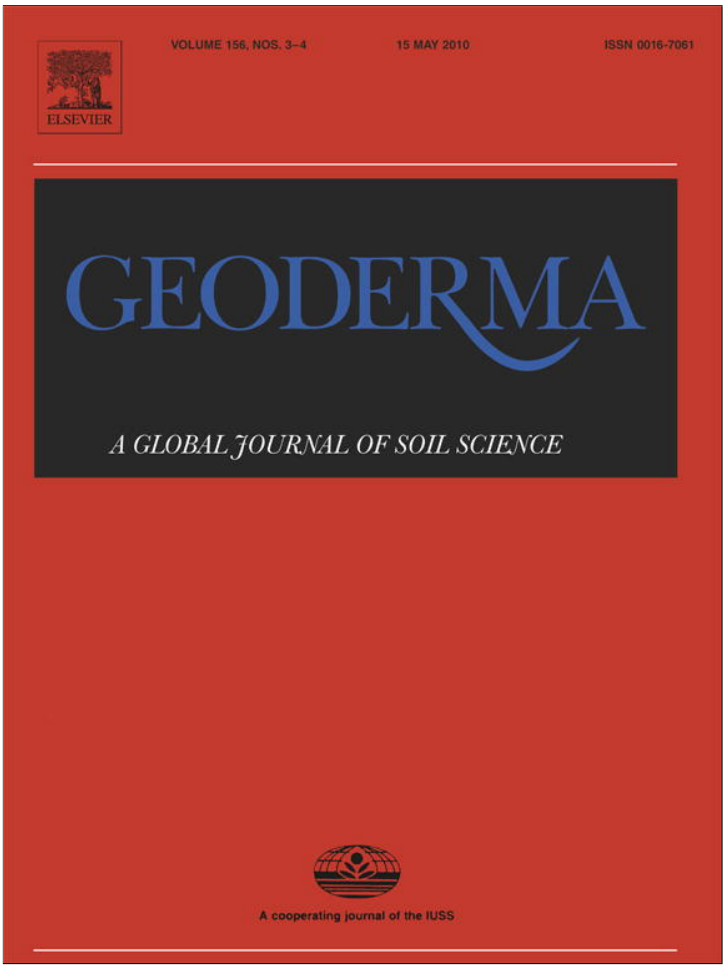

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Source determination of lipids in bulk soil and soil density fractions after four years of wheat cropping

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ABSTRACT

Preservation of soil organic matter (SOM) is strongly affected by occlusion within aggregates and by association of SOM with minerals. Protection of organic carbon (C) due to adsorption to mineral surfaces can be assessed by investigation of SOM in soil density fractions. Apart from the physical properties the preservation of SOM is affected by its chemical composition. While for bulk organic C this was demonstrated for numerous soils, SOM density fractions have been scarcely studied regarding their molecular composition. Lipids as a compound class that can derive from plants, microorganisms and contamination by products from incomplete combustion or fossil carbon were not investigated in density fractions so far. We hypothesized that molecular proxies deriving from lipid composition yield a large potential to elucidate the sources of organic matter entering soil, and in combination with density fractions they enable the identification of incorporation and preservation pathways of SOM.

We determined distribution patterns of aliphatic hydrocarbons and fatty acids as two representative groups for total lipids in soil density fractions. The fatty acids showed a predominant input of plant-derived poly unsaturated short chain and saturated long chain fatty acids in free particulate organic matter (fPOM). The microorganism-derived compounds such as unsaturated short chain fatty acids were largely abundant in fPOM and especially in occluded particulate organic matter (oPOM 1.6). The proportion of plant-derived components like long chain fatty acids increased with increasing density of the fractions, whereas the abundance of short chain fatty acids decreased in the same direction as indicated by the ratio of long chain vs. short chain fatty acids. The main portion of soil lipids (60% of total lipids) was recovered in the mineral (Min) fraction, which denotes the strongest protection of lipids adsorbed to mineral surfaces.

For the aliphatic hydrocarbons the contribution of plant- and microorganism-derived components was the largest in fPOM. Short chain alkanes as part of the aliphatic hydrocarbons showed contamination of soil by an incompletely burned plant biomass or fossil carbon. These contaminants were the most abundant in fPOM and subsequently attributed to particles with a low density, which derived probably from soot. However, a large contribution of fossil C was found in the Min fraction as well, which is thought to be attributed to degraded soot particles being adsorbed to minerals.

We demonstrated at the molecular level that the incorporation of individual C sources varies with the density fractions. It was found that microorganism-derived compounds were most abundant in fPOM and oPOM 1.6 fractions, whereas plant-derived long chain biopolymers were enriched in mineral dominated fractions (oPOM 2.0 and especially Min). Thus, preservation of plant-derived lipids in soil is strongly attributed to the association with minerals. Based on lipid composition in density fractions we evaluated several molecular proxies, which help to elucidate the sources of SOM.

1. Introduction

Physical fractionation techniques of soil organic matter (SOM) such as density fractionation have been applied to determine the association of SOM with primary particles and to quantify the amount of particulate organic matter (POM) between and within soil aggregates (Beare and Hendrix, 1994; Puget et al., 2000; John et al., 2005). Golchin et al. (1997) proposed a conceptual model linking POM decomposition and stabilization. They assumed that the protection of organic matter increases with the increasing density of SOM fractions, i.e. with the increasing association with mineral particles. Several recent studies supported this model showing several distinct trends within soil density fractions in the order free particulate organic matter (fPOM) > occluded particulate organic matter (oPOM) > mineral soil (Min) (e.g. John et al.,

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2005). In this direction contents of organic carbon (C) and nitrogen (N) as well as C:N ratios decrease within individual fractions, thus leaving minerals associated SOM rich in N. In the opposite direction the contribution of the individual fractions to bulk soil by weight strongly increases from fPOM towards Min. Hence, contribution to bulk SOM increases with increasing association to mineral particles. The mineral associated fractions have the largest potential for SOM stabilization, since they are (i) the largest fractions by weight and so, yield largest SOM portions, and (ii) contain the degraded chemically recalcitrant organic material bound to mineral surfaces (e.g. John et al., 2005).

While SOM fractions including density fractions have been characterized e.g. for their total and organic C and N contents, such investigations are limited for individual compound classes like soil lipids. Lipids are major organic components of fresh plant biomass and soils (e.g. Gregorich et al., 1996; Kögel-Knabner, 2002), which yield potential to be preserved in soils for decades (Wiesenberg et al., 2004a) until millennia (Huang et al., 1996). They play an important role for the incorporation and transformation of plant residues into soil and stabilization of soil organic matter (SOM). While the characterization of the lipid composition is available for particle-size separates of agricultural soils (Cayet and Lichtfouse, 2001; Quènèa et al., 2006; Wiesenberg et al., 2006) and forest soils (e.g. Marseille et al., 1999), this information is missing from aggregate and density fractions. Despite these studies describing the main storage of lipidic compounds associated with the silt and clay size fractions, it remains unclear, whether the lipids are predominantly bound to mineral surfaces or protected within soil aggregates. Furthermore, the documentation of lipid contribution to density fractions is currently not available. Especially due to the variety of sources for soil lipids including plant and microbial biomass (Harwood and Russell, 1984), or contamination by e.g. products from incomplete combustion processes (Wiesenberg et al., 2004a), lipids provide a potential to answer several questions related to the protection of the mineral associated SOM at the molecular level. We expected a predominant contribution of plant biomass-related lipids within the fPOM fraction and a large contribution of degraded plant- and microorganism-derived components to the Min fraction. The oPOM fractions are thought to show transformation stages of primary plant- and microorganism-derived lipids towards more resistant, i.e. long chain, biopolymers.

Soil lipids contain molecular proxies that are specific for source determination and taxonomic classification of plants and microorganisms (Gleixner et al., 2001). Plant-derived lipids are characterized by large proportions of long chain fatty acids, aliphatic hydrocarbons, and alcohols (e.g. van Bergen et al., 1998), whereas microorganism-derived lipids are characterized by large proportions of short chain fatty acids (Harwood and Russell, 1984). Previous soil lipid analyses on the ploughed horizons of agricultural soils have focused on the distribution patterns of individual lipid fractions like *n*-alkanes (Lichtfouse et al., 1994, 1997, 1998a; Wiesenberg et al., 2004a,b), fatty acids (Wiesenberg et al., 2004a,b), or bulk lipid extracts (e.g. van Bergen et al., 1998). Dynamics of soil lipids have been studied on field sites, where a monoculture practice results in a uniform biomass input over several years and a vegetation change resulted in a notable change in the biomass input into soil. These studies imply a change of plants with C_3-/C_4-photosynthesis for agricultural soils (Lichtfouse et al., 1994; Wiesenberg et al., 2004a,b) and for forest soil converted to C_4 vegetation (Quènèa et al., 2006). C_3- and C_4-grasses as well as forest trees are all characterized by a different qualitative contribution to SOM due to the differences in lipid biosynthesis and lipid composition of the individual functional plant groups (Bianchi and Bianchi, 1990; Maffei, 1996; Rommerskirchen et al., 2006; Wiesenberg and Schwark, 2006). Hence, the modified plant biomass input resulted in soil lipid compositional changes that were detectable after a few years (Lichtfouse et al., 1994; Wiesenberg et al., 2004a,b). Alternative experiments for the investigation of soil lipid dynamics imply FACE (free air CO_2 enrichment) experiments, where plants have been kept under ambient and elevated atmospheric CO_2 concentrations (Wiesenberg et al., 2008a). In these experiments the added CO_2 carried a different isotopic ($\delta^{13}C$) label than natural air, which enabled turnover determination of plant-derived lipids in soils based on the compound-specific isotope values of plants and soils kept under natural and elevated CO_2 concentrations (Wiesenberg et al., 2008b). However, all these experiments implied a significant change in the quality of the incorporated biomass into soil (Bianchi and Bianchi, 1990; Maffei, 1996; Wiesenberg et al., 2004a; Rommerskirchen et al., 2006; Wiesenberg and Schwark, 2006; Wiesenberg et al., 2008a) and subsequently a modification in the degradability of the incorporated biomass. Other experiments with a change from one to another C_3-grass, i.e. a change from grassland towards a wheat cropped soil, are expected to lack substantial changes in the quality of plant biomass input due to a similar lipid composition of the incorporated biomass (Maffei, 1996). However, the determination of molecular changes has not been tested for C_3-perennial grass/C_3-crop conversion experiments so far. We hypothesized that the molecular changes in soil after a vegetation change from a perennial C_3-grass towards wheat cropping are low due to their similar lipid composition (Maffei, 1996). Additionally, according to changes in bulk C we expected some changes in the lipid composition like a decrease in the total abundance due to the modification of the ploughing technique applied. Whereas grassland soil is not ploughed, wheat soil is ploughed annually. We check the composition of plant-derived changes in soil affected by the conversion from C_3-grassland towards C_3-monoculture cropping.

Based on the described potential of molecular proxies to follow C incorporation from various sources in soil during long term inputs, we evaluated 1) the applicability of lipids to trace short term changes by moderate modification of C input, and 2) the contribution of plant-, microorganism-, and contamination-derived lipidic components to soil density fractions.

2. Materials and methods

2.1. Samples

Soil and plant samples were collected from the experimental station 'Heidfeldhof' of the University of Hohenheim, Stuttgart (Germany). The soil was described as a Gleyic Cambisol (WRB, 1998) developed from Loess (Marhan et al., 2008). The uppermost 10 cm of the A_p horizon were sampled for this study. The site was treated as grassland until a monoculture of spring wheat (*Triticum aestivum* cv Triso) was introduced in 2002. Since then, soil was tilled in spring before crop sowing. Beginning in 2003, inorganic NPK fertilizers were applied (140 kg N, 60 kg K, and 30 kg P ha^{-1}) to each plot (Erbs and Fangmeier, 2006). Soil sampled in 2002 from the permanent grassland converted thereafter to wheat monoculture was available as a reference. From 2006 soil samples were taken after four years of wheat cropping. While for 2002 only one soil sample for the whole plot was available, for 2006 two replicate samples were taken representing five individual subsamples, each. The soil samples were air dried at room temperature and sieved through a 2 mm mesh. All visible root and plant remains were carefully removed with tweezers. Samples of wheat straw were collected in 2006 during harvest from the same two subplots where soil samples were taken. Straw samples were divided into leaf and stem tissues. All samples were air dried. Two replicates were analysed for each plant tissue.

2.2. Separation of density fractions

For the soil sample from 2002 only a low amount of soil was available which was not sufficient for density fractionation followed by lipid analyses. Hence, only soil samples of the year 2006 were separated for density fractions. The fractionation procedure by John et al. (2005) was used with some marginal modifications. 8 g of air dried soil were placed in a centrifugation tube and 35 ml of sodium

polytungstate solution (Sometu, Berlin, Germany) with a density of 1.6 g cm^{-3} were added. The tube was gently turned upside down by hand about 5 times and the solution was centrifuged at 4000 rpm for 1 h in Eppendorf Centrifuge 5810R at room temperature. The supernatant with floating particles was filtered (cellulose acetate, 0.45 µm; Sartorius) using a pressurized filtration cylinder (Sartorius) and washed with distilled water to gain the free particulate organic matter <1.6 g cm^{-3} (fPOM). Then, the residue was dispersed with 35 ml of sodium polytungstate solution (1.6 g cm^{-3}). 5 glass beads with a diameter of 5 mm were added and the solution was shaken for 16 h with a frequency of 60 movements per minute to crack the soil aggregates (Balesdent et al., 1991). After disaggregation, the soil suspension was centrifuged for 1 h at 4000 rpm. The supernatant with floating particles (light occluded particulate organic matter with a density <1.6 g cm^{-3}, oPOM 1.6) was filtered (0.45 µm) using a pressurized filtration cylinder and washed with distilled water. Thereafter, the residue was dispersed using sodium polytungstate solution of a density of 2.0 g cm^{-3}. The supernatant with floating particles (dense occluded particulate organic matter with a density of 1.6–2.0 g cm^{-3}, oPOM 2.0) was filtered and washed as described above. To remove the salt, the residue containing the mineral fraction (>2.0 g cm^{-3}, Min fraction) was washed four times with distilled water, each time centrifuging and discarding the supernatant. All fractions were dried at 40 °C, weighed and ground to powder by means of a ball mill (MM2, Retsch) for 15 s. To achieve sufficient material for lipid analysis, 160 g of soil were fractionated from two replicate soils from 2006.

2.3. Lipid analysis

Individual plant, bulk soil, and density fraction samples were extracted followed by a sequential separation scheme. For plant samples 1–5 g dry weight biomass were used for lipid analyses, while for bulk soil 30 g were extracted. Depending on the amount of soil density fractions after replicate separation 30–45 mg were available for extraction of fPOM, 100–130 mg of oPOM 1.6, 750–800 mg of oPOM 2.0, and 55 g of Min fractions, respectively. Extraction was performed via Soxhlet extraction for at least 36 h using a mixture of CH_2Cl_2/CH_3OH (93/7; v/v) providing almost identical results as the automated extraction procedure (Wiesenberg et al., 2004b). After drying samples via rotary evaporation, they were separated for fatty acids and low polarity lipids using solid phase extraction (Wiesenberg et al., 2009a). After drying of the low polarity fraction, this fraction was separated into aliphatic and aromatic hydrocarbons as well as low polar heterocompounds using automated medium pressure liquid chromatography (Radke et al., 1980; Wiesenberg et al., 2004b). In this study, we discuss only aliphatic hydrocarbon and fatty acid fractions in detail as representatives for the diverse group of soil lipids. For quantification deuteriated standards (d_{39}-*n*-C_{20} fatty acid, d_{50}-*n*-C_{24} alkane) were added to the fatty acid and aliphatic hydrocarbon fractions, respectively. Compound identification and quantification was performed via an Agilent 7890 Series gas chromatograph using reference standards. Fatty acids were silylated with BSTFA [N,O-bis(trimethylsilyl)trifluoroacetamide] and detected as silyl-esters, while aliphatic hydrocarbons were directly amenable to gas chromatography.

2.4. Calculations and statistics

In general all carbon as well as total and individual lipid contents are calculated for 1 g bulk soil or plant material, respectively. For replicate analyses always the mean values and standard errors of the mean are presented.

The determination of statistically significant differences between wheat leaves and stems as well as between soil from 2002 to 2006 was performed via *T*-test for dependent samples using the STATISTICA 7.0 software. Different significance values are indicated by an asterisk ($p<0.05$: *).

3. Results

3.1. Density fractionation

The weight distribution of density fractions was the following: fPOM ≤ oPOM 1.6 < oPOM 2.0 < Min (Table 1). The sum of all particulate organic matter fractions (POM) comprised about 2% from the initial weight of bulk soil, whereas the weight of the Min fraction reached 94% (Table 1).

The soil organic carbon (SOC) content in density fractions was the lowest for fPOM and oPOM 1.6 fractions (0.6 mg g^{-1} bulk soil each; Table 1), larger for oPOM 2.0 (3.3 mg g^{-1} bulk soil) and the largest for the Min fraction (8.6 mg g^{-1} bulk soil). Hence, the Min fraction as the heaviest fraction by weight comprised 65% of total SOC content, whereas all POM fractions contributed together 28% to total SOC. The losses of 4% by weight and 7% from total SOC content could be related to dissolved organic matter that was flushed through filters, materials adsorbed to glassware or losses during decantation.

3.2. Amount of total lipids and lipid fractions

Wheat plants revealed lipid contents in a range of 21–72 mg g^{-1} biomass (Table 1). For leaves the lipid contents were 3.5 times higher than for stems. The proportion of quantified fatty acids out of the total lipids was 33% in both leaf and stem tissues. While the proportion of fatty acids was identical in both plant tissues, the proportion of alkanes related to total lipids was 7% for stem biomass and only 3% for leaf biomass (Table 1).

The amount of total lipids in bulk soil increased within four years of wheat cultivation by 15% from 339 to 391 mg g^{-1} soil (Table 1). The total amounts of quantified fatty acids and alkanes remained constant during four years in a range of 150 µg g^{-1} bulk soil for fatty acids, and 6 µg g^{-1} bulk soil for alkanes.

Lipid extract yields in density fractions revealed a decrease from 36 mg g^{-1} in the fPOM fraction towards 0.24 mg g^{-1} in the Min fraction (Table 1). The sum of quantified alkanes in SOM density fractions

Table 1
Lipid yields and summarized contents of identified fatty acids and alkanes. Arrows indicate increasing or decreasing values for plant samples between leaves and stems, for bulk soils between 2002 and 2006, and for density fractions with increasing or decreasing density, respectively.

			Relative amount of density fractions [% of bulk soil]		Soil organic carbon content [mg g^{-1} bulk soil]		Lipid extract yield [µg g^{-1} density fraction]		Lipid extract yield [µg g^{-1} plant/ bulk soil]		Sum of quantified fatty acids [µg g^{-1} bulk soil/plant]		Sum of quantified alkanes [µg g^{-1} bulk soil/plant]	
Plant	Leaves								72,191 ± 3429	↗	24,225 ± 1966	↗	2216 ± 28	↗
	Stems								20,884 ± 192		6790 ± 772		1468 ± 88	
Soil	Bulk	2002			15.0	↘			339	↘	150	–	6.0	–
		2006			16.0 ± 0.6				391 ± 19		149 ± 18		6.2 ± 0.4	
	fPOM		0.2 ± 0.0	↘	0.6 ± 0.0	↘	36,350 ± 3612	↗	52 ± 9	↘	11 ± 1	↘	0.2 ± 0.0	↘
	oPOM1.6		0.2 ± 0.0		0.6 ± 0.0		19,079 ± 211		42 ± 0		25 ± 3	↗	0.1 ± 0.0	
	oPOM2.0		1.5 ± 0.0		3.3 ± 0.1		5825 ± 70		84 ± 9		18 ± 1	↘	0.6 ± 0.0	
	Mineral		93.7 ± 0.0		8.6 ± 0.8		238 ± 3		222 ± 3		53 ± 1		3.0 ± 0.0	

accounted for 0.1–3.0 μg g^{-1} bulk soil (Table 1). While the alkane contents for POM fractions were always low in a range of 0.1–0.6 μg g^{-1} bulk soil, the largest value of 3.0 μg g^{-1} bulk soil was observed for the Min fraction. When these contents are related to total lipids, alkanes contributed between 0.2 and 1.3% to total lipids in bulk soil. This contribution increased from the fPOM towards the Min fraction. The sum of quantified fatty acids varied between 11 and 53 μg g^{-1} bulk soil for the density fractions and was the lowest for the fPOM fraction and the largest for the Min fraction. A comparatively high value of 25 μg g^{-1} bulk soil was determined for the oPOM 1.6 fraction. When the fatty acid contents are related to total lipids in the individual density fractions, the sum of fatty acids in fPOM, oPOM 2.0, and Min fractions contributed 21% to total lipids. For oPOM 1.6 this contribution reached 60%.

3.3. Distribution patterns of fatty acids

Distribution patterns of fatty acids in wheat leaves and stems were characterized by a predominance of even fatty acids (Fig. 1). The content of individual fatty acids was approximately three times higher in leaves than in stems. Most abundant acids were $C_{16:0}$ and $C_{18:1+2}$ acids, followed by even homologues with a chain length between $C_{20:0}$ and $C_{30:0}$. The variability between replicate samples in general is low (<5%), except for a larger variability of primary cell membrane lipids ($C_{16:0}$ and $C_{18:1+2}$). Especially $C_{20:0}$ and $C_{28:0}$ were significantly ($p<0.05$) enriched in leaf biomass, when compared to stem biomass.

Fatty acids in bulk soil were predominated by the ubiquitous $C_{16:0}$ and even long chain fatty acids ($C_{24:0-28:0}$), each in an amount of 20 μg g^{-1} soil. The predominance of even over odd fatty acids was lower for bulk soils than for plant tissues. While for most abundant short chain acids ($C_{16:0}$, $C_{18:1a}$) that can derive from microorganisms and plants (Harwood and Russell, 1984) no changes occurred from the year 2002 to 2006, the content of several short chain acids slightly increased ($C_{16:1+2}$, $C_{18:1b}$, $C_{18:0}$). For plant-derived long chain fatty acids ($C_{20:0}$–$C_{25:0}$) no change was observed, whereas even long chain acids with a chain length of 26 or more carbons decreased in abundance without any statistical significance ($p>0.05$).

The distribution patterns of fatty acids in density fractions in general were similar to distribution patterns in bulk soils. When related to bulk soil, all saturated fatty acids were most abundant in the Min fraction, while contribution of unsaturated acids to bulk soil fatty acids varied between density fractions. Especially unsaturated $C_{16:1+2}$ and $C_{18:1}$ fatty acids were most abundant in the oPOM 1.6 fraction. Compared to fPOM and oPOM 1.6 fractions a larger amount of long chain fatty acids ($C_{24:0}$–$C_{28:0}$) occurred in oPOM 2.0 and Min fractions.

3.4. Distribution patterns of alkanes

The distribution patterns of wheat leaf and stem alkanes were strongly predominated by odd long chain *n*-alkanes maximizing at C_{29} alkane (Fig. 2). The mass-normalized abundance of stem alkanes was 10% lower than that of leaf alkanes, but was statistically significant ($p<0.05$) only for the most abundant *n*-C_{29} alkane.

Within bulk soil odd long chain alkanes were most abundant maximizing at *n*-C_{31} (2002) or *n*-C_{29} alkane (2006), whereas the general

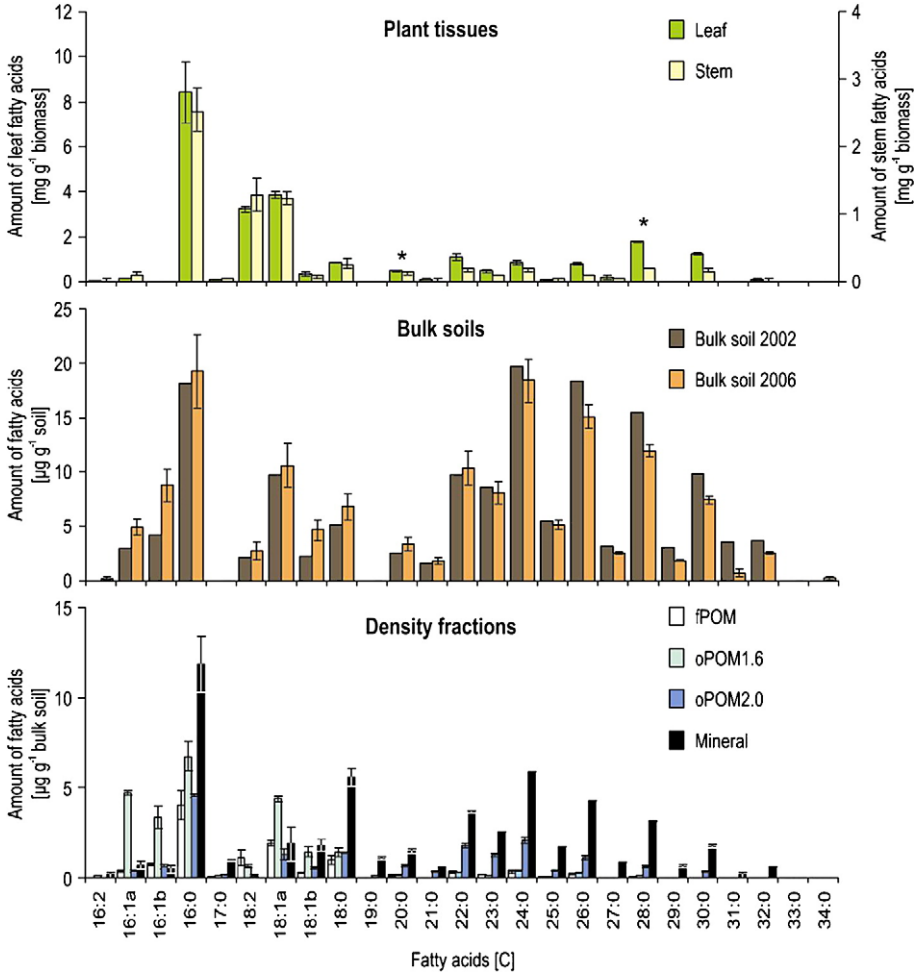

Fig. 1. Amount of fatty acids determined in wheat leaf and stem biomass, bulk soils from 2002 to 2006 as well as density fractions from the year 2006. Leaf fatty acids refer to the left *y*-axis and stem fatty acids to the right *y*-axis. Fatty acids are given as carbon numbers and double bonds within the carbon chain after the colon. Significant differences between sample pairs for leaf vs. stem biomass or soils from 2002 vs. 2006, respectively, are marked by an asterisk ($p<0.05$: *).

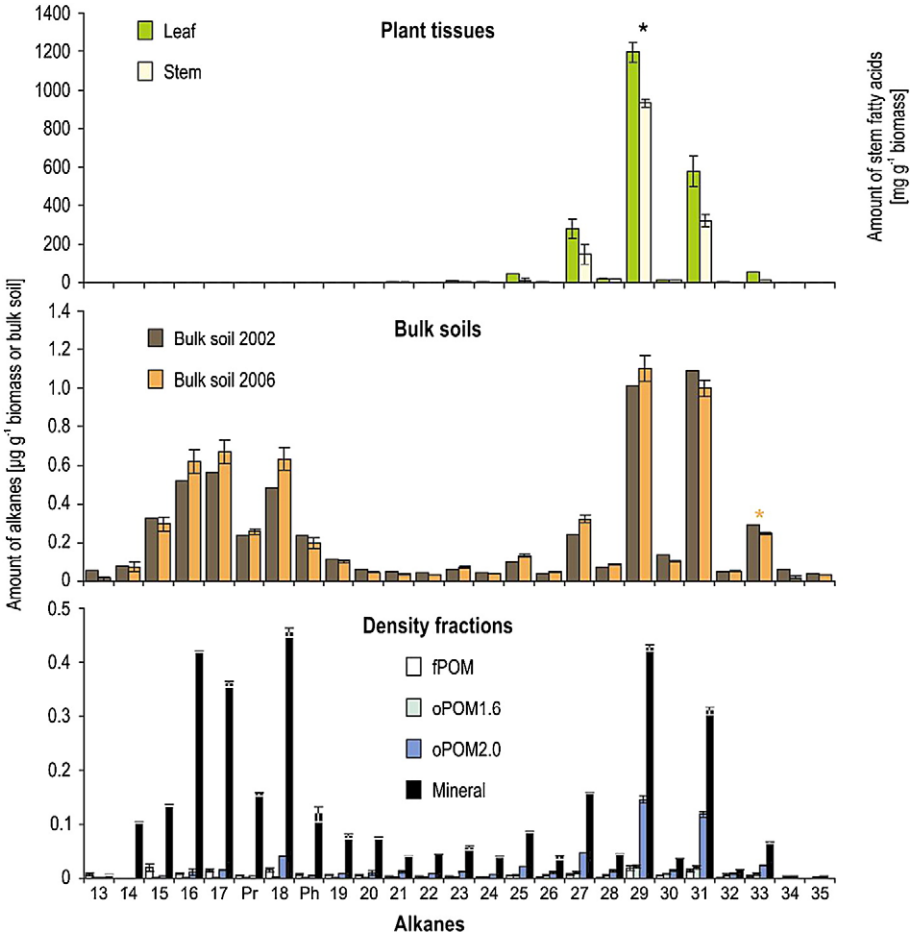

Fig. 2. Amount of alkanes determined in wheat leaf and stem biomass, bulk soils from 2002 to 2006 as well as density fractions from the year 2006. Numbers on the *x*-axis indicate carbon numbers of n-alkanes, while isoprenoid alkanes pristane (Pr) and phytane (Ph) are marked separately. Significant differences between sample pairs for leaf vs. stem biomass or soils from 2002 vs. 2006, respectively, are marked by an asterisk ($p<0.05$: *).

distribution pattern of long chain alkanes is similar to plant biomass (Fig. 2). Within short chain alkanes ($<C_{23}$) that can derive from micro-organisms or contamination (Wiesenberg et al., 2009b) a preference of even or odd homologues was missing. After introduction of wheat monoculture cropping the total abundance of short chain alkanes increased in soil with a stronger predominance of C_{16-18} alkanes. A significant ($p>0.05$) change in the distribution pattern of long chain alkanes was not observed after four years of wheat cultivation.

The alkane distribution patterns of density fractions generally revealed the same distribution pattern like bulk soil with a low variability especially for long chain alkanes (Fig. 2). For all fractions n-C_{29} alkane was the most abundant homologue. The abundance of the other odd long chain homologues was lower than that of n-C_{29} and almost similar in density fractions and bulk soil. In spite of some slight variability between the long chain alkane composition of density fractions, the differences were statistically not significant ($p>0.05$). Larger differences occurred especially for short chain alkanes. The abundance of short chain alkanes was lower than that of long chain alkanes within the individual oPOM fractions, whereas for fPOM and Min fractions short and long chain alkanes were abundant in similar quantities within each fraction.

4. Discussion

4.1. Density fractionation

The weight of density fractions and their SOC contents obtained in our study are comparable with the results of other studies on similar arable and grassland soils (Table 1; Yamashita et al., 2006). Compared to agricultural soil, forest soils were characterized by much larger proportions of POM fractions related to bulk soil and contents of SOC in POM fractions (John et al., 2005). The increased SOC content with increasing density of fractions is in accordance with the conceptual model of Golchin et al. (1997) who assumed that the protection of organic matter in soil increases with increasing density, i.e. with the increasing association with soil mineral particles. Thus, the level of association between the free light POM (fPOM) and the soil mineral matrix is low and fPOM corresponding to fresh plant litter is the most available for decomposition. In turn, oPOM 1.6 and oPOM 2.0 fractions derive from organic matter occluded in microaggregates, and the oPOM 2.0 fraction also partly derives from organic matter occluded in macroaggregates where its components act as binding agents between microaggregates. Organic matter in the Min fraction following oPOM 2.0 fraction has the largest association with mineral particles, i.e. the highest protection against decomposition.

In the investigated soil, the Min fraction has the highest contribution to the total SOC content indicating the relative importance for storage of C in this fraction. This is in agreement with results presented by Flessa et al. (2008). Despite a longer turnover time (~30%) for oPOM 1.6 than for Min fraction associated SOC, the very low mass of the oPOM 1.6 fraction (<3% of bulk soil) indicates the low relevance of this fraction for storage of total organic matter in soil (Flessa et al., 2008). Additionally, the comparatively low amount of fPOM fraction (Flessa et al., 2008) could be explained by the agricultural practice, when the whole aboveground biomass is harvested resulting in a low input of fresh plant litter into the soil thereafter.

4.2. Content of total lipids and lipid fractions

4.2.1. Plants

The lipid extract yields in a range of 21–72 mg g^{-1} biomass (Table 1) with a larger amount in wheat leaf than in wheat stem tissues are in accordance with other studies (Dove et al., 1996; Wiesenberg et al., 2009a). The high lipid content in leaves is related to a large amount of epicuticular and internal wax components (Kolattukudy et al., 1976), whereas in stem biomass fewer waxes are abundant due to a larger amount of other components including e.g. starch. The total fatty acid proportion of bulk lipids (33%) is related to the fact that fatty acids are main components of lipids and are abundant in epicuticular waxes and cell membranes of plants (Kolattukudy et al., 1976). In literature large proportions of total alkanes in leaves are typically attributed to epicuticular waxes, where alkanes are main components of the hydrophobic wax layer (Kolattukudy et al., 1976; Maffei, 1996). The larger contribution of total alkanes to bulk lipids in stem when compared to leaf biomass as in this study has not been described so far. However, the current total amount and the function of lipids like fatty acids and alkanes in stem and root tissues are currently not available. The comparatively large contribution of alkanes to stem lipids and the common property of alkanes as main epicuticular wax components result in an improved protection of stems against environmental influences when compared to leaf biomass. The current study cannot contribute to the discussion whether alkanes are mainly found in external waxes of stems, or whether they occur internally in large amounts with a different function than external lipids. Nevertheless, the larger proportion of alkanes similarly like lignin proportions in stems than in leaves might be responsible for the lower degradability of stem than leaf biomasses resulting from a larger turnover time of alkanes in soil when compared to other lipids (Wiesenberg et al., 2004a, 2008b).

4.2.2. Bulk soil and SOM density fractions

The content of total lipids in bulk soil in a range of 339–391 μg g^{-1} soil (Table 1) is typical of agricultural soils (Wiesenberg et al., 2004a, 2006). As the amount of quantified fatty acids and alkanes remained constant during the experiment, the increase of total lipids from 2002 to 2006 must be due to an increase in other lipids than the analysed ones, probably related to alcohols, ketones, or esterified compounds.

The decrease of lipid contents with increasing association with minerals from 36 mg g^{-1} in the fPOM fraction towards 238 μg g^{-1} in the Min fraction (Table 1) is according to the conception of soil aggregate formation and decomposition of organic matter (Oades, 1984; Golchin et al., 1997; Paustian et al., 2000). Thus, the highest lipid content within the fPOM fraction (Table 1) was similar to the lipid content of plant tissues and clearly indicates the source of this fraction mainly consisting of freshly incorporated plant debris. With ongoing degradation of plant debris the organic matter is occluded within aggregates of different sizes (Golchin et al., 1997; Paustian et al., 2000). Accordingly, lipid contents of oPOM decreased with increasing fraction density from 1.6 to 2.0 g cm^{-3}, whereas they were comparatively high to lipid contents of bulk soil and Min fraction (Table 1). The Min fraction had the lowest lipid content normalized to the mass of this fraction, which is due to the comparatively low amount of organic matter related to the mass of minerals in this fraction. In contrast to the specific lipid contents of density fractions, the total lipid contents considering the weight of those fractions showed the reverse trend (Table 1). Thus, the largest amount of lipids was determined for the Min fraction (more than 50% of total lipids), accounting for 93% of the weight of bulk soil (Table 1). The decrease of total lipid contents related to the bulk soil from dense Min fraction to light oPOM 1.6 indicates a decreasing storage of organic compounds, e.g. lipids. Hence, the allocation of organic compounds like lipids in soil is strongly dependent on the adsorption of lipids to minerals.

The increase of total alkanes normalized to bulk soil from fPOM to the Min fraction indicates an improved association with soil mineral particles as described for total lipids in this direction. The uniform contribution of fatty acids to total lipids in most of the density fractions except for the oPOM 1.6 fraction cannot be explained by the presence of plant-derived lipids, exclusively. Furthermore, lipids in soil derive not only from plant biomass, but additionally from microbial biomass and contamination (Harwood and Russell, 1984). As fatty acids in soil are not main parts of potential contamination sources like residues from incomplete combustion processes or dust (Rethemeyer et al., 2004; Wiesenberg et al., 2004a), the strong enrichment of fatty acids in the oPOM 1.6 fraction related to fPOM and oPOM 2.0 fractions due to contamination can be excluded. The incorporation of plant-derived fatty acids into soil follows a degradation pathway from plant residues in fPOM fraction towards more mineral associated lipids in oPOM and Min fractions (John et al., 2005). A selective enrichment of plant-derived fatty acids in oPOM 1.6 fraction is not expectable, because plant-derived fatty acids degrade faster than other lipids in soil (Wiesenberg et al., 2004a). Finally, fatty acids in soils are largely abundant in microorganisms and used as molecular markers of living tissues as phospholipid fatty acids (PLFA; Zelles et al., 1994; Zelles, 1999). The density fractionation and lipid extraction with several drying steps and the destruction of aggregates by shaking with glass beads must have destroyed living microbial tissues releasing free extractable fatty acids as degradation products of PLFA. Hence, the enrichment in fatty acids in light oPOM 1.6 can be associated with the presence of large amounts of microorganisms in soil microaggregates, where oPOM 1.6 fraction is mostly related to. The preferable location of microorganisms in soil aggregates of different sizes was confirmed by findings in other studies (Postma and van Veen, 1990; Guggenberger et al., 1999; Dorodnikov et al., 2009a,b).

4.3. Distribution pattern of fatty acids

4.3.1. Plants

The observed distribution pattern of wheat fatty acids with a predominance of even fatty acids (Fig. 1) is typical of biomasses of higher plants (Harwood and Russell, 1984). The higher content of lipids in leaf than in stem tissues is related to a higher content of cuticular waxes in leaf when compared to stem biomasses (Dove et al., 1996; Wiesenberg et al., 2004b). The bimodal distribution pattern of fatty acids with the first maximum at $C_{16:0}$ and $C_{18:1+2}$ and the second maximum at long chains ($C_{20:0-30:0}$) is in agreement with literature results for wheat biomass fatty acids (Wiesenberg et al., 2004a,b; Wiesenberg and Schwark, 2006). Long chain acids ($C_{22:0-26:0}$) confirm typical patterns for temperate C_3-grasses as indicated by low values (<0.67) of the Carboxylic Acid Ratio ($CAR = C_{24:0}\ (C_{22:0} + C_{26:0})^{-1}$ fatty acids; Wiesenberg and Schwark, 2006) with 0.44 ± 0.01 for the leaf and 0.66 ± 0.11 for stem biomass (Table 2). In general, the distribution patterns of stem and leaf fatty acids are similar and can be differentiated within long chain acids. Lower relative amount of long chain and very long chain fatty acids ($>C_{26:0}$) in stem biomass as compared to leaf biomass could be observed due to a more effective carbon chain elongation mechanism during fatty acid biosynthesis in leaf tissues (Kolattukudy et al., 1976) when compared to stem biomass. The high abundance of very long chain acids ($\geq C_{28:0}$) in leaf biomass has not been described previously for wheat plants, but was observed for other grasses like maize (Wiesenberg et al., 2004a) and *Miscanthus* (Wiesenberg et al., 2009a), or other plants including coffee (Stocker and Wanner, 1975) and clover (Wiesenberg et al., 2008a). The variability of primary cell membrane fatty acids ($C_{16:0}$ and $C_{18:1+2}$) between replicates is common for samples deriving from natural environments. This is related to differences in micro-environmental and nutritional conditions between sampled plots, reflected in a slight variability of the plant lipid composition (Baker, 1974). Due to the

Table 2
Fatty acid molecular proxies. Arrows indicate increasing or decreasing values for plant samples between leaves and stems, for bulk soils between 2002 and 2006, and for density fractions with increasing or decreasing density, respectively.

			$C_{16:1-2} \times C_{16:0}^{-1}$		$C_{18:1-3} \times C_{18:0}^{-1}$		ACL_{FA}		CPI		$\Sigma C_{20-34} \times \Sigma C_{16-19}^{-1}$		CAR	
Plant	Leaves		<0.1	–	8.6 ± 0.1	↘	19.6 ± 0.1	↗	4.1 ± 0.1	↗	0.4 ± 0.0	↗	0.44 ± 0.01	↘
	Stems		0.1 ± 0.0		10.5 ± 1.0		18.5 ± 0.2		3.1 ± 0.2		0.2 ± 0.0		0.66 ± 0.11	
Soil	Bulk	2002	0.4	↘	2.7	–	23.2	↗	2.9		2.3	↗	0.70	↘
		2006	0.8 ± 0.0		2.6 ± 0.1		22.2 ± 0.3		2.9 ± 0.0		1.6 ± 0.2		0.72 ± 0.00	
	fPOM		0.3 ± 0.1	↘	5.6 ± 2.1	↗	17.7 ± 0.0	↗	3.8 ± 0.8	↗	0.1 ± 0.0	↘	0.70 ± 0.00	↘
	oPOM1.6		1.3 ± 0.0	↗	4.9 ± 0.5		16.4 ± 0.0		7.5 ± 0.7		0.1 ± 0.0		0.69 ± 0.00	
	oPOM2.0		0.2 ± 0.0	–	1.4 ± 0.3		20.1 ± 0.1	↘	2.7 ± 0.0		0.9 ± 0.0		0.72 ± 0.00	
	Mineral		0.2 ± 0.1		0.7 ± 0.3		21.1 ± 0.0		2.6 ± 0.0		1.1 ± 0.0		0.74 ± 0.00	

similarity of wheat stem and leaf biomass observed in this study and a similar composition in roots as described previously (Wiesenberg et al., 2004a; Wiesenberg and Schwark, 2006), the fatty acid distribution patterns of wheat aboveground biomass can be used to assess plant biomass incorporation, as root tissues were not available.

4.3.2. Bulk soil and density fractions

The distribution pattern of fatty acids in bulk soil (Fig. 1) was typical of rural agricultural soils without significant contribution of fossil fuel- or combustion product-derived contamination and at the same time with a large abundance of microorganism-derived components (Wiesenberg et al., 2004a,b). The constant amount of most fatty acids from 2002 to 2006 is related to the uniform incorporation of plant biomass during the experiment. The increased abundance of $C_{16:1+2}$ fatty acids reflected a larger amount of microorganism-derived components (Harwood and Russell, 1984) present in soil from 2006 than four years before. The decrease of long chain fatty acids ($\geq C_{26:0}$) within four years of wheat cropping is due to the degradation of plant-derived long chain acids in soil, whose contribution to soil decreased since the introduction of the wheat cultivation. This decrease is due to the predominant incorporation of wheat root biomass and stubbles, while aboveground biomass is removed during harvest. As root tissues are depleted in long chain fatty acids ($\geq C_{26:0}$) when compared to aboveground biomass (Wiesenberg et al., 2004a), the depletion of $\geq C_{26:0}$ in bulk soil from 2006 can be explained by the lower contribution of aboveground tissues since the introduction of the experiment.

Generally, the distribution patterns of fatty acids in density fractions confirm the pattern of fatty acids in bulk soils (Fig. 1). The large abundance of $C_{16:1+2}$ and $C_{18:1}$ acids in oPOM 1.6 fraction clearly indicates the strongest contribution of fatty acids derived from the microorganism to this fraction (Harwood and Russell, 1984) when compared to other fractions. The larger amount of long chain fatty acids ($>C_{20:0}$) in oPOM 2.0 and Min compared to the other density fractions is related to the improved adsorption of long chain fatty acids to mineral surfaces in these fractions.

4.3.3. Molecular proxies

Several molecular indicators can be used to assess the source of organic matter at the molecular level (Gleixner et al., 2001) and to determine the protection of fatty acids against degradation in the organic matter adsorbed to the mineral particles. A selection of molecular ratios that are useful to trace e.g. microorganism- vs. plant-derived contribution and stage of degradation are discussed in the following.

High values (>0.1) of the ratio $C_{16:1+2} \times C_{16:0}^{-1}$ indicate the contribution of microorganism-derived compounds to the total amount of fatty acids (Table 2) due to the common presence of $C_{16:1+2}$ fatty acids in microbial tissues (Harwood and Russell, 1984). Contrary, plant tissues are commonly depleted in $C_{16:1+2}$ (ratio<0.1) when compared to microbial biomass (Table 2; Harwood and Russell, 1984). Within bulk soils an increase in the portion of unsaturated $C_{16:1+2}$ fatty acids occurred from 2002 to 2006, which can be related to the increasing amount of microorganism remains in the soil or a decreasing contribution of plant-derived $C_{16:0}$ fatty acids. The latter must be more responsible for the time-related increase of the $C_{16:1+2} \times C_{16:0}^{-1}$ ratio as the microorganism activity and hence microorganism remains are commonly lower under cropland than under grassland cultivation (Zelles et al., 1994). For most density fractions the $C_{16:1+2} \times C_{16:0}^{-1}$ ratio revealed low values, except for the oPOM 1.6 fraction. Therein, contribution of microorganism-derived fatty acids is most likely the reason for the large proportion of unsaturated $C_{16:1+2}$ acids. This confirms NMR spectroscopy (Golchin et al., 1994) showing strong enrichment of alkyl groups in oPOM 1.6 fractions. This is related to the contribution of microorganism- or plant-related compounds present in these fractions. Thus, the proposed $C_{16:1+2} \times C_{16:0}^{-1}$ ratio could be used to identify the contribution of microorganism-derived compounds to the density fractions.

The increase or decrease of the ratio of unsaturated vs. saturated fatty acids $C_{18:1-3} \times C_{18:0}^{-1}$ reflects the ongoing degradation from plant biomass towards soil organic matter (Wiesenberg et al., 2010). While plant tissues are enriched in $C_{18:1-3}$ fatty acids, successive degradation decreases $C_{18:1-3}$ fatty acid contents, but $C_{18:0}$ acids are selectively preserved. This is related to the higher functionality of unsaturated compared to saturated fatty acids during degradation and a further contribution of saturated short chain acids during degradation of esterified molecules with fatty acid side chains and degradation of long chain fatty acids (e.g. Kolattukudy et al., 1976). Consequently, wheat leaf and stem tissues are characterized by the largest values of the $C_{18:1-3} \times C_{18:0}^{-1}$ ratio (Table 2). The decrease of this ratio from fPOM towards Min fraction indicates the degradation of plant-derived fatty acids with increasing association to minerals. Hence, the decrease of this ratio clearly indicates the degradation stage of fatty acids.

As second molecular proxy reflecting the source and the degradation stage of fatty acids in soil the Average Chain Length (ACL_{FA}) can be used:

$$ACL_{FA} = \sum(z_n \times n) / \sum(z_n),$$

with z_n as the relative amount of the fatty acids with n C atoms, whereby n was 16 to 34 C atoms. The strongest abundance of $C_{16:0}$ and $C_{18:0-3}$ fatty acids in plant tissues and a large abundance in even $C_{20:0-30:0}$ fatty acids results in intermediate ACL_{FA} with a range between 18–20 for plant tissues (Fig. 2). Organic matter derived from microorganisms is characterized by lower ACL_{FA} due to the absence of any long chain fatty acids ($>C_{22:0}$) and must be related to the range of the predominant fatty acids between 16 and 18 carbons. With increasing degradation a decrease of easily degradable short chain and a selective enrichment of long chain fatty acids can be expected resulting in an increasing ACL_{FA} with increasing degradation. The ACL_{FA} was in a range of 18.5–19.6 for wheat tissues confirming the theory. ACL_{FA} values were lower for fPOM fraction than for plant materials due to a contribution of microorganism remains. The lowest ACL_{FA} was observed in oPOM 1.6 fraction indicating the strongest contribution of microorganism-related fatty acids to this fraction. With increasing binding to mineral particles, the ACL_{FA} increased from oPOM 1.6 towards oPOM 2.0 and Min fractions. This is related to a depletion of short chain fatty acids and a predominant preservation of

long chain fatty acids in mineral associated organic matter. Hence, the increased contribution of degraded fatty acids to heavier than to lighter SOM fractions indicate the increasing recalcitrance of the former and in accordance with the conception of the SOM decomposition process (Paustian et al., 2000; Jastrow et al., 2007).

The third molecular proxy – the carbon preference index (CPI) – characterizes the predominance of even over odd chain acids:

$$CPI = [(\Sigma C_{20+22+24+26+28+30} / \Sigma C_{19+21+23+25+27+29}) + (\Sigma C_{20+22+24+26+28+30} / \Sigma C_{21+23+25+27+29+31})] / 2$$

The CPI is largest in living plant tissues due to the preferential biosynthesis of even homologues (Kolattukudy et al., 1976) and lowest in degraded plant-derived SOM due to an equilibration of even and odd homologues during degradation. The CPI of plant materials showed intermediate values between soil fractions and no significant increase in biomass samples (Table 2, Fig. 2). Commonly, large CPI values (>3) are present in plant biomass and values decrease with ongoing degradation. The comparatively low CPI values of plant samples must be due to sampling of plants during harvest and thus these values do not represent living plant biomass, where a stronger enrichment of even fatty acid homologues can be expected. While lowest values were present in Min and oPOM 2.0 fractions the CPI value was largest in the oPOM 1.6 fraction (Table 2). The CPI value in fPOM fractions were in a range of plant materials. Hence, freshly incorporated plant litter is attributed to fPOM fractions, which is common knowledge (John et al., 2005) for bulk carbon, but was not previously demonstrated for soil lipids. The large CPI value in the oPOM 1.6 fraction is attributed to the degradation of plant-derived organic matter including wax esters, which are enriched in even long chain acids (Kolattukudy et al., 1976). This results in a predominance of these components in this density fraction. With further degradation and association with minerals in oPOM 2.0 and Min fractions the CPI value decreases due to an equilibration of even and odd homologues of long chain fatty acids. Bulk soil CPI values revealed a composite of fraction values.

The fourth molecular proxy – the ratio of long chain vs. short chain acids ($\Sigma C_{20-34} \times \Sigma C_{16-19}^{-1}$) – indicates the contribution of fresh plant- and microbial-derived fatty acids vs. a degradation pattern. Fresh plant materials are expected to be characterized by low values due to the predominance of fatty acids with 16 and 18 carbons (Kolattukudy et al., 1976; Harwood and Russell, 1984). Microorganism-derived fatty acids must reveal extremely low values due to the absence of long chain acids (Harwood and Russell, 1984). Hence, fresh plant- and microorganism-derived biomass is indicated by low values of the ratio of long chain vs. short chain acids. Contrastingly, degraded organic matter like brown coal is depleted in short chain acids (Wiesenberg et al., 2004a,b) and thus large values of the ratio can be expected. Observed long chain vs. short chain fatty acid ratios (Table 2) were low (0.2–0.4) for plant tissues and lowest for fPOM and oPOM 1.6 fractions (0.1). The extremely low values in the latter ones are related to the plant-dominated input and the contribution of microorganism-derived components, both characterized by low amounts of long chain acids. The values significantly increased from oPOM 1.6 towards oPOM 2.0 and Min fractions, indicating an increasing association of old, degraded biomass with increasing association with minerals. This is slightly different to the ^{14}C ages determined in density fractions (Rethemeyer et al., 2005). They obtained lowest ^{14}C ages in oPOM 2.0 fraction and increasing ^{14}C ages over fPOM, Min, towards oPOM 1.6 fractions. However, they did not investigate the sources of organic matter in the density fractions. Probably, the contribution of contaminants like ^{14}C free combustion residues or dust present in oPOM 1.6 and fPOM fractions resulted in their lower ^{14}C content and subsequently higher ^{14}C ages. Our data clearly indicates the stronger degradation of organic matter in the density fractions following the direction fPOM>oPOM 1.6>oPOM 2.0>Min. The increasing association with minerals (Golchin et al., 1997) and simultaneously the degradation of plant- and microorganism-derived organic matter determined in the oPOM 2.0 and Min fractions result from a selective degradation of short chain fatty acids and preferential preservation of resistant long chain biopolymers (Lichtfouse et al., 1998b) in the SOM determined in these fractions.

4.4. Distribution patterns of alkanes

4.4.1. Plants

The alkane distribution patterns maximizing at *n*-C_{29} (Fig. 3) confirm previous results determined for wheat straw, leaf, and stem tissues obtained by Bianchi and Corbellini (1977), Bianchi and Bianchi (1990), Lichtfouse et al. (1994), Maffei (1996), and Wiesenberg et al. (2004a,b).

4.4.2. Bulk soil and SOM density fractions

The change of the most abundant alkane in bulk soil from *n*-C_{31} in 2002 towards *n*-C_{29} in 2006 (Fig. 3) reflects the modified contribution of plant biomass after vegetation change from grassland to wheat cultivation. The grassland vegetation in advance of wheat introduction is supposed to be dominated by *n*-C_{31} alkane. In spite of missing reference samples from the vegetation that existed in former times on the plot, this grassland-derived pattern with a predominance of *n*-C_{31} alkane can be a true indicator for the former vegetation, because grasses are often characterized by a predominance of *n*-C_{31} alkanes (Maffei, 1996). Hence, the introduction of wheat monoculture changed the alkane distribution pattern towards a predominance of *n*-C_{29} alkane. The missing preference of even or odd homologues from short chain alkanes (<C_{23}) in soil can be attributed to the presence of degraded materials, which derived either from the input of degraded alkanes e.g. from a contamination by remains of fossil fuel burning or fossil fuel itself, or a microbial degradation of lipids within soil (Wiesenberg et al., 2004a,b). While during microbial degradation predominantly odd short chain alkanes are produced and abundant in soil, such predominance is commonly missing in fossil fuel-derived alkanes. Additionally, the large abundance of pristane and phytane in soil confirms the fossil fuel source of these alkanes (Peters et al., 2005). Hence, the short chain *n*-alkanes and isoprenoid alkanes indicate a contamination by fossil fuel as previously determined for other soils (Lichtfouse et al., 1997; Wiesenberg et al., 2004a,b). The enrichment of short chain alkanes (C_{16-18}) after introduction of wheat

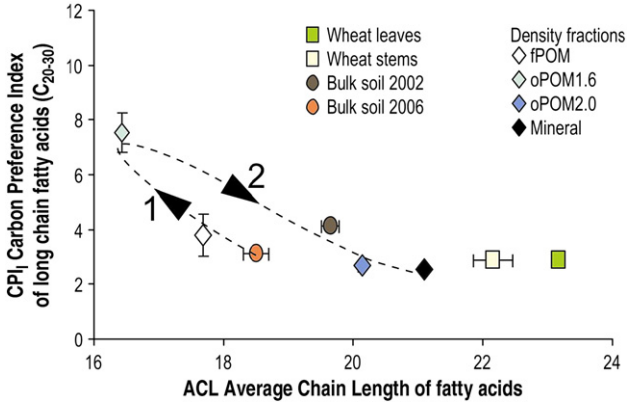

Fig. 3. Carbon preference index (CPI) of long chain acids vs. average chain length (ACL_{car}) indicating the degradation pathway (broken line) of plant biomass entering soil via organic matter fractions. Numbers beneath the broken line indicate the first degradation step of plant biomass over fPOM towards microbial biomass dominated oPOM1.6 fraction. Thereafter the second degradation step leads to a successional degradation of both plant and microbial biomass in oPOM2.0 towards Min fractions.

cropping can be attributed to the ploughing applied to the soil annually after experimental beginning, leading to an improved aeration of the soil compared to previously not ploughed grassland soil. This effect together with a breakup of aggregates as a result of ploughing release more organic components from organic matter bound to mineral surfaces and within aggregates and probably improved the desorption of organic compounds like short chain alkanes from mineral surfaces.

Within density fractions equal quantities of short and long chain alkanes in the fPOM and Min fractions were observed. Plant fragments and black particles were main parts of the fPOM fraction. The black particles might be attributed to black carbon deriving from incomplete combustion processes or probably derived from the input of coal dust (e.g. from charcoal; Wiesenberg et al., 2009b). This could explain the large abundances of short chain n-C_{16} and n-C_{18} alkanes with predominance of even homologues in the fPOM fraction. Similarly, the large abundance of pristane and phytane as degradation proxies for organic matter (Peters et al., 2005) indicated the allochthonous origin of these components in the fPOM fraction according to observations made by Lichtfouse et al. (1997) on short chain alkanes. This is in contradiction with observations made by Rethemeyer et al. (2005), who reported a larger proportion of fossil fuel-derived C in oPOM 1.6 than in fPOM fraction. However, they did not describe the molecular composition of these fractions. Probably, a larger amount of plant litter in fPOM, denser brown coal dust (Rethemeyer et al., 2004) in the study of Rethemeyer et al. (2005) and a larger contribution of fossil fuel derived components to fPOM in our study might be the reason for the effect. The similar distribution pattern of short chain alkanes in the Min fraction like in the fPOM fraction was probably related to a natural degradation of dust and adsorption of degradation products to minerals. Alternatively, an artificial degradation of black carbon during the density fractionation procedure and subsequently an adsorption of black carbon fragments to mineral surfaces could be the reason for this similarity with respect to short chain alkanes in both fractions.

4.4.3. Molecular proxies

Several diagnostic ratios were determined for alkanes to attribute the source (autochthonous plant- and microbial-derived vs. allochthonous-derived from dust and incomplete burned biomass) and the degree of degradation of lipids within the different density fractions. The average chain length (ACL_{alk}) is commonly used to differentiate microbial (ACL_{alk} ~16–19) from higher plant (ACL_{alk} ~27–31) biomass origin of alkanes in soils:

$$ACL_{alk} = \sum(z_n \times n) / \sum(z_n),$$

with z_n as the relative amount of the n-alkane with n carbons, whereby n was 13 to 35 carbons. For all plant samples ACL_{alk} (Table 3) was always high (29.2) due to the missing short chain alkanes and the large abundance of long chain homologues. Bulk soils showed comparatively low values of ACL_{alk} with 22.5 (2002) and 22.3 (2006) as a consequence of largely abundant short chain alkanes. The ACL_{alk} decreasing by time was related to the larger abundance of short chain homologues, whereas the amount of long chain homologues remained unchanged. However, this trend was not statistically significant ($p>0.05$). As a consequence of the presence of allochthonous short chain alkanes deriving from contamination, ACL_{alk} was lowest in fPOM and Min fractions and was attributed to contamination rather than to a biological source of alkanes in the density fractions.

The carbon preference index was calculated for short chain (CPI_s) and for long chain (CPI_l) alkanes separately, because of different sources for short and long chain alkanes:

$$CPI_s = [(C_{15} + C_{17} + C_{19}) \times (C_{14} + C_{16} + C_{18})^{-1} + (C_{15} + C_{17} + C_{19}) \times (C_{16} + C_{18} + C_{20})^{-1}] \times 0.5$$

$$CPI_l = [(\Sigma C_{27-33odd} / \Sigma C_{26-32even}) + (\Sigma C_{27-33odd} / \Sigma C_{28-34even})] \times 0.5$$

Generally, both CPI values indicate the preference of odd alkane homologues over even counterparts. The CPI is stronger in living biological tissues and decreases with degradation of organic matter (Peters et al., 2005). Charred biomass is indicated by low CPI_s due to an enrichment of even homologues during thermal degradation process (Wiesenberg et al., 2009b). The CPI_s was high for wheat and fPOM (Table 3). The higher CPI_s value in fPOM than in plant biomass is attributed to the abundance of microorganism-derived odd alkanes in fPOM. The oPOM 1.6 fraction, which was observed to yield a large amount of microbial biomarkers as derived from the fatty acid distribution pattern, comprised a large CPI_s value due to the presence of degraded plant residues and especially microbial biomass. oPOM 2.0 and Min fractions were characterized by very low values due to the presence of bound residues of thermally degraded biomass as previously found to be predominated by even short chain alkanes (Wiesenberg et al., 2009b). The CPI_l indicates the degree of degradation for fresh plant biomass with values commonly $\gg 20$ entering bulk soil and soil fractions, where in strongly degraded samples CPI_l values ~1 can be expected (Peters et al., 2005). Hence, wheat samples revealed large CPI_l values (>40; Table 3), which are typical of fresh plant biomass. The values for bulk soil with CPI_l ~8 reflect a large proportion of plant-derived organic matter present in the soil. The density fractions showed some surprising results. Especially the fPOM fraction was expected to be in a range between plant biomass and bulk soil due to a large amount of freshly incorporated plant biomass. However, the CPI_l value was comparatively low (4.3), which is to be related to the presence of fossil fuel-derived organic matter either as dust particles or as residues from incomplete combustion processes (Wiesenberg et al., 2009b). The lowest value (2.8) was observed for oPOM 1.6 fraction, where a large contribution from microorganism-derived components was related to the presence of microbial biomarkers. Degradation products of plant-derived organic matter predominated in fPOM and oPOM 1.6 fractions. The other fractions showed larger values (oPOM 2.0: 7.4, Min: 6.7) related to the improved protection of alkanes with

Table 3
Alkane molecular proxies. Arrows indicate increasing or decreasing values for plant samples between leaves and stems, for bulk soils between 2002 and 2006, and for density fractions with increasing or decreasing density, respectively.

			ACL_{alk}		CPI_s		CPI_l		$CPI_l \times CPI_s^{-1}$	
Plant	Leaves		29.2 ± 0.0	–	1.2 ± 0.1	–	47.0 ± 2.9	↗	38.5 ± 4.2	↗
	Stems		29.2 ± 0.0		1.3 ± 0.0		42.1 ± 0.6		32.4 ± 0.1	
Soil	Bulk	2002	22.5	–	0.9	–	7.9	↘	8.4	↘
		2006	22.3 ± 0.2		0.8 ± 0.0		8.8 ± 0.3		10.8 ± 0.4	
	fPOM		22.0 ± 0.2	↘	1.8 ± 0.4	↗	4.3 ± 0.5	↗	3.0 ± 0.9	–
	oPOM1.6		27.7 ± 0.2		1.0 ± 0.0		2.8 ± 0.4	↘	3.0 ± 0.4	↘
	oPOM2.0		26.9 ± 0.3	↗	0.5 ± 0.1		7.4 ± 2.6	↗	14.1 ± 3.5	↗
	Mineral		22.1 ± 0.1		0.6 ± 0.0		6.7 ± 0.1		11.3 ± 0.1	

increasing association with mineral particles when compared to the lower protection in fPOM and oPOM 1.6 fractions.

The value of $CPI_l \times CPI_s^{-1}$ is supposed to be a measure for the predominant source of alkanes in soil samples. The value was expected to be high ($\gg 10$) in plant-derived samples due to the strong predominance of odd long chain alkanes and a low CPI_s value. In contrast, low $CPI_l \times CPI_s^{-1}$ values (<1) can be related to a low CPI_l in microorganism-derived SOM and contaminated samples. Largest values were observed in plant materials (>30) confirming this hypothesis (Table 3). Vice versa fPOM and oPOM 1.6 fractions were dominated by microbial components and contamination products and hence revealed lowest results (3) with a present proportion of plant-derived alkanes. oPOM 2.0 and Min fractions showed intermediate values due to the presence of some contamination products (Min) and predominantly plant biomass-derived components. Bulk soil revealed intermediate values, confirming the admixture of plant biomass input and contamination to SOM. The significantly ($p<0.05$) increasing $CPI_l \times CPI_s^{-1}$ value from 2002 to 2006 for bulk soil might be related to an improved preservation of plant-derived alkanes during the experiment. Hence, this diagnostic proxy can be recommended to elucidate plant- and microorganism-derived contributions in soil (fractions).

5. Conclusion

This is the first study describing the lipid composition of soil density fractions and the association of lipids with mineral particles in soil. The molecular composition of lipids in density fractions in general confirms the composition for bulk organic carbon, whereas several new findings have been made concerning the contribution of plant- and microbial-, as well as contamination-derived lipids to soil density fractions.

With increasing association to mineral particles the stabilization of plant-derived fatty acids and alkanes in SOM increases. Plant litter dominates free particulate organic matter (fPOM), where microbial degradation starts. Within occluded POM the contribution of microorganism-derived compounds, like unsaturated $C_{16:1}$ fatty acids, was largest in oPOM 1.6 fractions. The degradation of plant-derived organic matter and subsequently the attribution of degradation products to the low density oPOM 1.6 fractions resulted in an enrichment of even long chain fatty acids that derive from the microbial degradation of wax esters. In dense occluded POM 2.0 the contribution of microbial-derived components was lower than in fPOM and oPOM 1.6 and protection of plant-derived long chain lipids occurs in this fraction. The mineral particle dominated Min fraction is the largest pool of lipids in soil due to the strongest binding of lipids on mineral surfaces or organo-mineral complexes.

Several molecular parameters like the ratio of long chain vs. short chain fatty acids, the carbon preference index (CPI) and the average chain length of fatty acids (ACL_{FA}) indicate a stronger contribution of old, degraded plant-derived organic matter in Min when compared to POM fractions. Aliphatic hydrocarbons indicated the presence of fossil fuel-derived carbon especially to the fPOM and Min fractions. While in fPOM dust and soot particles with a low density occurred, in the Min fraction binding of soot and dust degradation products to minerals occurred. Though the distribution of lipids in density fractions was shown in this study, further data is required concerning the dynamics and preservation of lipids in aggregates of different sizes to clearly elucidate the protection of SOM at the molecular level with respect to lipids.

The vegetation change from green fallow vs. wheat cropping led to a change of soil lipid composition even after four years. Some molecular proxies were sensitive enough to trace the replacement of one C_3 grass by another C_3 grass even after this short period. However, the changes were mostly not significant probably related to this short duration. Hence, further data is required concerning long term effects of such vegetation change on SOM.

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