

Source and turnover of organic matter in agricultural soils derived from *n*-alkane/*n*-carboxylic acid compositions and C-isotope signatures

Guido L.B. Wiesenberg^a, Jan Schwarzbauer^b, Michael W.I. Schmidt^c,
Lorenz Schwark^{a,*}

^a Department of Geology, University of Cologne, Zùlpicher Str. 49a, 50674 Cologne, Germany

^b Institute of Geology and Geochemistry of Petroleum and Coal, RWTH Aachen, Lochner Str. 4-20, 52056 Aachen, Germany

^c Department of Geography, University of Zurich, Winterthurerstr. 190, 8057 Zurich, Switzerland

Received 20 October 2003; accepted 9 March 2004

(returned to author for revision 8 March 2004)

Available online 30 July 2004

Abstract

Agricultural soils are regarded as one potential sink for atmospheric CO₂ via photosynthetic fixation in plant biomass and subsequent transformation into soil organic matter upon soil diagenesis. The difference in C-isotope signatures of C₃- vs. C₄-plants allows for a natural isotopic labelling of soil organic matter after changes from C₃- to C₄-cropping. In this study, we demonstrate that isotopic shifts are paralleled by molecular signatures of C₃- vs. C₄-crop alkyl lipids. Turnover times vary significantly, based on cropping techniques. For grain-maize cropped soils at steady state average turnover times of 40 years for bulk SOC, 35 years for *n*-alkanes and 21 years for *n*-carboxylic acids were determined. Turnover times for silage-maize cropped soil at steady state were on average 250 years for bulk SOC, 60 years for *n*-alkanes and 49 years for *n*-carboxylic acids. Turnover times reported here for silage-maize cropped soils may be taken as maximum values only, because they derive from a single trial, which was affected by addition of anthropogenic refractory carbon. Discrimination of input from various plant parts (roots, stems and leaves) based on bulk C-isotopes is not feasible but can easily be achieved using compositions of carboxylic acids, especially the ratio of *n*-C₂₄ vs. *n*-C₂₂₊₂₆ and their respective C-isotope values. This enables delineation of the influence of different cropping techniques, e.g., silage- or grain-maize, on carbon storage in soils. Admixture of external sources of organic matter to the soil organic carbon pool of an urban site in Halle, Germany was identified based on alkyl lipid distributions. Nearby lignite mining was identified as a source for non-crop-derived alkyl lipids, primarily based on the elevated *n*-C₂₆-carboxylic acid content and heavier carbon isotopic signatures.

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1. Introduction

Increasing amounts of anthropogenic emissions of CO₂ into the atmosphere have led to an intensive de-

bate about the potential environmental consequences and have initiated activities as defined in the Kyoto Protocol of the United Nations Organization (IGBP, 1998; Prentice et al., 2001). Controversial discussions demonstrate the need for an improved knowledge of the CO₂ sequestration processes. In particular, a better quantification of sources and sinks of CO₂ and of turnover-times of carbon in the geobiosphere is

* Corresponding author. Tel.: +49-221-470-2542; fax: +49-221-470-5149.

E-mail address: lorenz.schwark@uni-koeln.de (L. Schwark).

required. Soils are regarded as one potential sink for atmospheric CO₂ via photosynthetic fixation in plant biomass, which is then transformed into soil organic matter upon soil diagenesis. Soil organic matter studies intend to differentiate whether soils act as sources of or sinks for CO₂ and to unravel the incorporation and stabilisation processes of plant biomass (Kögel-Knabner, 2002). Recent studies report very controversial results. Nieder and Richter (2000) showed an enrichment of carbon in soils over the last 30 years within Germany as a consequence of the cropping and fertilization techniques applied. In contrast, Janssens et al. (2003) on a European scale described, that agricultural soils export carbon into the atmosphere.

With our analyses we try to show, that turnover of plant to soil biomass strongly depends on cropping procedures, even on a molecular level, and that proper planning and crop management might offer significant potential for controlled sequestration of atmospheric CO₂ into soil organic matter. Long-term field experiments are of great value for such analytical approaches, particularly if well-documented crop changes from C₃- to C₄-monocultures occurred (Balesdent et al., 1988; Gregorich et al., 1996a; Liang et al., 1998; Collins et al., 1999; Fortuna et al., 2003). These experiments use the differences in isotope fractionation within C₃- and C₄-plants during photosynthesis (e.g., O'Leary, 1981; Hayes, 1993).

It is well known that lipids constitute a major part of the organic components of fresh plant materials and soils (e.g., Gregorich et al., 1996b). They play an important role in the incorporation of plant material into soil organic carbon (SOC; Kögel-Knabner, 2002) and contain several diagnostic markers for source apportionment and turnover rate determinations. Most molecular studies of agricultural soils utilized single lipid fractions (Lichtfouse et al., 1994, 1997, 1998), or alternatively total lipid extracts were investigated (e.g., van Bergen et al., 1998). Parallel analyses of several lipid fractions obtained from SOM in agricultural soils are still scarce (Stevenson, 1994; Bull et al., 1998), whereas numerous studies of molecular composition of SOM and plant litter in forest soils and peats exist (Jambu et al., 1991, 1993; Amblès et al., 1993, 1994; Almendros et al., 1996; Bol et al., 1996; Marseille et al., 1999). Furthermore, in the existing studies usually only one plant part (e.g., leaves, or stems) was used for analyses of transformation of plant residues into SOM (Lichtfouse et al., 1994; van Bergen et al., 1998). Combinations or comparisons of several plant organs for turnover rate measurements as performed by Gregorich et al. (1996b) are still very scarce. However, such differentiated approaches would be necessary for the investigation of the effects of different harvesting techniques like silage- and grain-maize.

Recently, stable carbon isotope analyses have been used in combination with lipid analyses, first as bulk isotopic measurements of total SOC and thereafter as compound-specific isotopic analyses of individual lipids (Lichtfouse and Budzinski, 1995). This combination allows differentiation of compound sources, to assess how fast they are incorporated into soils and calculation of their residence times in the pedosphere.

Until now comparisons between different sites as well as different harvesting techniques have not been available. Different harvesting techniques, like silage- and grain-cropping for maize, lead to variable proportions of shoot versus root biomass incorporation into soils (Anderson, 1988; Bolinder et al., 1997). Molecular signatures may allow discrimination of between shoot and root biomass. For a better understanding of SOC-stabilization molecular and compound-specific isotope data are thus of crucial importance because they allow for an assessment of how different cropping methods may affect CO₂ sequestration rates and soil carbon fluxes.

2. Materials and methods

2.1. Sampling sites

Soils and plants originated from different long-term agricultural trials, two in Germany (Rotthalmünster near Passau and 'Eternal Rye' trial in Halle/Saale) and one in France (Boigneville). From Boigneville only soil samples were available, whereas for German locations plant and soil samples could be obtained. While the site at Halle/Saale is situated in an urban area and thus prone to pollutant input, the Rotthalmünster and Boigneville sites are situated in rural areas. Upon macroscopic inspection soils from the Halle plot were found to contain brick, charcoal and brown coal fragments. Soils from experimental plots Boigneville and Halle were previously described in detail (Bala-bane and Balesdent, 1992; Flessa et al., 2000; Merbach et al., 1999, 2000).

2.2. Soils

We investigated the ploughed A horizons (A_{xp}) of eight arable soils of three sampling sites (Table 1). From all sampling sites monocultures of C₄-crops [maize, *Zea mays* (L.)] were introduced on previously C₃-cropped (wheat, *Triticum aestivum* (L.); or rye, *Secale cereale* (L.)) soils. For all plots a reference site was kept under C₃-monoculture.

Investigation of differences in cropping techniques comprised a major objective of this study. Grain cropping leaves all biomass except for grains on the plots and leads to a high biomass incorporation. In contrast upon silage cropping, most of the above-ground biomass is

Table 1
Soil characteristics

Locality	Sampling year	Soil type ^a	Mono-culture cropping	Years after maize introduction	Horizon	Depth (cm)	TOC ^b (g kg ⁻¹)	TN ^c (g kg ⁻¹)
Boigneville	1993	Dystric Cambisol	Wheat		Ap	0–20	11.6	1.0
Boigneville	1993	Dystric Cambisol	Grain-maize	23	Ap	0–20	9.8	0.9
Halle	2000	Haplic Phaeozem	Rye		Ap	0–25	12.4	1.1
Halle	2000	Haplic Phaeozem	Silage-maize	39	Ap	0–25	11.6	1.4
Halle	2001	Haplic Phaeozem	Grain-maize	0	Ap	0–25	12.3	1.1
Halle	2002	Haplic Phaeozem	Grain-maize	1	Ap	0–25	11.5	0.9
Rotthalmünster	2002	Stagnic Luvisol	Wheat		Ap	0–30	11.3	1.4
Rotthalmünster	2002	Stagnic Luvisol	Grain-maize	23	Ap	0–30	12.5	1.5

^a According to FAO (1994).

^b Total organic carbon.

^c Total nitrogen.

removed, leaving only the lowermost parts of stems (up to 15 cm height) and the complete root biomass on the field.

Two soil samples were taken in 1993 at Boigneville; one permanently cropped with wheat, the other with grain-maize for 23 years (Table 1). Two soils from Rotthalmünster were taken in 2002 with one soil permanently cropped with wheat while the other was cropped with grain-maize for 23 years (Table 1). Soils derived from the 'Eternal Rye' trial in Halle/Saale, were either permanently cropped with rye (reference site), or for the silage-maize plot corn was introduced on a part of the 'Eternal Rye' plot 39 years ago. Additionally, a second part of the 'Eternal Rye' plot was converted to grain-maize cropping in 2001 (Table 1). In addition to the other long-term trials this provides the advantage for recognising short-term effects of carbon incorporation into SOC. Fresh soil samples were stored in a freezer (–27 °C) until further treatment. After freeze-drying (Steris Lyovac GT-2), samples were crushed with a pestle and mortar and dry-sieved over a 2 mm sieve.

2.3. Plants

All samples from fresh plants (rye and maize from Halle as well as wheat and maize from Rotthalmünster) were taken between one and two weeks prior to harvesting. Molecular and isotopic compositions of plants at this stage were assumed to be identical to those of senescent plants when converted to soil organic matter (SOM). For all plants roots, stems and leaves were stored and analysed separately. In addition to fresh plants wheat straw was collected in September from Rotthalmünster wheat cropped soil. Degraded lowermost parts of stems as well as uppermost parts of roots were collected on maize cropped soils in Halle in March of the following year. These samples were analysed to monitor plant degradation in top soils within the first months after senescence. All plant samples were first air dried at ambient temperature and then freeze-dried (Steris Lyovac GT-2).

2.4. Brown coal

As described above the 'Eternal Rye' plot is situated in an urban area. A railway line where brown coal was transported throughout the 1990s runs parallel to the plots. To study the input of brown coal and coke fragments into soils, potential source materials were obtained. A brown coal taken from the main seam of the nearby lignite deposits in Bitterfeld/Beuna and a brown coal briquette from Beuna were analysed.

2.5. Bulk elemental analyses

All soil samples were measured for total organic carbon content (TOC, Table 1) and total nitrogen

content (TN, Table 1). TOC of dried samples was measured after decarbonisation with HCl (10% v/v) using a Leco CS-225 analyser. TN was measured using a Heraeus Vario EL analyser.

All samples were analysed for isotopic carbon composition of the CO₂ obtained by combustion in a continuous flow sample preparation system (Robo Prep-CN). The purified CO₂ from the Robo Prep-CN was analysed with a Europa Scientific Tracer Mass Classic mass spectrometer. Carbon isotope compositions are expressed in permil relative to the Vienna Pee Dee Belenite 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 $^{13}\text{C}/^{12}\text{C}_{\text{std}} = 0.0112372$.

2.6. Lipid extraction and separation

The complete extraction and separation procedure for soil lipids was previously described in detail (Wiesenberg et al., 2004). For the extraction of soil lipids, an accelerated solvent extractor (Dionex ASE 200) was used. Stainless-steel extraction vessels were filled with 30 g samples of dried soil or with a maximum of 10 g dried plant material or brown coal. For soil samples 3–5 ASE vessels were extracted and extracts combined thereafter. Free lipids were extracted with dichloromethane/methanol (93/7; v/v) at 5×10^6 Pa and a temperature of 75 °C. The heating phase was 5 min and static extraction time was 20 min. Extraction was then repeated under identical conditions except for a higher temperature (140 °C) and both extracts were combined. Rye and silage-maize cropped soils from Halle were extracted, separated and analysed in duplicate, named sample set *A* and *B*.

Total lipids were sequentially separated into eight fractions of different polarity (Wiesenberg et al., 2004). A hetero-compound medium-pressure liquid chromatography separation (H-MPLC) described by Willsch et al. (1997) yielded 6 fractions: (i) a low polarity fraction containing aliphatic and aromatic hydrocarbons as well as acyclic ketones; (ii) an intermediate polarity fraction comprising straight-chain and branched alcohols and sterols; (iii) a carboxylic acid fraction; (iv) a fraction of organic bases; (v) a high polarity and/or HMW-fraction containing very long-chain wax esters and (vi) a polar fraction of still undefined content. The low polarity fraction was then re-chromatographed using the medium-pressure liquid chromatography separation scheme (MPLC) described by Radke et al. (1980) to yield three additional fractions: (i) aliphatic hydrocarbons, (ii) aromatic hydrocarbons, and (iii) low polarity hetero-compounds. Volume reduction was performed via a turbo vapouriser (ZyMark) or rotary evaporation.

2.7. Analysis using gas chromatography–mass spectrometry

For identification and quantification, defined amounts of deuteriated standards (*d*_{50-*n*}-C₂₄ alkane, *d*_{39-*n*}-C₂₀ carboxylic acid) were added to the corresponding MPLC and H-MPLC lipid fractions. Compound identification was performed on a HP 5890 Series II gas chromatograph coupled to a HP 5989A mass spectrometer. For quantification, a HP 5890 Series II GC equipped with a flame ionisation detector (FID) was used. Carboxylic acids were derivatised with diazomethane and detected as methyl esters.

2.8. Compound-specific isotope analysis

Isotopic analysis of individual aliphatic hydrocarbons and carboxylic acids was carried out under a continuous helium 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). Carboxylic acids were derivatised with BF₃-methanol (10%, w/w) and corrected for the isotopic signature of the introduced methyl group. All measurements were done at least in triplicate. The displayed compound-specific isotope analyses (CSIA) results represent the mean of the three most abundant compounds (δ_M) normalised to the proportion of each compound:

$$\delta_M = (A \times \delta_A) + (B \times \delta_B) + (C \times \delta_C), \quad (2)$$

with *A*, *B*, *C* as the relative proportions of the three most abundant compounds and δ_A , δ_B , δ_C as the $\delta^{13}\text{C}$ isotope values of the most abundant compounds.

2.9. Calculating new maize proportions and turnover times

The introduction of continuous C₄-cropping on previously exclusively C₃-cropped soils allows for calculation of new C₄-proportions in originally C₃-marked soils. Turnover time calculations are based on turnover rates under assumed steady state conditions, i.e., the carbon content (net balance of input and degradation) is constant in the soils (Balesdent and Mariotti, 1996). After C₄-crop introduction on C₃-cropped soils the admixture of the carbon fraction originating from the new C₄-vegetation (*F*_{C4}) can be calculated as follows (Balesdent et al., 1987):

$$F_{C4} = (\delta_{C4\text{soil}} - \delta_{C3\text{soil}}) / (\delta_{C4\text{plant}} - \delta_{C3\text{plant}}), \quad (3)$$

where $\delta_{C4\text{plant}}$ and $\delta_{C3\text{plant}}$ are the isotopic signatures of C₄- and C₃-plants. $\delta_{C4\text{soil}}$ and $\delta_{C3\text{soil}}$ are the isotopic signatures of the C₄-cropped soil and the original C₃-cropped soil. Alternatively, $\delta_{C3\text{soil}}$ is taken equivalent to a reference site kept under the initial vegetation (Bales-

dent and Mariotti, 1996). The residual fraction of C₃-derived carbon (F_{C_3}) in the C₄-cropped soil can be expressed as

$$F_{C_3} = F_{C_3,0} - F_{C_4}, \quad (4)$$

with $F_{C_3,0}$ as the fraction of C₃-carbon at the time of conversion (t_0) to C₄-cropping. Because all plots presented in this study were managed for several decades with C₃-crops prior to continuous corn cropping, it could be assumed that SOC was completely C₃-labeled (Balesdent and Mariotti, 1996) and thus $F_{C_3,0} = 1$.

Calculations of new C₄-C proportions based on natural stable isotope labelling in soils are 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 (e.g., Balesdent et al., 1987). In contrast models with several carbon pools of different turnover exist (e.g., Jenkinson and Rayner, 1977; Huggins et al., 1998; Paul et al., 2001). SOC decomposition is assumed in most models to follow first-order kinetics at steady state conditions (Balesdent and Mariotti, 1996; Huggins et al., 1998). The decomposition of SOC per time unit 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:

$$k = \ln(F_{C_3}/F_{C_3,0})/(t - t_0), \quad (5)$$

with the remaining F_{C_3} in the soil at time t . Based on this calculation the turnover time (T), which is used synonymously to the mean residence time (MRT) of organic carbon in soils, can be calculated (Huggins et al., 1998; Collins et al., 1999) as:

$$T = 1/k. \quad (6)$$

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

3. Results and discussion

In the following, the molecular composition of soil and plant n -alkanes and n -carboxylic acids is described first. Thereafter, the compound-specific isotope signatures of the most abundant long chain alkyl lipids are presented. Based on bulk and compound-specific isotope ^{13}C -data the proportions of maize-derived carbon into formerly C₃-cropped soils are calculated. These data are then used to calculate turnover times for bulk plant carbon and to the best of our knowledge for the first time for individual plant lipid fractions.

3.1. Aliphatic hydrocarbons

The aliphatic hydrocarbon fraction in all samples mainly consisted of n -alkanes and lower amounts of isoprenoid alkanes (pristane and phytane) and very low amounts of pentacyclic triterpenoids (mainly hopanes). Hopanes are not shown due to low amounts and the low significance of bacterial triterpenoid biomass input into soils (Ries-Kautt and Albrecht, 1989; Bull et al., 1998).

Generally soils from all sites and different croppings showed similar alkane distribution patterns (Fig. 1). All soils were dominated by long-chain, odd-numbered n -alkanes with chain lengths between n -C₂₅ and C₃₅ characteristic for biomass input from grasses (Marseille et al., 1999; Lichtfouse and Budzinski, 1995; van Bergen et al., 1998). In comparison to our analyses, Lichtfouse (1998) found equivalent free n -alkane distribution patterns in maize-cropped soil from Boigneville. The high amounts of n -C₂₉ and n -C₃₁ are related to a distinctive crop input (Lichtfouse et al., 1997) with the maize-cropped soils containing lower portions of n -C₂₉ relative to n -C₃₁. The linear regression observed by Lichtfouse et al. (1994) for n -C₂₇/ n -C₂₉ vs. $^{13}C_{27}-\delta^{13}C_{29}$ proposed as molecular marker for differentiation of wheat vs. maize cropping could not be verified. Soils from Halle contained higher proportions of even-numbered long-chain n -alkanes (n -C₂₆ to n -C₃₄). When compared with n -alkanes from brown coal [Fig. 1(e)] the n -alkane distribution patterns of Halle soils were virtually identical to those of the coal samples. Brown coal differed only marginally from the brown coal briquette. Thus the higher proportions of even-numbered and in part of the odd-numbered n -alkanes in Halle soils must originate from brown coal pollution. Higher proportions of n -alkanes in Boigneville soils with chain length from C₁₆ to C₁₈ were previously related to anthropogenic pollution (Lichtfouse et al., 1997), although a microbial origin cannot be excluded. In Roththalmünster soils n -alkanes with chain length around C₂₀ were slightly enriched. This might be attributed to either a microbial origin (Dinel et al., 1990) or input of root biomass or biodegraded material.

Great differences could be observed between different plants, plant organs and sampling sites (Fig. 2). In all plants above-ground biomass including stems and leaves was dominated either by n -C₃₁ or n -C₂₉. Equivalent distribution patterns for agricultural C₃-grasses were observed by Bianchi and Corbellini (1977) and van Bergen et al. (1998). Our results in general confirmed a n -C₃₁ maximum for C₄-grasses as previously reported (Boom et al., 2002). When investigated for different plant parts, the n -C₃₁ dominance was most pronounced in leaves. n -C₃₃ showed low amounts when compared to n -C₂₉ in C₃-plants and equivalent abundance in maize plants. Degraded above-ground biomass showed an enrichment of alkanes with shorter chain lengths, but

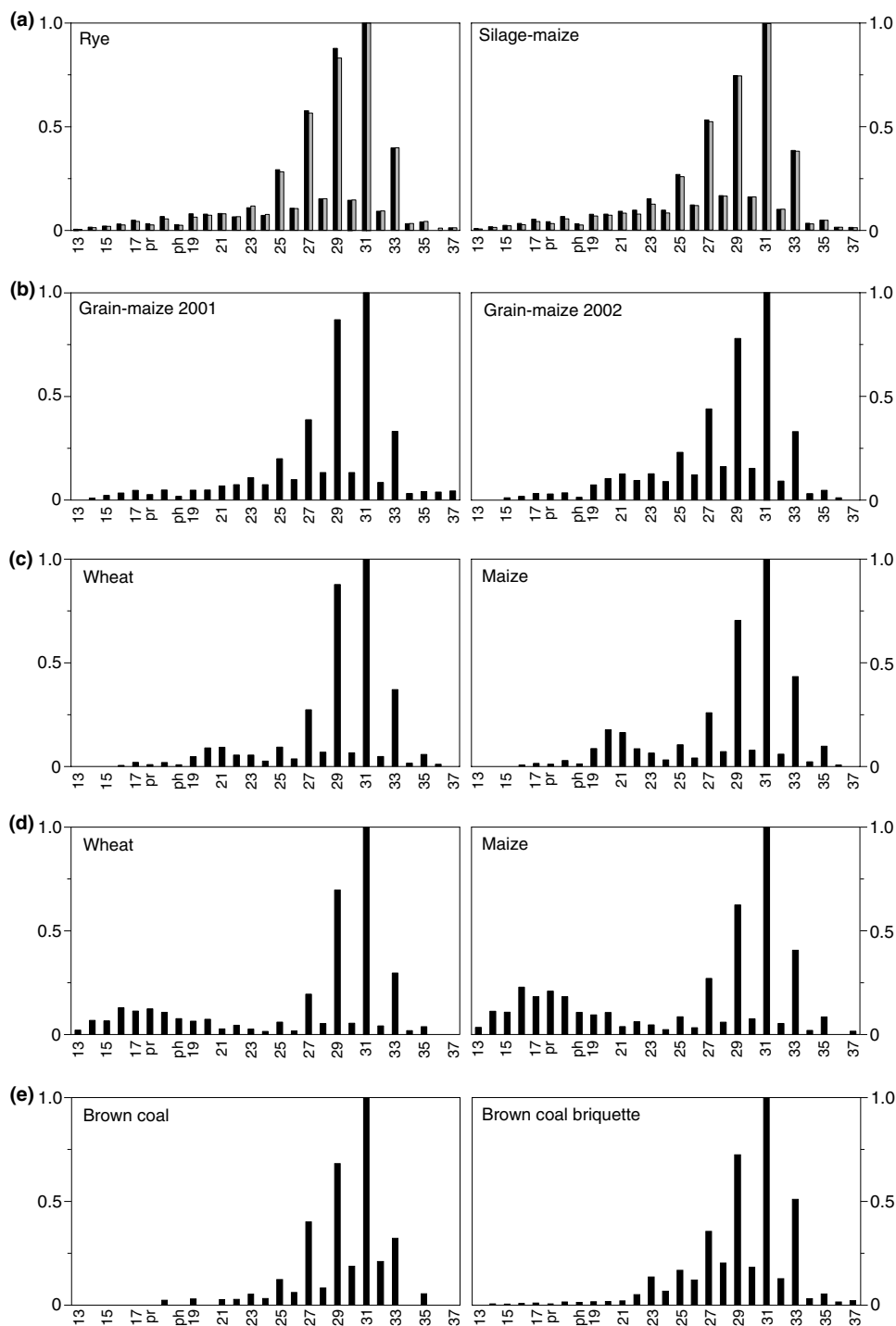


Fig. 1. Relative distributions of *n*-alkanes and isoprenoid-alkanes extracted from soils and brown coals analysed, normalized to the most abundant compound. Numbers beneath bars indicate carbon numbers of *n*-alkanes. Abbreviations for isoprenoid-alkanes: pr = pristane, ph = phytane. Soil samples from top to bottom originated from Halle (a,b), Roththalmünster (c), and Boigneville sites (d). Halle rye and silage-maize cropped soils were analysed in duplicate (black and gray bars). Brown coal and brown coal briquette (e) potentially contributing to Halle soils were derived from the nearby Beuna mine. Soil alkane distribution patterns were dominated by long-chain, odd-numbered *n*-alkanes, derived from terrestrial plant biomass. After introduction of maize plants on previously C3-cropped soils a relative enrichment of *n*-C₃₁ occurred.

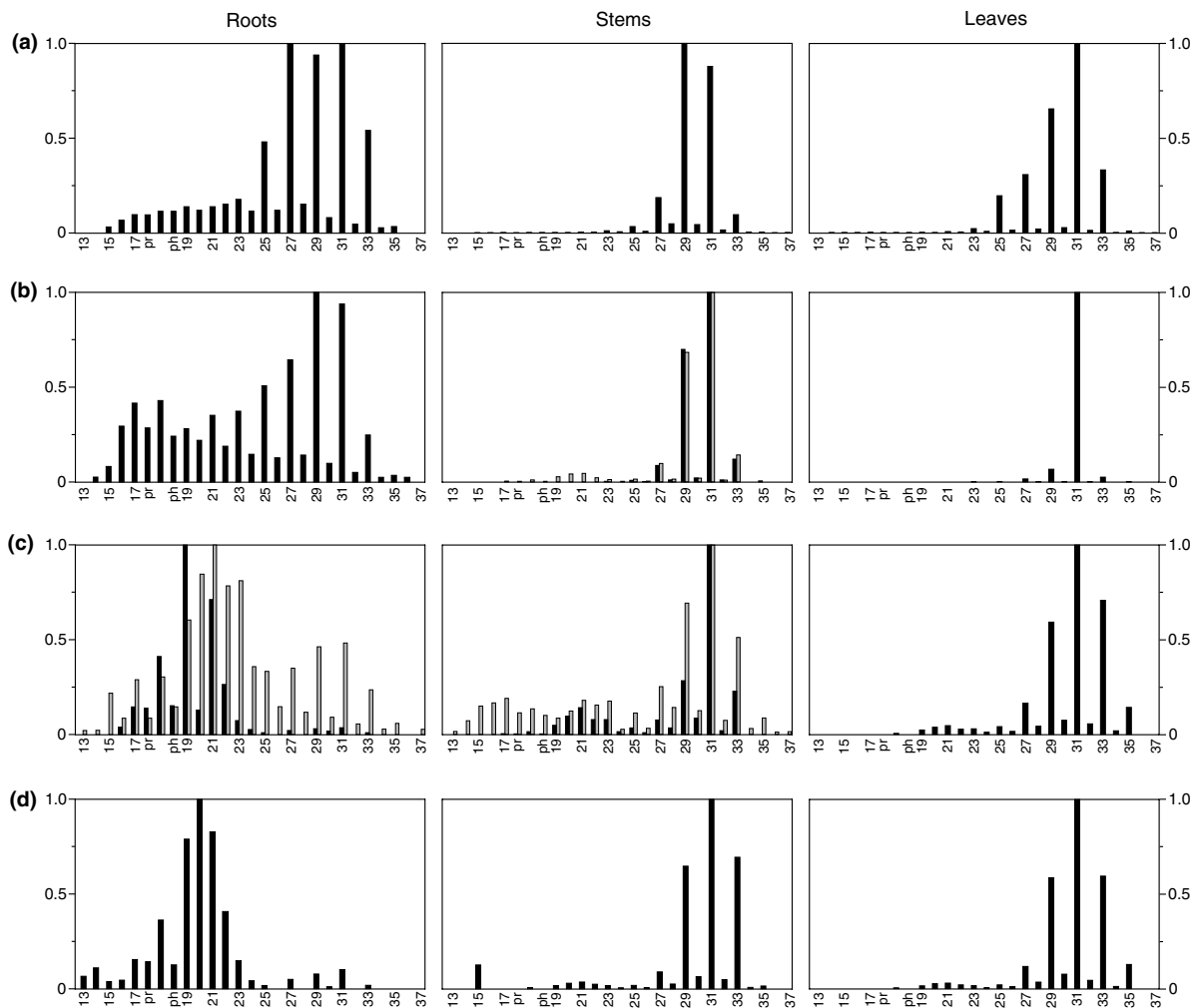


Fig. 2. Alkane distribution patterns normalized to most abundant compound discriminated for different plant parts (roots, stems and leaves). From top to bottom samples derived from Halle rye (a), Rotthalmünster wheat (b), Halle maize (c), and Rotthalmünster maize (d). Black bars represent plant compositions shortly before harvesting. Gray bars represent samples of degraded plant parts left on the Rotthalmünster site until September of the same year (b) and the Halle site March of the following year (c). For abbreviations beneath bars see Fig. 1. Above-ground biomass (stems and leaves) showed relatively similar distribution patterns of alkanes with a dominance of long-chain, odd-numbered *n*-alkanes. While C₃-plants contained higher amounts of *n*-C₂₉, C₄-plants contained higher values of *n*-C₃₃ alkanes. Root biomass consists of high amounts of mid-chain *n*-alkanes (around *n*-C₂₀) and is depleted in long-chain *n*-alkanes when compared with corresponding above-ground biomass. The depletion of long-chain *n*-alkanes in root biomass was most obvious for maize plant biomass. Degraded biomass was characterised by more balanced alkane distribution patterns in comparison to fresh biomass.

the predominance of long-chain alkanes was still present. Root material of C₃-plants contained relatively large amounts of long-chain, odd-numbered *n*-alkanes, as previously observed in other studies (Marseille et al., 1999). In contrast, maize roots were dominated by mid-chain alkanes (maximising around *n*-C₂₀). The presence of higher amounts of mid-chain length *n*-alkanes in Rotthalmünster soils thus seems to result from root biomass input. During degradation the roots from the

Halle site got progressively depleted in mid-chain alkanes.

Within most soils and plant parts odd-numbered *n*-alkanes with a chain-length of C₂₉–C₃₃ were most abundant and their relative distributions are shown in Fig. 3 and Table 2. For wheat a trend from root biomass with nearly identical amounts of *n*-C₂₉ and *n*-C₃₁ to a dominance of more than 90% *n*-C₃₁ in leaves was recognizable (Table 2). For rye and maize plants different

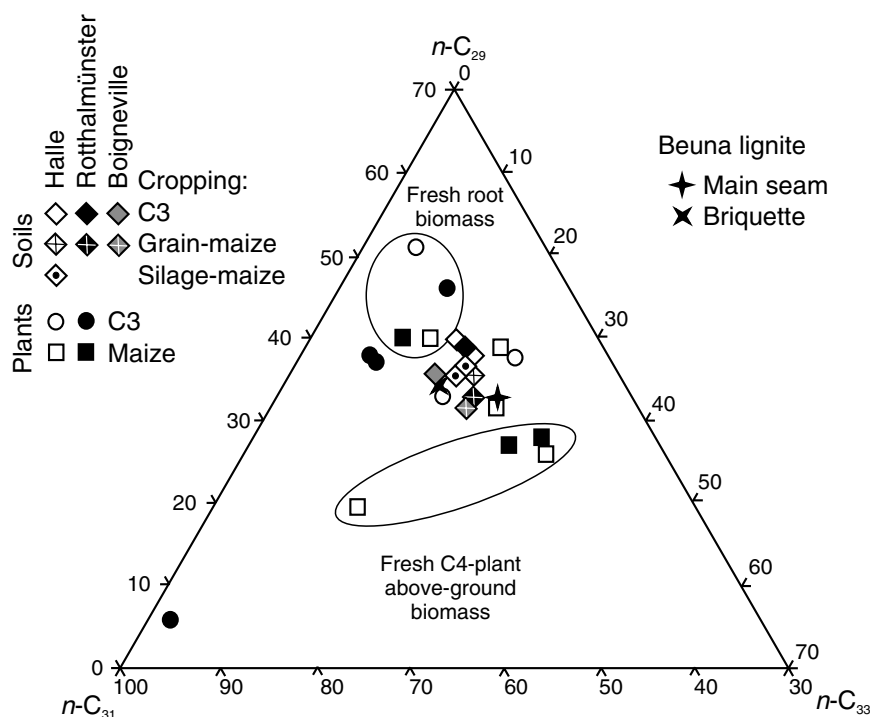


Fig. 3. Ternary diagram showing the relative composition of the three most abundant n -alkanes (C_{29} , C_{31} , C_{33}) in plants and soils. Fresh root biomass of all plants is characterised by low amounts of n - C_{33} and equal amounts of n - C_{29} and n - C_{31} . Above-ground biomass of C4-plants contains the highest amounts of n - C_{33} , while degraded maize biomass has intermediate compositions. All soils and brown coal samples show intermediate compositions. C3-cropped soils are slightly depleted in n - C_{33} , when compared to the corresponding grain-maize cropped soils. Silage-maize cropped soils from Halle show a composition intermediate between brown coal and fresh root biomass.

trends were observed. High amounts of n - C_{29} and n - C_{31} occurred in roots whereas higher contributions of n - C_{33} were characteristic of above-ground biomass. Within all soils similar trends from low (C_3 -cropped soils) to higher amounts of n - C_{33} could be observed after introduction of maize crops (Table 2). This tendency was more pronounced in grain-maize cropped soil and for the Halle site already observed after one year, because of exceptionally high incorporation of above-ground biomass. The Halle silage cropped site revealed an intermediate increase in relative abundance of n - C_{33} (Fig. 3 and Table 2). This was due to the presence of brown coal pollution and the low abundance of above-ground biomass left on the plot after silage harvesting.

3.2. Carboxylic acids

The carboxylic acid fraction consisted mainly of saturated and mono- as well as di-unsaturated straight-chain n -carboxylic acids. Polyunsaturated and branched acids occurred in trace amounts only and are not discussed further.

All soils (Fig. 4) contained high proportions of even-numbered mid-chain carboxylic acids (between n - C_{16} and

n - C_{18}) and their unsaturated counterparts as well as high values of even-numbered long-chain carboxylic acids (n - C_{22+}). For the latter, the n - C_{22} , n - C_{24} and n - C_{26} were the most abundant homologues as previously observed for forest soils (Almendros et al., 1996) and for grassland soils (van Bergen et al., 1998). In Halle soils long-chain acids predominated over mid-chain carboxylic acids. Soils from Rotthalmünster and Boigneville were dominated by carboxylic acids with 16 and/or 18 carbon atoms. These mid-chain compounds could be derived from several sources including fungi, bacteria, algae and higher terrestrial plants. Because they are ubiquitous in living organisms (Boeschker and Middelburg, 2002; Bossio et al., 1998; Lichtfouse et al., 1995a; Marseille et al., 1999) they are not suitable for plant biomass turnover determinations. Higher amounts of long-chain carboxylic acids with a predominance of even carbon numbers are preferentially due to carbon input from terrestrial biomass. Brown coal samples of Beuna [Fig. 4(e)] had compositions similar to soils from the Halle site, characterised by lower amounts of mid-chain carboxylic acids and high amounts of long-chain acids (n - C_{22+}). This served as an additional indication for the high input of brown coal particles into soils at the urban Halle site.

Table 2
Relative abundance of long chain *n*-alkanes and *n*-carboxylic acids as percent of each lipid fraction

Sampling site/sample	Sampling time	<i>n</i> -C ₂₇ alkane (%)	<i>n</i> -C ₂₉ alkane (%)	<i>n</i> -C ₃₁ alkane (%)	<i>n</i> -C ₃₃ alkane (%)	<i>n</i> -C ₂₂ carboxylic acid (%)	<i>n</i> -C ₂₄ carboxylic acid (%)	<i>n</i> -C ₂₆ carboxylic acid (%)
<i>Soils</i>								
Halle								
Rye	2000a	12.9	19.7	22.4	8.9	8.5	12.7	11.7
	2000b	12.8	18.9	22.7	9.0	7.9	12.0	11.5
Silage maize	2000a	11.8	16.6	22.2	8.6	5.8	13.0	10.7
	2000b	12.1	17.3	23.1	8.8	5.5	11.6	10.5
Grain maize	2001	9.4	21.1	24.3	8.0	6.4	11.8	10.6
	2002	10.6	18.9	24.3	8.0	7.1	12.5	9.2
Rotthalmünster								
Wheat	2002	8.2	26.4	30.1	11.1	5.8	7.6	5.0
Grain maize	2002	7.3	19.9	28.4	12.2	5.8	8.4	3.8
Boigneville								
Wheat	1993	5.7	20.5	29.4	8.7	5.3	4.1	1.7
Grain maize	1993	6.4	14.7	23.7	9.6	6.1	8.1	3.1
Brown coal from Beuna								
Main seam		12.1	20.6	30.3	9.7	1.2	6.0	15.0
Briquette		9.1	18.7	25.8	13.1	1.5	8.0	11.1
<i>Plants</i>								
Rye (Halle)								
Roots		17.3	16.3	17.3	9.4	4.9	3.6	3.8
Stems		7.9	42.3	37.2	4.1	7.2	5.4	3.2
Leaves		11.0	23.4	35.7	11.9	5.7	2.2	2.4
Wheat (Rotthalmünster)								
Roots		9.0	13.9	12.1	3.5	2.5	1.4	0.6
Stems		4.4	35.5	50.8	6.1	4.3	2.6	2.5
Leaves		1.4	6.0	89.5	2.3	3.4	1.1	0.7
Straw (mainly stems)		4.5	31.7	46.3	6.6	10.3	5.9	3.3
Maize (Halle)								
Roots	Fresh	0.7	0.9	1.1	0.3	0.6	1.8	0.7
	Degraded	4.4	5.8	6.1	3.0	4.4	12.1	3.6
Stems	Fresh	3.4	12.6	44.4	10.1	2.2	4.6	1.3
	Degraded	5.3	14.5	20.9	10.7	5.2	14.6	4.7
Leaves	Fresh	5.4	19.5	33.0	23.3	1.1	2.9	1.4
Maize (Rotthalmünster)								
Roots		1.1	1.8	2.3	0.4	1.8	4.1	1.6
Stems		3.1	22.5	34.8	24.2	1.6	2.0	0.4
Leaves		4.3	21.4	36.5	21.7	2.1	4.4	1.7

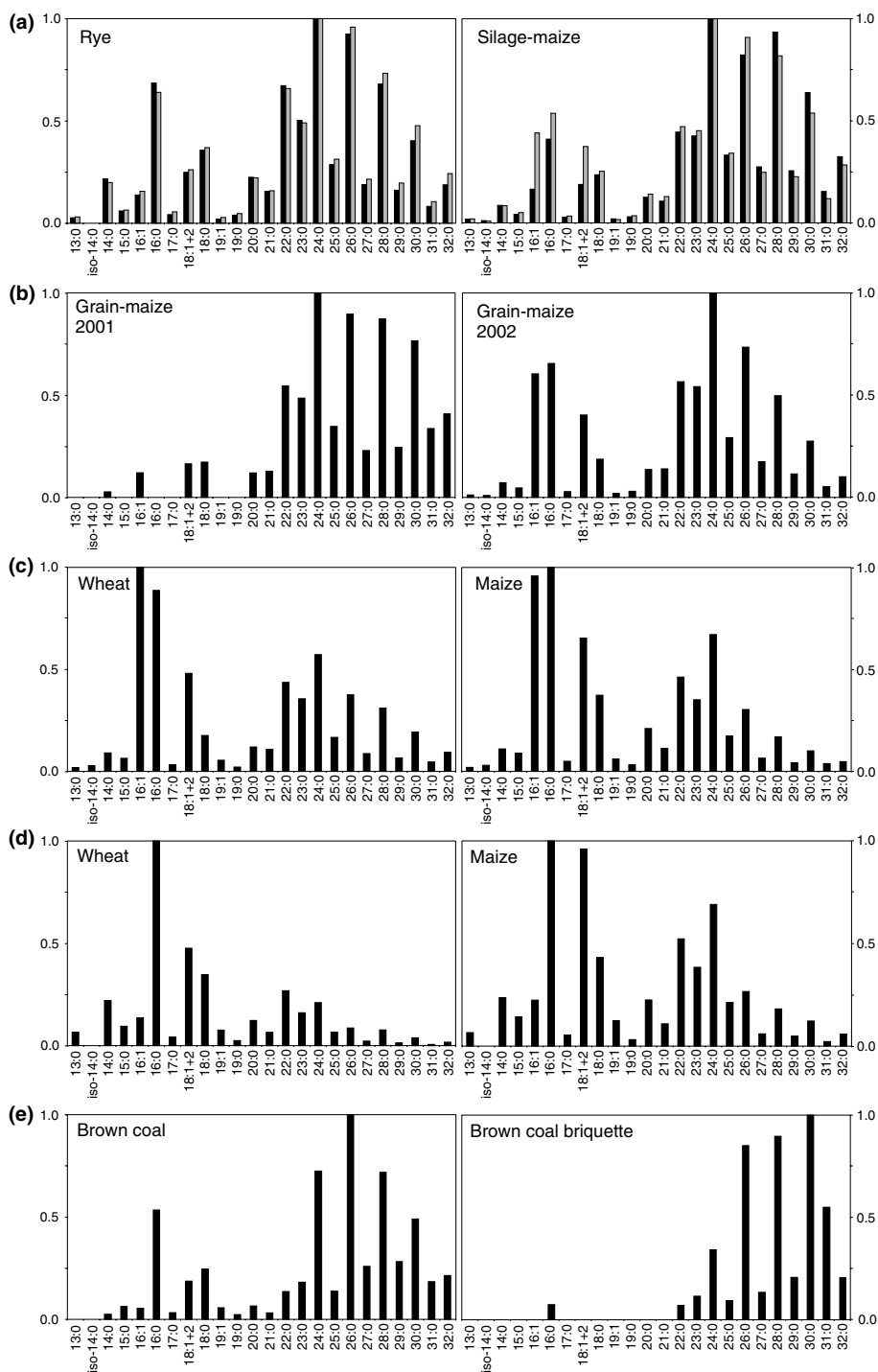


Fig. 4. Relative distributions of carboxylic acids extracted from soils and brown coals analysed, normalized to the most abundant compound. Numbers beneath bars indicate carbon numbers of carboxylic acids and number behind the colon depict the number of double bonds within the molecule. Soils originated from Halle (a,b), Roththalmünster (c) and Boigneville (d) sites with C_3 -reference plots on the left and new introduced maize cropped plots on the right hand. Brown coal and brown coal briquette (e) were derived from the Beuna mine near Halle. Halle silage cropped soils were analysed in duplicate. Soils from Halle are characterised by higher amounts of long-chain carboxylic acids. All other soils contain higher amounts of short-chain (C_{16} and C_{18}) compounds with site-specific signatures. Brown coal samples yield carboxylic acid distribution patterns comparable to Halle soils.

For plant samples, a dominance of carboxylic acids with 16 and/or 18 carbon atoms could be observed. Long-chain acids were present in minor amounts with a slight dominance of even carbon numbers (Fig. 5), which is in agreement with previous studies for plant biomass (Bianchi and Corbellini, 1977; Guil-Guerrero and Rodríguez-García, 1999). In other studies, however, no long-chain carboxylic acids were detected in fresh plant material (van Bergen et al., 1998). C_3 -plants contained more n - C_{16} than maize plants and the highest proportions were found in rye plants with increasing proportions from roots to leaves. Wheat generally yield-

ded higher contents of unsaturated carboxylic acids with 18 carbon atoms. Wheat leaves contained similar proportions of n - C_{16} and $C_{18:1+2}$ carboxylic acids. Maize plants from both sampling sites were dominated by unsaturated C_{18} -carboxylic acids. Higher portions of n - C_{18} and lower contents of n - C_{16} allowed differentiating between maize- and C_3 -plants based on carboxylic acid fingerprints. Degraded plant biomass was depleted in mid-chain and enriched in long-chain carboxylic acids. The presence of even-numbered long-chain acids in all plant parts and in soils, justifies their use as diagnostic indicators for plant biomass input into soils.

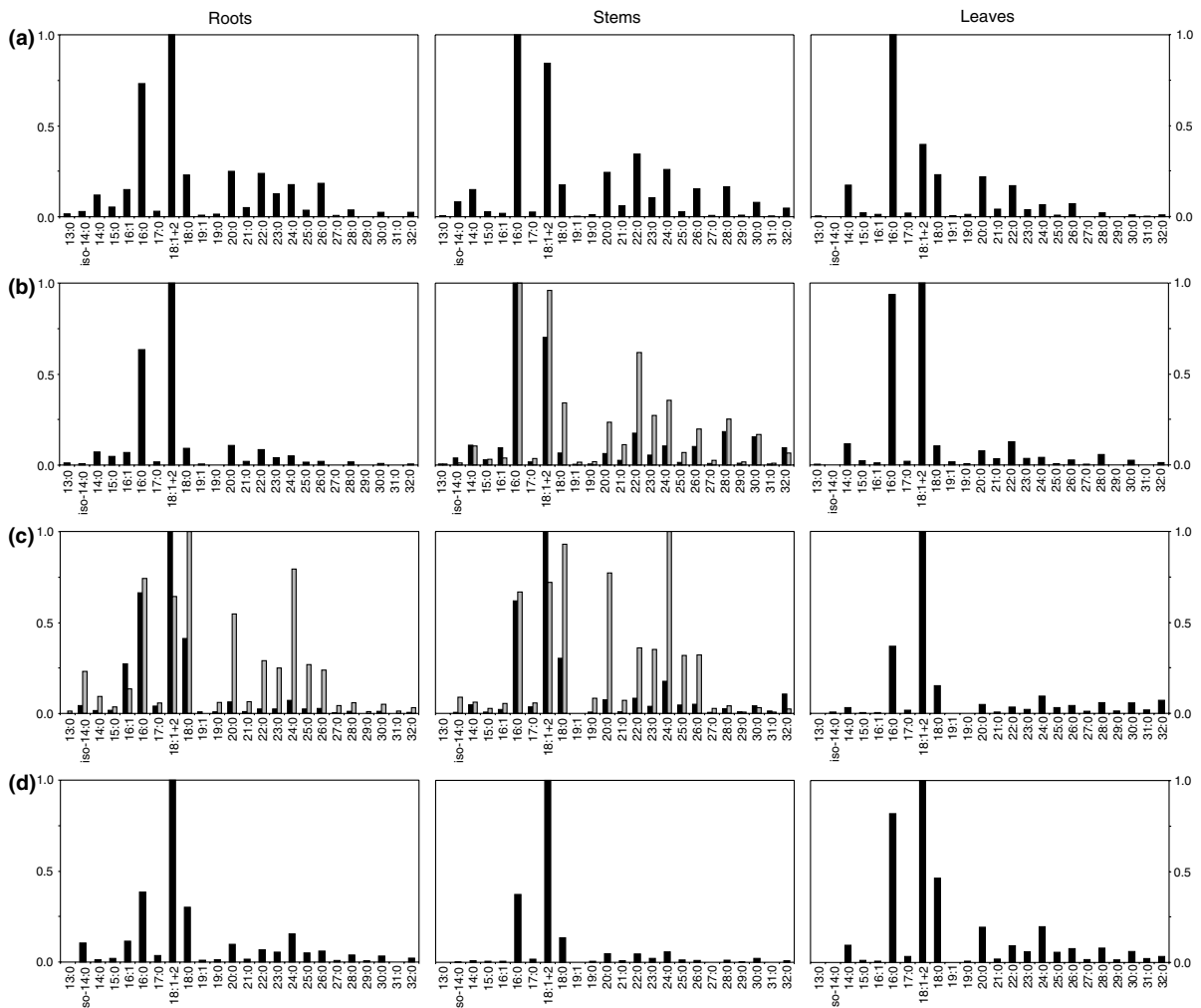


Fig. 5. Carboxylic acid distribution patterns normalized to most abundant compound discriminated for different plant parts (roots, stems and leaves). From top to bottom samples derived from Halle rye (a), Rotthalmünster wheat (b), Halle maize (c), and Rotthalmünster maize (d). Black bars represent plants shortly before harvesting. Gray bars represent samples of degraded plant parts left on the Rotthalmünster site until September of the same year (b) and the Halle site March of the following year (c). For abbreviations beneath bars see Fig. 4. All plant samples are characterised by high amounts of n - $C_{16:0}$ and n - $C_{18:1+2}$ and lesser amounts of n - $C_{18:0}$. Long-chain n -carboxylic acids with a predomination of even numbered homologues are present in all samples. Degraded plant material shows an enrichment of long-chain carboxylic acids when compared to fresh plant material.

Discrimination between C_3 - and C_4 -crop plants could be achieved by plotting long-chain n -carboxylic acid composition in a ternary diagram (Fig. 6). In agreement with observations of Bianchi and Corbellini (1977) C_3 -plants were characterised by the highest amounts of n - C_{22} (>40%), whereas in C_4 -plants n - C_{24} was most abundant (>40%). Brown coal samples revealed lower amounts of n - C_{22} and n - C_{24} and increased n - C_{26} carboxylic acid concentrations (Table 2). C_3 - and C_4 -cropped soils from Rothalmünster and Boigneville plotted on a mixing line between C_3 - and C_4 -plant biomass (Fig. 6). For Halle soils a higher amount of n - C_{26} was observed, indicative of a significant brown coal contamination (Fig. 6). Silage-maize cropped soils were only slightly enriched in n - C_{24} , compared to rye-cropped soils at the Halle site. These small differences between the rye and silage-maize cropping were due to the above-ground biomass removal upon silage harvesting. A shift of equal magnitude in the long-chain carboxylic acid composition was observed between silage- and grain-maize cropping after only one year for the Halle site. The grain harvesting technique led to a significant depletion in the n - C_{26} and a preferential increase in n - C_{24} carboxylic acid.

3.3. Compound-specific isotope analyses

The isotopic composition ($\delta^{13}C$) of the most abundant n -alkanes generally had an offset of approximately 9‰ against the bulk isotopic composition (Fig. 7 and Table 3) as a result of biosynthetic fractionation (Hayes, 1993). C_3 -plants were depleted in ^{13}C as a result of the Hatch/Slack-photosynthesis metabolism, leading to bulk isotope values of $-25‰$ to $-33‰$ and corresponding n -alkane isotope values between $-36‰$ and $-42‰$ (Table 3).

For wheat plants the isotopic depletion of bulk samples and n -alkanes was larger for leaves and lower in roots (Table 3). These results are in excellent agreement with bulk isotopic compositions of wheat as reported by Lichtfouse et al. (1995b). Similarly, for bulk wheat plants Balesdent et al. (1988) gave $\delta^{13}C$ values of $-27‰$ and for wheat grain and straw Zhao et al. (2001) determined values ranging from $-25‰$ to $-28‰$. Rye plants did not show the tendency for larger isotopic depletion in root n -alkanes or bulk ^{13}C composition (Table 3).

C_4 plants were relatively enriched in ^{13}C , leading to bulk isotopic values between $-12.4‰$ and $-14.1‰$ (Table 3). This is in agreement with results reported for

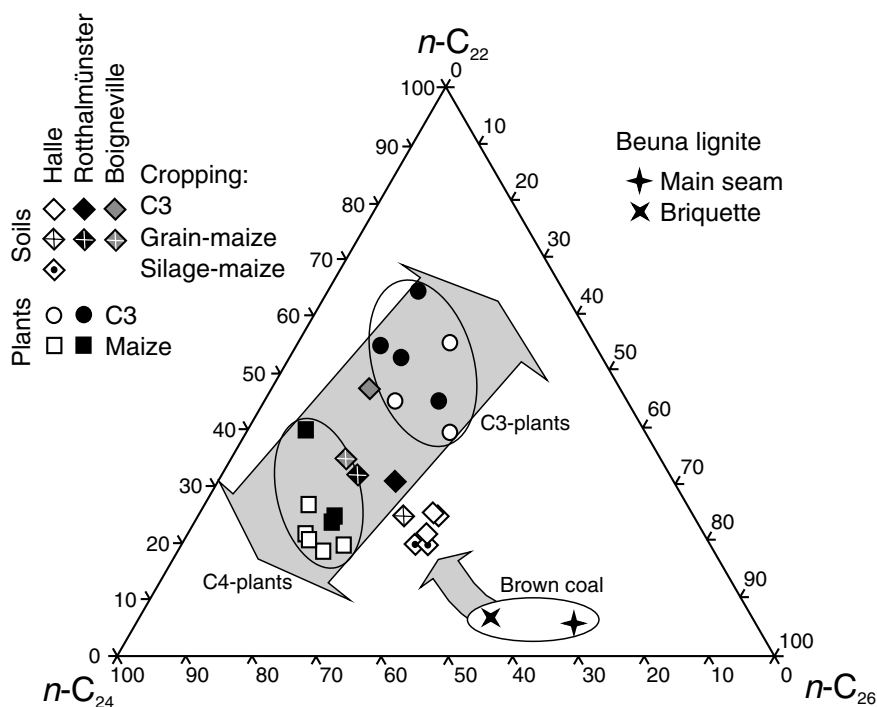


Fig. 6. Ternary diagram showing the relative composition of the three most abundant long-chain n -carboxylic acids (n - C_{22} , n - C_{24} , n - C_{26}) as molecular indicators for plant biomass input. Generally, plant biomass is characterised by low amounts of n - C_{26} in contrast to brown coal. C_3 -plants contain highest proportions of n - C_{22} , while maize plants are enriched in n - C_{24} . C_3 -cropped soils and C_3 -plants have similar long-chain carboxylic acid compositions. C_4 -cropped soils plot on a mixing line between C_3 - and C_4 -plants. High proportions of n - C_{26} carboxylic acid indicate brown coal pollution in Halle soils. One year after introduction of grain-maize cropping this soil from Halle site shows a higher tendency to C_4 -plants than the other Halle soil, which has been silage-cropped for 23 years.

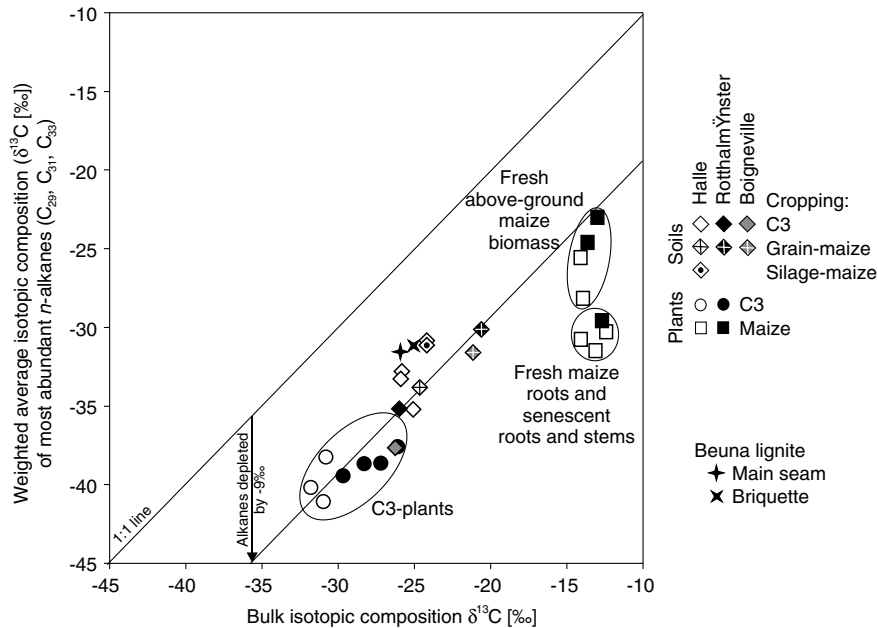


Fig. 7. Weighted average isotopic composition of most abundant *n*-alkanes (C_{29} , C_{31} , C_{33}) compared to the bulk isotopic composition. Long-chain *n*-alkanes are depleted by 9‰ in comparison to bulk isotopic values. C_3 -plants are most depleted in ^{13}C . C_4 -plants are relatively enriched in ^{13}C , but fresh roots, degraded roots, and stem biomass show a ^{13}C -depletion in comparison to fresh above-ground maize biomass. C_3 -cropped soils show isotope ratios similar to C_3 -plants and a mixing trend towards C_4 -biomass is observed in C_4 -cropped soils. High proportions of brown coal cause an enrichment of ^{13}C in Halle soils and deviations from C_4 -plant biomass.

maize biomass (Balesdent et al., 1987, 1988; Marino and McElroy, 1991). For different parts of maize plants we observed relatively constant isotope values for the bulk biomass but highly variable isotope values for *n*-alkanes (Table 3). While *n*-alkanes from fresh leaves were enriched in ^{13}C (average -24.2‰), those from fresh stems were slightly depleted (average -25.6‰). The *n*-alkanes of fresh and degraded maize roots as well as degraded stems gave the lightest isotopic ^{13}C -signatures (average -30.1‰), for details see Table 3. These large variations between different maize plant parts might be a result of plant internal biosynthetic fractionation during C-transport and fixation (Hobbie and Werner, 2004). Microbial colonization of plant surfaces may also be considered as a process to alter the isotopic composition of long-chain alkyl lipids. In contrast to fresh plant material, brown coal samples had intermediate bulk and *n*-alkane isotopic compositions (Table 3).

As a result of biomass input C_3 -cropped soils had the lowest bulk ^{13}C contents, but were still slightly enriched when compared with fresh plant material. This effect was previously described for in situ preservation of resistant biopolymers and their stabilization through condensation reactions (Lichtfouse et al., 1995b). Lichtfouse et al. (1994, 1995b) reported an enrichment of about 3.0 ‰ in ^{13}C for wheat cropped soils when compared to above-ground biomass in Boigneville. This

could also be observed for plants and wheat cropped soils from Rotthalmünster. Halle rye cropped soils showed nearly equivalent bulk ^{13}C signatures, when compared with C_3 -cropped soils from Rotthalmünster and Boigneville. The difference in bulk ^{13}C between C_3 -cropped soils from Halle and fresh above-ground plant biomass was 5.1‰ (Fig. 7 and Table 3) and thus significantly larger than for the other sites.

For all sites the bulk isotopic composition of SOC gave values intermediate between those of C_3 - and C_4 -plant biomass (Fig. 7). In general isotopic differences between C_3 - and C_4 -cropped soils were larger, if the C_4 -cropped soil had been under grain-maize as compared to silage-maize harvesting. This was also observed in Halle where silage-maize cropping was practised for a longer time than grain-maize cropping on the other plots. In 2001, a new plot was established in Halle for grain-maize cropping on a previously rye cropped soil. Prior to introduction of maize this soil showed a slightly larger isotopic depletion in ^{13}C of *n*-alkanes in comparison to other C_3 -cropped soils in Halle, due to inhomogeneities in the soil properties of this trial. A significant enrichment in ^{13}C of *n*-alkanes and bulk SOC of the grain-maize plot in Halle could be observed after only one year.

Soil at the Halle site received input of lignite-derived lipids as shown by molecular compositions (Fig. 6).

Table 3
Isotopic ($\delta^{13}\text{C}$) signatures of bulk samples and predominant long-chain *n*-alkanes and *n*-carboxylic acids

Sampling site/sample	Sampling time	Bulk sample (‰) ^a	<i>n</i> -C ₂₇ alkane (‰)	<i>n</i> -C ₂₉ alkane (‰)	<i>n</i> -C ₃₁ alkane (‰)	<i>n</i> -C ₃₃ alkane (‰)	<i>n</i> -C ₂₂ carboxylic acid (‰)	<i>n</i> -C ₂₄ carboxylic acid (‰)	<i>n</i> -C ₂₆ carboxylic acid (‰)
<i>Soils</i>									
Halle									
Rye	2000a	-25.8 ± 0.1	-31.2 ± 0.0	-32.3 ± 0.0	-33.1 ± 0.1	-33.2 ± 0.2	-33.3 ± 0.4	-31.6 ± 0.2	-30.7 ± 0.2
	2000b	-25.8 ± 0.1	-31.9 ± 0.3	-32.7 ± 0.3	-33.7 ± 0.3	-33.8 ± 0.3	-32.5 ± 0.1	-31.8 ± 0.5	-31.4 ± 0.2
Silage maize	2000a	-24.1 ± 0.1	-29.1 ± 0.2	-30.9 ± 0.1	-30.7 ± 0.1	-31.5 ± 0.2	-27.0 ± 0.4	-26.2 ± 0.4	-28.3 ± 0.6
	2000b	-24.1 ± 0.1	-28.9 ± 0.2	-30.8 ± 0.3	-31.3 ± 0.5	-31.7 ± 0.2	-25.4 ± 0.9	-26.6 ± 0.2	-28.1 ± 0.4
Grain maize	2001	-25.1 ± 0.1	-32.2 ± 0.2	-35.1 ± 0.1	-35.6 ± 0.0	-34.3 ± 0.4	-30.8 ± 0.8	-33.3 ± 0.5	-33.5 ± 0.6
	2002	-24.6 ± 0.1	-30.8 ± 0.4	-34.0 ± 0.2	-34.4 ± 0.3	-32.1 ± 0.6	-29.8 ± 0.6	-30.4 ± 0.0	-31.0 ± 0.3
Rotthalmuenster									
Wheat	2002	-26.0 ± 0.1	-32.2 ± 0.2	-34.1 ± 0.3	-34.6 ± 0.1	-39.4 ± 0.3	-32.4 ± 0.1	-32.5 ± 0.1	-33.9 ± 0.3
Grain maize	2002	-20.6 ± 0.1	-25.6 ± 0.1	-29.4 ± 0.1	-29.4 ± 0.1	-33.1 ± 0.6	-26.1 ± 0.2	-27.5 ± 0.6	-30.9 ± 0.5
Boigneville									
Wheat	1993	-25.9 ± 0.1	-33.6 ± 0.3	-37.4 ± 0.2	-38.6 ± 0.4	-35.2 ± 0.4	-32.5 ± 0.0	-32.2 ± 0.0	-32.5 ± 0.4
Grain maize	1993	-20.8 ± 0.1	-30.2 ± 0.5	-32.8 ± 0.1	-32.7 ± 0.2	-26.9 ± 0.3	-25.8 ± 0.5	-25.9 ± 0.6	-28.4 ± 0.8
Brown coal from Beuna									
Main seam ^b		-25.8 ± 0.1	-30.8 ± 0.5	-31.4 ± 0.5	-32.2 ± 0.5	-30.3 ± 0.5	-29.6 ± 0.5	-29.4 ± 0.5	-32.7 ± 0.5
Briquette ^b		-25.0 ± 0.1	-29.6 ± 0.5	-30.4 ± 0.5	-31.3 ± 0.5	-32.3 ± 0.5	- ^c	-29.3 ± 0.3	-29.6 ± 0.7
<i>Plants</i>									
Rye (Halle)									
Roots		-31.8 ± 0.1	-37.0 ± 0.7	-37.6 ± 0.1	-41.5 ± 0.3	-42.3 ± 0.3	-37.5 ± 0.3	-37.0 ± 0.1	-38.1 ± 0.2
Stems		-30.8 ± 0.1	-37.6 ± 0.2	-37.8 ± 0.3	-38.5 ± 0.4	-40.4 ± 0.3	-37.1 ± 0.1	-37.5 ± 0.4	-38.2 ± 0.3
Leaves		-31.0 ± 0.1	-36.5 ± 0.5	-39.8 ± 0.6	-41.6 ± 0.2	-42.1 ± 0.7	-38.6 ± 0.3	-38.4 ± 0.4	-39.3 ± 0.4
Wheat (Rotthalmünster)									
Roots		-26.1 ± 0.1	-33.8 ± 0.5	-36.1 ± 0.3	-39.5 ± 1.2	-36.3 ± 1.2	-34.6 ± 0.0	-35.5 ± 0.1	-35.7 ± 0.8
Stems		-28.3 ± 0.1	-36.7 ± 0.3	-38.0 ± 0.2	-39.0 ± 0.2	-39.7 ± 0.8	-35.9 ± 0.1	-36.8 ± 0.2	-37.0 ± 0.4
Leaves		-29.7 ± 0.1	-36.6 ± 0.4	-38.6 ± 0.3	-39.5 ± 0.5	-39.0 ± 0.6	-37.9 ± 0.0	-38.0 ± 0.2	-37.5 ± 0.5
Straw (mainly stems)		-27.2 ± 0.1	-36.7 ± 0.2	-38.3 ± 0.4	-38.7 ± 0.2	-40.0 ± 0.5	-35.9 ± 0.1	-36.0 ± 0.2	-36.7 ± 0.3
Maize (Halle)									
Roots	Fresh	-14.1 ± 0.1	-31.0 ± 0.3	-31.3 ± 0.3	-30.6 ± 0.3	-29.6 ± 1.1	-20.6 ± 0.4	-21.8 ± 0.4	-20.2 ± 1.7
	Degraded	-12.4 ± 0.2	-31.6 ± 0.0	-30.3 ± 0.5	-32.1 ± 0.2	-26.7 ± 0.5	-17.7 ± 0.3	-18.7 ± 0.2	-19.8 ± 0.1
Stems	Fresh	-13.9 ± 0.1	-26.5 ± 1.2	-25.5 ± 0.3	-29.7 ± 0.0	-24.7 ± 0.5	-20.7 ± 0.2	-24.6 ± 0.1	-24.1 ± 0.3
	Degraded	-13.1 ± 0.0	-28.5 ± 0.3	-31.2 ± 1.0	-34.1 ± 1.2	-26.8 ± 1.0	-19.0 ± 0.5	-20.0 ± 0.2	-20.9 ± 0.3
Leaves	Fresh	-14.0 ± 0.1	-26.9 ± 0.5	-27.3 ± 0.3	-25.1 ± 0.5	-24.6 ± 0.0	-26.5 ± 0.3	-27.1 ± 0.3	-24.9 ± 0.1
Maize (Rotthalmünster)									
Roots		-12.8 ± 0.1	-29.2 ± 0.1	-30.1 ± 0.3	-29.1 ± 0.3	-29.6 ± 0.7	-19.1 ± 0.2	-20.4 ± 0.3	-23.2 ± 0.5
Stems		-13.7 ± 0.1	-24.9 ± 0.2	-23.7 ± 0.7	-24.8 ± 0.2	-24.7 ± 0.1	-21.7 ± 0.2	-23.8 ± 0.2	-24.0 ± 0.3
Leaves		-13.0 ± 0.1	-23.0 ± 0.6	-22.8 ± 0.1	-23.1 ± 0.6	-24.6 ± 0.2	-23.8 ± 0.2	-27.2 ± 0.4	-27.2 ± 0.8

^a For single determinations of bulk isotopes a standard deviation of $\pm 0.1\text{‰}$ was assumed.

^b Compound-specific isotope analysis based on a single analysis with an assumed standard deviation of $\pm 0.5\text{‰}$.

^c not detected.

Lignite bulk isotopic composition was identical to rye cropped soil whereas of $\delta^{13}\text{C}$ of lignite *n*-alkanes was slightly enriched when compared to soils (Fig. 7 and Table 3). Input of lignite-derived organic matter thus has diluted the incorporation of C_4 -plant lipids and total biomass into Halle soils for several decades.

In soils most abundant *n*-carboxylic acids generally gave results identical to those of *n*-alkanes, when compared with bulk isotopic composition (Fig. 8). The mean biosynthetic fractionation was slightly lower (7‰) than for *n*-alkanes. Fractionation within carboxylic acids of C_3 -plants was similar to those observed for *n*-alkanes and yielded results typical for C_3 -plants (Ballentine et al., 1998). Much larger isotopic differences occurred between various C_4 -plant parts. In contrast to *n*-alkanes the ^{13}C -content of *n*-carboxylic acids of maize plants was lowest for fresh leaves, slightly higher for fresh stems and highest for fresh and degraded roots as well as for degraded stems (Fig. 8 and Table 3). Results for leaves were similar to other C_4 -plants described by Ballentine et al. (1998). Reasons for this contrast in isotopic composition between *n*-alkanes and *n*-carboxylic acids are still unknown but may be attributed to biosynthetic pathways (Kolattukudy et al., 1976; Lichtfouse, 1998).

The observations made on *n*-alkanes in soils and brown coal samples were also valid for *n*-carboxylic acids. Only Boigneville soils were enriched in ^{13}C , which

might depend on deviating soil properties or microbial communities.

Comparison of isotopic compositions of most abundant *n*-carboxylic acids with those of most abundant *n*-alkanes (Fig. 9) revealed only marginal differences between both lipid classes, except for maize plant biomass. The combination of results from both lipid classes improved discrimination between different fresh plant organs of maize and degraded biomass. As a result of the intra-plant isotopic variability of maize plants, the various C_4 -cropped soils showed different isotopic behaviour when compared with C_3 -cropped soils. This was due to the lack of significant intra-plant isotopic variability observed for C_3 -plants. The intra-plant isotopic variation was amplified by application of different cropping techniques, which selectively removed the above-ground biomass and its corresponding isotopic signatures from the plot (silage-maize) or preserved the isotopic signature of the above-ground biomass (grain-maize).

Grain-maize cropped soils were enriched in ^{13}C in both the *n*-alkane and *n*-carboxylic acid fractions as a result of high above-ground biomass input with ^{13}C -enriched stems and leaves. Only 15–17% of the total biomass input to these plots resulted from roots (Anderson, 1988), because nearly all biomass was left on the plot after harvesting. Contrastingly, on silage-maize

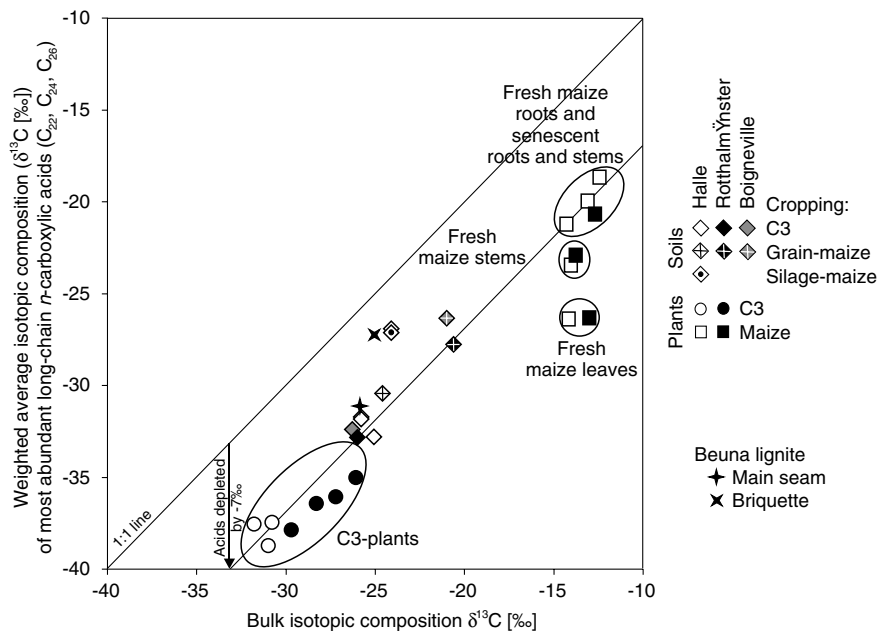


Fig. 8. Weighted average isotopic composition of most abundant long-chain *n*-carboxylic acids (C_{22} , C_{24} , C_{26}) compared with bulk isotopic composition. Isotopic depletion of carboxylic acids in comparison with bulk isotope signature is 7‰. While C_3 -plants show similar ^{13}C -depletions, C_4 -plants are generally enriched in ^{13}C . The compound-specific isotopic depletion in ^{13}C within maize plants is lowest in fresh roots, degraded roots, and stems, but larger in fresh stems and largest in leaves. Soils show a mixing trend from isotopically depleted C_3 -cropped soils to isotopically enriched C_4 -cropped soils.

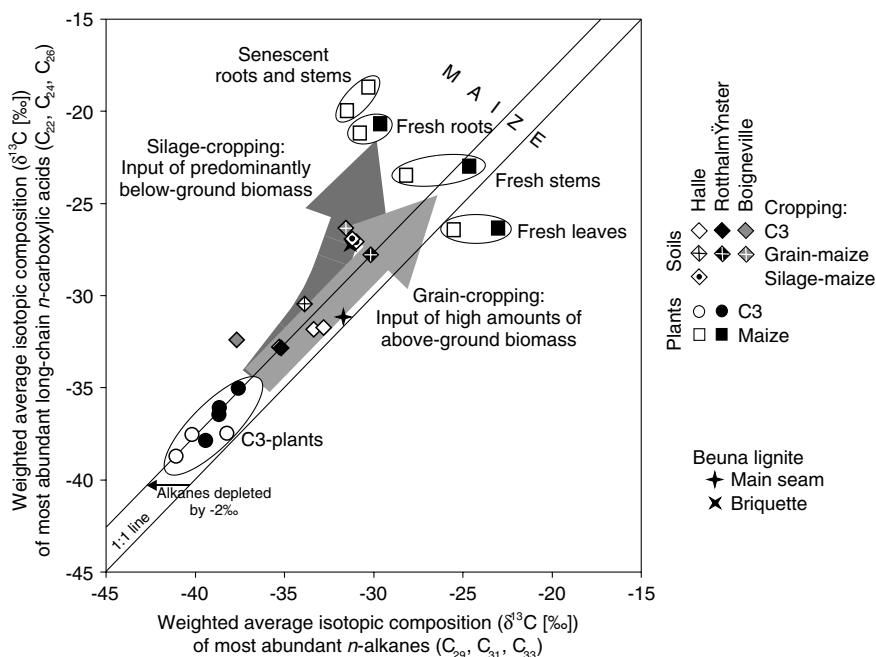


Fig. 9. Weighted average composition of most abundant long-chain *n*-carboxylic acids plotted against weighted average isotopic composition of most abundant *n*-alkanes. In comparison with carboxylic acids the alkanes are slightly depleted in ^{13}C . C_3 -plants show the highest isotopic depletion and nearly homogeneous results for all plant parts. Within maize plants an isotopic differentiation between different fresh and degraded plant parts occurs. Depending on harvesting techniques discrete incorporation pathways are observable: i) high above-ground biomass input of grain-cropping leads to similar isotopic enrichment in soils for alkanes and carboxylic acids (light gray arrow); ii) silage-cropping with preferential incorporation of below-ground biomass leads to an isotopic enrichment of carboxylic acids (dark gray arrow).

cropped soils nearly all above-ground biomass was removed during harvesting and mainly roots and the lowermost parts of the stems (up to 15 cm) remained on the field. Thus, a ratio between shoot and root (S:R-ratio) of 1:1 must be assumed for plant biomass incorporation on this plot. This contrasting biomass input led to a preferential ^{13}C -enrichment in the *n*-carboxylic acids, whereas *n*-alkanes got only slightly enriched.

3.4. New maize-C proportions

We demonstrated for *n*-alkanes and *n*-carboxylic acids that C_3 - and C_4 -plants had different molecular and isotopic compositions and that organic matter of maize plants differed significantly between plant parts. Consequently, we separately calculated the input of new maize soil organic carbon, assuming an exclusive input of several plant parts (roots, stems and leaves) and different shoot to root ratios (S:R-ratio). Adequate S:R-ratios were selected for each cropping technique. For grain-maize cropping a S:R-ratio of 5.7:1 or 15% root biomass (Anderson, 1988), and for silage-cropping a S:R-ratio of 1:1 was used. For all soils above-ground biomass was assumed to have leaf to stem biomass ratios of ap-

proximately 1:1. Proportions of new maize-C (Fig. 10) in previously C_3 -cropped soils were calculated as:

$$M = 100 \times F_{\text{C}_4}, \quad (7)$$

with M as the percentage of newly introduced maize-derived carbon into total soil carbon, δ as the isotopic composition of soil carbon at a given time of cultivation, δ_0 representing the original isotopic composition of soil carbon before maize cultivation, and δ_m the isotopic composition of crop plant carbon.

For calculations based on bulk isotopic values [Fig. 10(a)] only marginal differences (<5%) between various plant parts could be observed. Hence, input of separate plant parts was not significantly different for bulk isotopic signatures and each plant part may be used for calculations. Halle silage-maize cropped soils contained only 15% of new maize-C after 39 years of continuous cropping. In contrast after only one year grain-maize cropping the soil from Halle contained 5% new biomass. This fast turnover was firstly caused by high biomass incorporation and secondly by the incorporation of labile carbon from, e.g., sugars within the first year. In Rotthalmünster and Boigneville soils, the proportions of new maize-C were around 41%

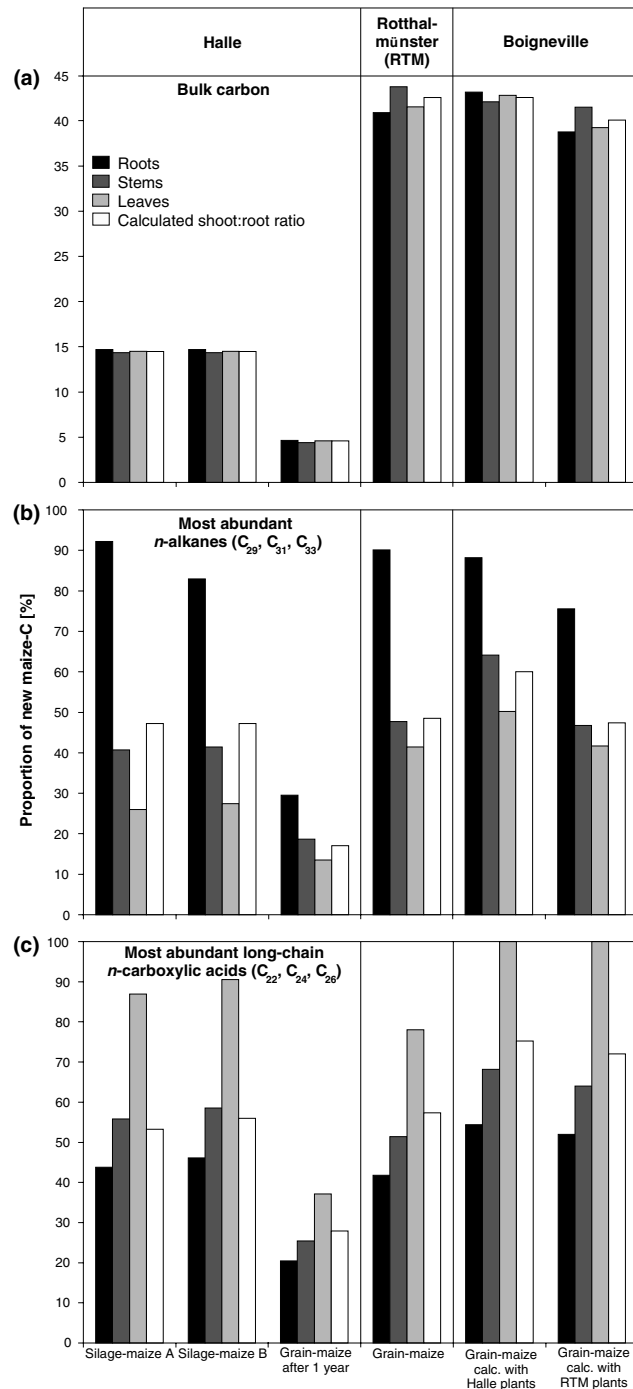


Fig. 10. Scenarios showing the calculated effect of incorporation of different plant parts on apparent proportions of new maize carbon in previously C₃-cropped soils. Scenarios were calculated for: only roots, only stems, only leaves, or a mixture of all. For the latter a shoot:root ratio of 1:1 for silage-maize in Halle and 5.7:1 for all other plots is used. A stem:leaf ratio of 1:1 is assumed to represent shoot biomass. New maize carbon was calculated for bulk soil (a), most abundant *n*-alkanes (b; C₂₉, C₃₁, C₃₃) and most abundant long-chain *n*-carboxylic acids (c; C₂₂, C₂₄, C₂₆). For bulk carbon slightly lower proportions of new maize carbon occur in comparison to new maize contributions calculated for alkanes and carboxylic acids. Calculations based on bulk isotope signatures show similar results for all plant parts and after shoot:root correction. For *n*-alkanes highest proportions of new maize carbon are observed for the scenario with exclusively root input, while for *n*-carboxylic acids the highest proportions of new maize carbon is calculated for exclusively leaf input.

(Fig. 10(a)). Because fresh plant material for the Boigneville site was not available for analysis, calculation of biomass input was made using ^{13}C -signatures of plant material measured from the Halle and Rothalmünster sites. Differences between calculations for biomass input into Boigneville soils based on Halle and Rothalmünster plant isotopic values varied only marginally. For the Boigneville site Puget et al. (1995) and Cayet and Lichtfouse (2001) indicated proportions of new maize-derived carbon of 45% and 41%, respectively. The close match between three different analyses lends credibility to the analytical approach used.

Gregorich et al. (1996a) determined 20% and 30% new maize-C incorporation into agricultural soils after 35 years continuous maize cropping with less incorporation corresponding to non-fertilized plots. Slightly higher proportions of new maize-C were determined by Collins et al. (1999) ranging from 30% to 58% after 16–35 years of fertilized continuous maize cropping. Liang et al. (1998) observed lower proportions of new maize-C incorporation between 5% and 19% due to shorter duration of fertilized continuous maize cropping ranging between 3 and 11 years. Balesdent et al. (1987) reported new maize-C proportions of 30% after 13 years of corn-cropping on previously C3-utilized soils. Similarly, Accoe et al. (2002) calculated 33% new maize-C after 19 years since the switch from C3-cropping occurred. The incorporation of maize-derived carbon into agricultural soils, managed by grain- and silage-cropping, as determined in this study is in good agreement with data reported in the literature. The low proportions of maize-C in Halle silage-cropped soils are attributed to low biomass input due to the harvesting technique applied and the dilution of crop-plant carbon by lignite derived carbon.

On a molecular level significant differences between various modes of plant biomass incorporation into soils [Figs. 10(b) and (c)] were observed. For *n*-alkanes [Fig. 10(b)] the maize-C proportions were highest (around 90%) for an incorporation of exclusively root biomass. This is related to the ^{13}C -depletion within maize roots. Contrastingly, biomass incorporation exclusively from stems showed lower maize-C proportions of 40–60% depending on sampling site and were lowest for the grain-maize plot in Halle (20%). The lowest maize-C proportions were derived for an input of exclusively leaves. This may have led to an underestimation of new maize C-proportions calculated by Cayet and Lichtfouse (2001), who used exclusively leaf-derived *n*-alkane isotopic signatures in their calculations of new maize-C proportions. The largest differences between below- and above-ground biomass were observed in Halle soils related to silage-maize cropping. For Boigneville soils, differences up to a maximum of 20% were observed, when calculations were based on Halle or Rothalmünster plant biomass values. Thus, it was re-

quired to use the plant biomass properties from each individual plot for the isotopic assessment of maize-C turnover based on *n*-alkanes. Maize-C calculations corrected for the S:R-ratio again showed results comparable to those based on calculations using exclusively stem biomass. This implies that *n*-alkanes could be used as plot independent markers for SOC turnover. For silage-maize cropped soils from the Halle site similar results for maize-C incorporation could be observed compared to the other sites.

For *n*-carboxylic acids contrasting trends were observed [Fig. 10(c)] when compared with *n*-alkanes. Leaf-derived carboxylic acids seemed to be incorporated to a high degree, when compared with exclusively root or stem biomass. A proper correction had to be used for the *n*-carboxylic acid fraction, to obtain realistic proportions of new carbon incorporation and turnover rates. Calculations of S:R-ratio-corrected new carbon incorporation in soils led to similar maize-C yields in Halle silage-maize cropped soil and Rothalmünster soil, which were almost in the range of exclusively stem-biomass input. The highest proportions of maize-C could be observed in Boigneville soils with identical results based on calculations using Halle and Rothalmünster plant biomass properties. Grain-maize cropped soil in Halle had relatively high maize-derived carboxylic acid proportions of nearly 30% after only one year of C₄-cropping. This is related to the preferential occurrence of *n*-carboxylic acids in shoots, in contrast to the dominance of *n*-alkanes in roots. For the Halle sites enhanced proportion of new maize-derived carboxylic acids in the grain-maize cropped plot as compared to the silage-maize cropped plot is due to the harvesting technique leaving more biomass on the field. This increase in biomass input is accompanied by a reduction in SOM dilution by lignite due to a cease of open pit mining in the Halle-Bitterfeld-Beuna region.

Calculations of new biomass incorporation into soil based on stable carbon isotopes were viable for bulk isotopes without further corrections. Within lipid fractions corrections for different biomass incorporation into soils as a result of varying cropping and harvesting methods must be considered. For optimum accuracy, it was required to sample biomass directly before harvesting because variable conditions might lead to considerable deviations. For preliminary estimations of C-incorporation into soils it is recommended to use stem biomass properties, which compared to weighted S:R-ratio corrections revealed a degree of uncertainty of less than 10%.

3.5. Turnover times

In order to account for the variable duration of long-term field experiments with respect to the proportion of

new C₄-incorporated carbon into SOM, normalization to turnover times or mean residence times as defined in Eq. (6) is required. As defined under methods, this approach requires steady-state conditions in soil carbon balance and similar plant physiologies, e.g., vegetation periods (Balesdent and Mariotti, 1996).

Turnover times for the Rotthalmünster and Boigneville sites cropped with grain-maize were determined in this study and using data obtained from Puget et al. (1995) and Cayet and Lichtfouse (2001). As described in Section 3.4 above lack of plant samples for the Boigneville site required us to use approximate values for calculation of new maize-C proportions and resulting turnover times. Turnover times were calculated based on adequate S:R-ratios as outlined above.

For bulk SOC the obtained turnover times of 39–45 years are highly similar (Fig. 11). Turnover times for *n*-alkanes are virtually identical for the Rotthalmünster and Boigneville sites with 35 years, except when using

isotopic composition of plants from Halle, which resulted in slightly shorter turnover times of 26 years (Fig. 11). Turnover times of *n*-alkanes determined for Boigneville using data from averaged *n*-C₂₉ and *n*-C₃₁ as reported by Cayet and Lichtfouse (2001) led to results identical with our calculations. This is taken as evidence for the high reliability of the methodological approach due to the complex work-up of lipid fractions required for compound-specific isotope analysis. Carboxylic acid fractions gave consistently shorter turnover time of 18 years for the Boigneville site than for the Rotthalmünster site with 28 years, irrespective of the isotopic composition used for the parent plant material. Soil lipid degradation can be affected by soil acidity (Moucawi et al., 1981; Marseille et al., 1999; Bull et al., 2000) especially in forest or waterlogged soils. The near neutral conditions (pH 5.8–6.8) of the arable soils studied here exclude pH-influence on lipid turnover.

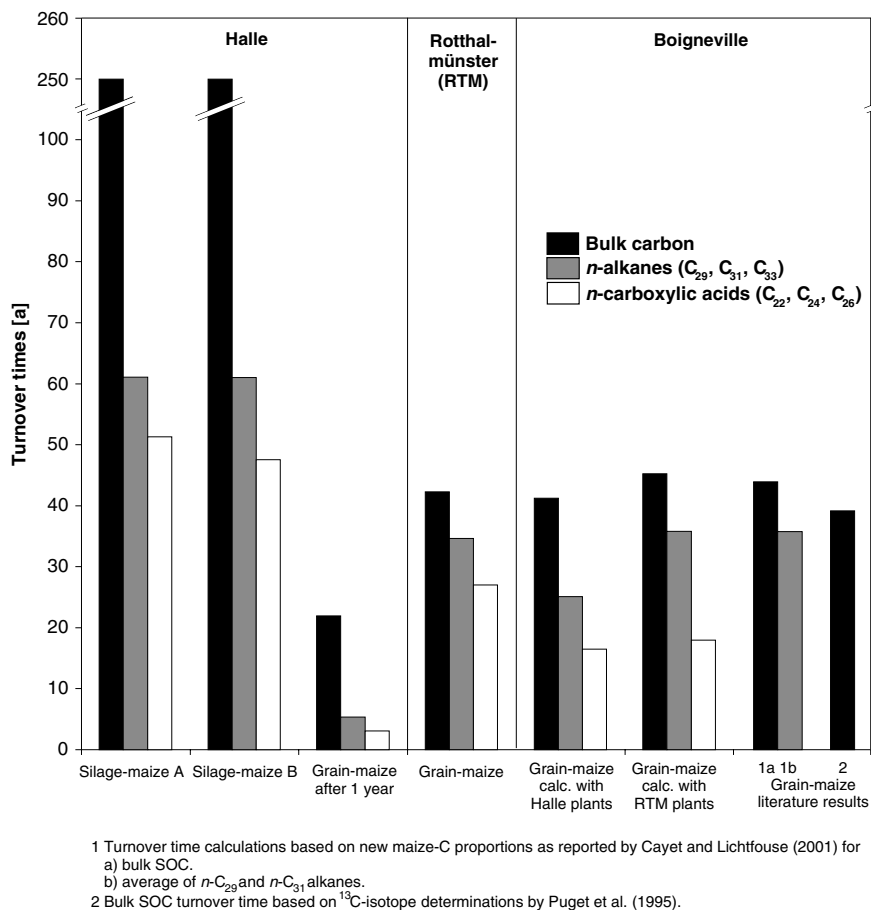


Fig. 11. Turnover times for bulk-C, most abundant *n*-alkanes and *n*-carboxylic acids, based on shoot:root-corrected maize-C proportions. Silage-cropping is only practised in Halle, all other sites employ grain-cropping. Alkanes and carboxylic acids yield lower turnover times than bulk carbon. Lower turnover times in carboxylic acids result from microbially degradable functional groups and a partial origin of alkanes from fossil sources. Turnover times increase with pollution level and silage-cropping.

The grain-maize experiment in Halle has been running for only one year and thus could not have approached steady state conditions augmented by low input of plant biomass prior to maize-cropping. Thus the short turnover times of 21 years for bulk SOC, 5 years for *n*-alkanes and 3 years for *n*-carboxylic acids, (Fig. 11) are not comparable to the other grain-maize plots.

Silage-maize plots in Halle show exceptionally long turnover times of 250 years for bulk carbon and 48–60 years for lipid fractions (Fig. 11). The long turnover times are related to two processes: (i) the lower direct plant biomass input as a consequence of the harvesting technique, which removes more half of the standing biomass from the plot and (ii) the presence of exceptionally high quantities of refractory fossil organic matter. The latter results from lignite input as described above (Fig. 6) and further anthropogenic contamination (Rethemeyer et al., 2004; Wiesenberg and Schwark, unpublished). Both of these two factors may have contributed to reducing microbial activity to a minimum in Halle soils (pers. communication with A. Miltner and S. Scheu), which in turn would contribute to longer turnover times.

At present it is not possible to apportion the effects of silage-maize cropping versus anthropogenic refractory organic matter input into soils at the Halle site. This is the subject of ongoing research based on study of non-contaminated sites with silage-harvesting and extension of work on grain-maize plots established at the Halle site in 2001.

Agricultural soils at all sites investigated show longer turnover times for total SOC than for lipid fractions. Within the lipid fractions *n*-alkanes had slightly longer turnover times than *n*-carboxylic acids. This corresponds to previous observations by Lichtfouse et al. (1998), who concluded that *n*-alkanes are more stable against chemical and biological degradation because of the lack of functional groups that may serve as sites for microbial attack.

Consequently, application of grain-cropping on existing arable soils would lead to higher atmospheric CO₂ sequestration via incorporation and fixation of crop biomass in soils. In addition to introduction of non-disruptive ploughing procedures the CO₂-emission character of cropped soils as described by Janssens et al. (2003) might be changed towards a CO₂-sink character.

4. Conclusions

For agricultural soils it was, to the best of our knowledge, demonstrated for the first time that both long-chain *n*-alkanes (C₂₉, C₃₁, C₃₃) and long-chain *n*-carboxylic acids (C₂₂, C₂₄, C₂₆) are suitable diagnostic markers for plant biomass incorporation into SOM. A

clear differentiation between C₃- and C₄-plants was achieved by their respective molecular and isotopic properties.

Based on the source signals variable contributions of C₃- and C₄-plant biomass to organic matter in agricultural soils could be determined. Molecular tracers allowed for a discrimination of organic carbon input from different plants parts (leaves, stems, roots). This facilitated to unravelling the influences of different cropping techniques via their selective incorporation of specific plant parts. Differences between silage- and grain-maize cropping led to preferential incorporation of root dominated or above-ground biomass dominated plant material, respectively. The differences in harvesting techniques necessitated proper corrections for different amounts of below- and above-ground biomass for maize-C calculations. If complete plant biomass was not available for proper correction, stem biomass would give the most realistic results for maize-C calculations based on *n*-alkanes and *n*-carboxylic acids.

While bulk soil-C had residence times up to 250 years, lipid fractions were less stable with turnover times of maximal 60 years. *N*-carboxylic acids were marginally less stable than *n*-alkanes. Hence, *n*-alkanes and *n*-carboxylic acids represented a portion of the slow carbon pool sensu Paul et al. (2001), which was mainly affected by terrestrial plant biomass incorporation as demonstrated for long-chain lipids. For grain-maize cropped soils at steady state average turnover times of 40 years for bulk SOC, 35 years for *n*-alkanes and 21 years for *n*-carboxylic acids were determined. Turnover times for silage-maize cropped soil at steady state were on average 250 years for bulk SOC, 60 years for *n*-alkanes and 49 years for *n*-carboxylic acids. The latter turnover times derive from only a single trial site (Halle) and may substantially overestimate the mean residence time due to anthropogenic contamination with fossil fuel carbon. Turnover times reported here for silage-maize cropped soils may thus be taken as maximum values only.

Grain-maize cropped soils with high turnover rates more likely act as sinks for atmospheric CO₂ than silage-cropped soils with associated low biomass incorporation. Optimised cropping techniques may thus enable targeted sequestration of atmospheric CO₂.

Acknowledgements

This project received funding from the German Research Foundation (DFG) within the Priority Program 1090 “Soils as sources and sinks for CO₂” under contract Schm438/3-1 and Schw554/14-2. We thank the Institut Technique des Céréales et des Fourages and C. Chenu (INRA, Paris) who made the Boigneville samples available. L. Schmidt and W. Merbach

(University Halle) provided soil and plant samples from Halle. Mr. Schnellhammer (Staatliche Höhere Landbauschule Rotthalmünster) provided plant samples and the opportunity to sample the Rotthalmünster site. K. Scheffler (University of Bonn) is thanked for bulk isotope determinations. B. Stapper, H. Cieszynski, A. Richter and E. Lehndorff (Geological Institute, University Cologne) provided excellent laboratory assistance. E. Lichtfouse, C. Largeau and an anonymous reviewer are thanked for their helpful comments.

Guest Associate Editor—Jose del Rio

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