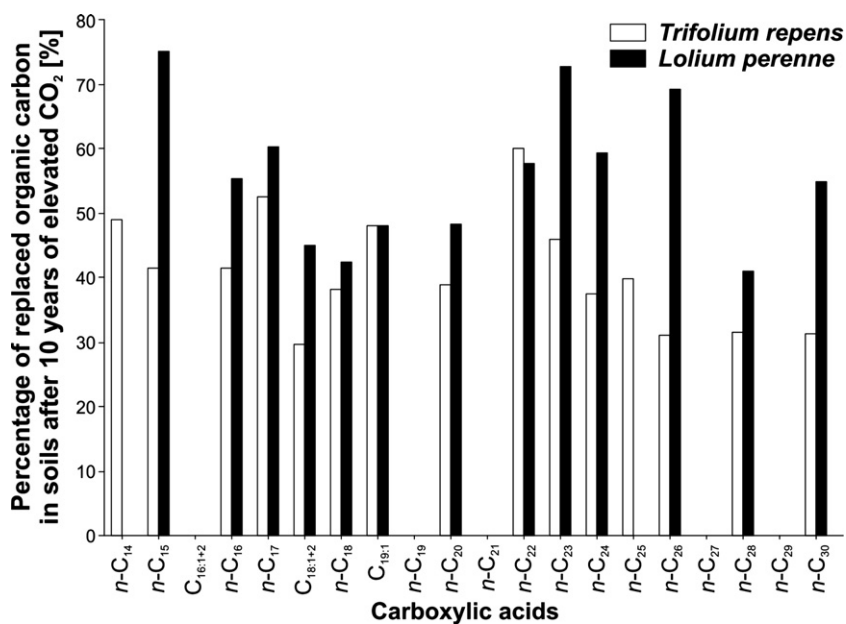


Fig. 6. Calculated replaced carbon proportions of individual carboxylic acids in soils under *T. repens* and *L. perenne*.

*L. perenne*. In contrast, high relative proportions of root biomass and exudates were reported by Six et al. (2001) for *T. repens*.

Alkanes in general were characterized by lower proportions of replaced carbon (13–45%, Fig. 7). As for the acids, larger amounts of long chain

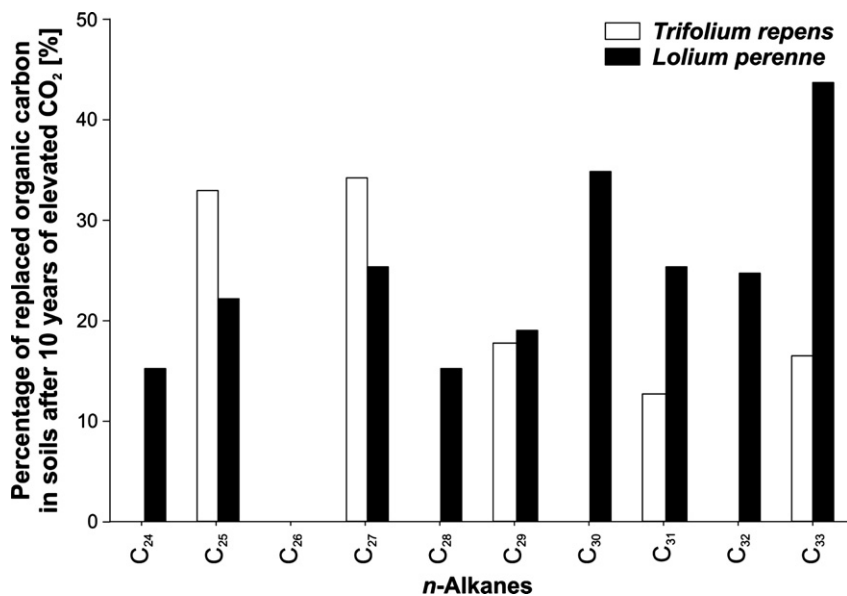


Fig. 7. Calculated replaced carbon proportions of individual *n*-alkanes in soils under *T. repens* and *L. perenne*.

*n*-alkanes ( $\geq C_{30}$ ) were replaced under *L. perenne* than *T. repens*. This enhanced replacement was strongly related to the high contribution of plant-derived compounds to soil *n*-alkanes, which was demonstrated to be of major importance for *L. perenne* (Wiesenberg et al., 2007). Contrastingly, equal or even larger amounts of the shorter chain *n*-alkanes were found to be replaced under *T. repens* than under *L. perenne*.

The heterogeneity of replaced carbon proportions for individual lipids does not argue in favour of turnover determinations on a molecular level based on individual compounds. Hence, a mean turnover calculation for a lipid class or total lipid fraction should be based on weighted average isotope values for the most abundant compounds in order to provide realistic estimates, as shown elsewhere (Wiesenberg et al., 2004b). Weighted aver-

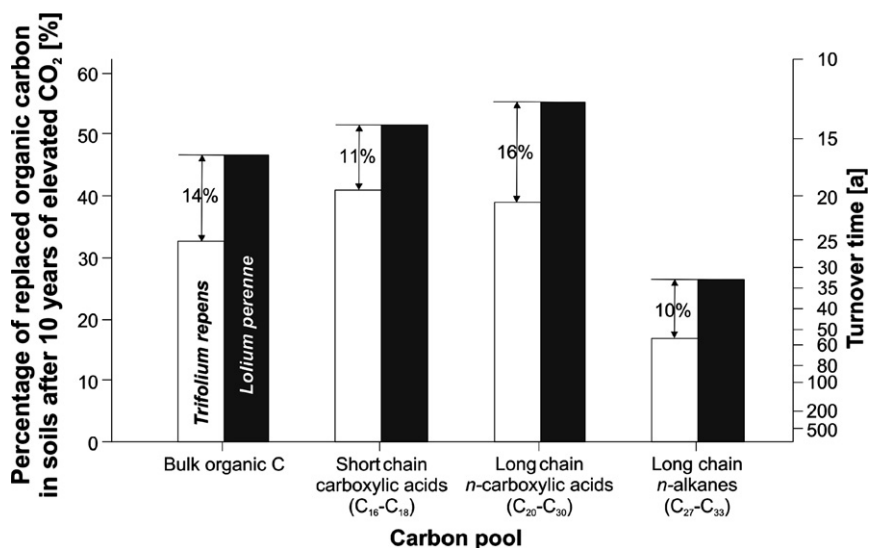


Fig. 8. Calculated replaced carbon proportions and turnover times for bulk carbon and individual lipid pools under *T. repens* and *L. perenne*.

ages of the mean isotopic composition of the most abundant even numbered compounds were used for the determination of the turnover of short ( $C_{16}$ – $C_{18}$ ) and long chain acids ( $C_{20}$ – $C_{30}$ ), as well as odd numbered compounds for the determination of the turnover of long chain alkanes ( $C_{27}$ – $C_{33}$ ). Generally, replaced carbon proportions of all determined lipid pools and bulk carbon were significantly higher (at least 10%), corresponding to lower turnover times in soils under *L. perenne* than under *T. repens*. This must be related to the structure of the incorporated biomass, which has been found to vary on a molecular level for both plants (Wiesenberg et al., 2007). Short chain and long chain acids of pasture plants were found to have a uniform turnover time of 12–21 years (Fig. 8), in agreement with stable carbon analyses for arable soils (Wiesenberg et al., 2004b) and  $^{14}C$  determinations for peaty soil (Bol et al., 1996). Long chain *n*-alkanes were characterized by significantly slower turnover times, between 32 and 60 years, which for *T. repens* pastures was similar or slightly lower than for arable soil (Wiesenberg et al., 2004b). In contrast to previous determinations (Wiesenberg et al., 2004b), bulk carbon showed turnover times for both FACE-treated pasture plots being intermediate between carboxylic acids and alkanes, suggesting that alkanes remain more stable than bulk organic carbon in these experiments. Hence, recalcitrance of alkanes in pasture soils seems to be enhanced in comparison to arable soils, as observed previously only for peaty soil (Bol et al., 1996).

#### 4. Conclusions

Elevated  $CO_2$  and depleted atmospheric  $\delta^{13}C$  values established in a FACE experiment resulted in a uniform depletion in bulk carbon and lipid isotope values of pasture plant biomass. Turnover of lipids was independent of chain length for carboxylic acids, whereas turnover under clover was slower for lipids and bulk carbon than under ryegrass, due to the different quality of the biomass incorporated into the pasture soil. Turnover of selected lipid fractions in pasture soils differed from that of the same lipids in arable and peaty soils. In pasture soil, turnover of lipids and bulk soil organic matter in the order from fast to slow is: carboxylic acids > soil organic carbon > *n*-alkanes. This suggests that *n*-alkanes may contribute part of the slow-reacting carbon fraction in pasture soils, as observed for peaty soil, whereas they are turned over faster in arable soil.

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