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COMPLEXITY OF SOIL ORGANIC MATTER: AMS ^{14}C ANALYSIS OF SOIL LIPID FRACTIONS AND INDIVIDUAL COMPOUNDS

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ABSTRACT. Radiocarbon measurements of different lipid fractions and individual compounds, isolated from soil samples collected on 2 different agricultural long-term study sites, located in the rural area of Rotthalmünster (Germany) and in the city of Halle/Saale (Germany), were analyzed to obtain information about sources and the stability of soil organic matter (SOM). Different lipid compound classes were isolated by automated solvent extraction and subsequent medium-pressure liquid chromatography. Generally, ^{14}C contents of lipid compound classes from topsoil samples of maize plots at Rotthalmünster are close to the modern atmospheric ^{14}C content. Lower ^{14}C values of aliphatic and aromatic hydrocarbons isolated from neutral lipids suggest a contribution of old carbon to these fractions. In contrast, ^{14}C values of bulk soil (52 pMC) as well as isolated lipid classes from Halle are highly depleted. This can be attributed to a significant contribution of fossil carbon at this site. Extremely low ^{14}C contents of aromatic (7 pMC) and aliphatic hydrocarbons (19 pMC) reflect the admixture of fossil hydrocarbons at the Halle site. Individual phospholipid fatty acids (PLFA), which are used as a proxy for viable microbial biomass, were isolated by preparative capillary gas chromatography (PCGC) from topsoils at Rotthalmünster and Halle. PLFA ^{14}C values are close to atmospheric ^{14}C values and, thus, indicate a clear microbial preference for relatively young SOM. At Rotthalmünster, the ^{14}C concentration of short-chain unsaturated PLFAs is not significantly different from that of the atmosphere, while the saturated PLFAs show a contribution of sub-recent SOM extending over the last decades. At Halle, up to 14% fossil carbon is incorporated in PLFAs *n*-C17:0 and *cy*-C18:0, which suggests the use of fossil carbon by soil microorganisms. Moreover, it can be concluded that the ^{14}C age of soil carbon is not indicative of its stability.

INTRODUCTION

Soil organic matter (SOM) is an important factor in the global carbon cycle. As SOM is composed of a complex mixture of organic components in various stages of decomposition and transformation, the objective of diverse studies is to differentiate between different organic matter pools or components to be able to follow carbon transformation and sequestration in the soil system.

Radiocarbon measurements have been helpful to classify bulk SOM separated into physically- or chemically-defined organic matter pools (O'Brien 1986; Balesdent 1987; Trumbore et al. 1990; Trumbore 1993; Trumbore and Zheng 1996). ^{14}C concentrations of SOM pools represent the mean residence time and, thus, the stability of the organic matter (Scharpenseel and Becker-Heidmann 1992; Trumbore 1996). However, most physical and chemical SOM fractions still consist of a complex mixture of organic molecules with different origin and decomposability. Additionally, SOM properties may be influenced by the contribution of anthropogenic pollutants, such as fossil fuel-derived carbon, which complicates the interpretation of the ^{14}C data (Rumpel et al. 2003; Rethemeyer et al., forthcoming). Our results on SOM samples from Halle showed this fossil contamination persisted to varying degrees in the usual physical and chemical fractions, including a lipid and phospholipid fraction (Rethemeyer et al., forthcoming). More specific separation methods are thus needed.

Lipids, representing 4–8% of SOM, are assumed to be of high importance for SOM stabilization. In soils, they mainly derive from plants and microorganisms, but they also can be of anthropogenic ori-

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gin (Gregorich et al. 1996; Lichtfouse et al. 1997). Soil lipids are a relatively stable organic carbon fraction that contain several biomarker compounds which can be related to the input of plant constituents to the soil and to their transformations (Lichtfouse et al. 1994, 1997; Bol et al. 1996; van Bergen et al. 1997).

Compound-specific accelerator mass spectrometry (AMS) ^{14}C analysis is a modern technique which can help to exclude contaminating carbon sources, and to obtain information on the origin and biodegradability of organic matter in soils and sediments (Eglinton et al. 1996, 1997; Uchida et al. 2000). Different biomarker compounds, which can be attributed to specific sources, have been used to study pathways of organic carbon in soils and sediments (Hedges 1991; Lichtfouse et al. 1997; Eglinton et al. 1997). In this study, we combined organic geochemical isolation methods with AMS ^{14}C measurements to identify sources of SOM and, moreover, to find fractions that are not influenced by anthropogenically-derived, fossil carbon contamination and, thus, can be used to study C dynamics in soils. Different chemically-defined lipid compound classes (Willsch et al. 1997; Wiesenberg et al., forthcoming a), separated by medium-pressure liquid chromatography, as well as individual phospholipid fatty acids isolated by preparative capillary gas chromatography, were analyzed. PLFAs are integral components of the cell membrane. They are degraded within days or weeks after cell death (White et al. 1979) and, thus, can be used as indicators of viable microbial biomass in soils (Frostegård and Bååth 1996; Zelles 1999; Petsch et al. 2001). The investigations were done on soil samples from 2 agricultural long-term field trials located in a rural and in an industrialized region of Germany.

MATERIALS AND METHODS

Study Sites and Soil Sampling

Samples from 2 study sites in Germany with agricultural long-term field experiments were used for the investigations. The study site at Rotthalmünster is located in a rural area in the south of Germany. The mean annual temperature in this region is 8.2 °C and the mean annual precipitation is 890 mm. The soil is a Haplic Luvisol (FAO 1990) derived from loess (10% sand, 73% silt, 17% clay). Samples were taken from a plot with continuously cropped maize since 1979. The field trials of the University of Halle are located in a heavily industrialized region in the middle eastern part of Germany. The mean annual temperature is 9.2 °C and the mean annual precipitation is 465 mm. The soil type is a Haplic Phaeozem (FAO 1990) derived from sandy loess consisting of about 70% sand, 20% silt, and 10% clay (Merbach et al. 1999). Soil samples from plots with continuous rye since 1878 and continuous maize since 1961 were used for this study.

Samples of the field trials in Rotthalmünster were collected in 2002, and from the experiments in Halle in 2000. Soil samples of the ploughed surface soil (0–30 cm) were taken with a spade from different locations of each plot and mixed to obtain a representative sample. Mixed subsoil samples, taken from 8–10 locations per plot, were collected by corer.

Extraction and Separation of Lipid Compound Classes

The extraction of total lipids from bulk soil samples was done via accelerated solvent extraction (Dionex ASE 200) at the University of Cologne. Each sample of 150 g of dried soil was distributed into 5 extraction vessels. They were extracted twice for 20 min at 50 bar and at a temperature of (1) 75 °C, and (2) 140 °C, using a mixture of dichloromethane and methanol (DCM:MeOH: 93:7 by volume). The 5 extracts and both isolation steps were combined resulting in the total lipid extract (Ex) (Wiesenberg et al., forthcoming a).

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Total lipids were separated by medium-pressure liquid chromatography (H-MPLC) into 6 fractions of different polarity (Willsch et al. 1997): neutral lipids (N), medium polar compounds (F), high molecular lipids (W), acid fraction (H), basic fraction (Q), and high polar fraction (V). The neutral fraction (N) was further separated by a second MPLC described by Radke et al. (1980), resulting in 3 additional fractions: aliphatic- (A) and aromatic hydrocarbons (B), and low polar heterocompounds (C). The extracts were evaporated to dryness using rotary evaporation.

Purity of the individual compound classes was checked by gas chromatography-mass spectrometry analysis (Wiesenberg et al., forthcoming a). A small proportion of the total extract (Ex) (<15%) remained insoluble in DCM or could not be recovered from the MPLC columns.

Isolation of Phospholipid Fatty Acids

Fresh soil samples were shaken for 3 hr with chloroform, methanol, and a phosphate buffer (2.5:5:2.5 by volume). The resulting total lipid extract was then separated into 3 fractions by chromatography using silica gel columns conditioned with chloroform. Neutral-, glyco-, and phospholipids were eluted with chloroform, acetone, and methanol (White et al. 1979; Zelles et al. 1993). The phospholipid fraction was derivatized to fatty acid methyl ester by mild alkaline methanolysis as described by White et al. (1979). Individual saturated- and monounsaturated-PLFA methyl esters were isolated at the Max Planck Institute for Biogeochemistry (Jena, Germany) by PCGC (HP 6890 GC, Gerstel preparative trapping device). To obtain sufficient quantities (about 100 µg carbon) for AMS ^{14}C measurements, only the most abundant PLFAs (*i/a*-C15:0, *n*-C16:0, *cy*-C16:0, *n*-C18:0, *cy*-C18:0, *n*-C16:1, *n*-C17:1, and *n*-C18:1) were isolated.

The isolation procedure and sample preparation for AMS were checked repeatedly using fatty acid methyl ester standards in the sample size range of the isolated PLFAs. The standards had ^{14}C contents of about 70 pMC (*n*-C28:0) and about 110 pMC (*n*-C12:0, *n*-C18:0), allowing the detection of both modern and fossil contamination (C Kramer, personal communication). No significant contamination by background was detected. Since 1 carbon atom is added during derivatization, ^{14}C results have to be corrected by isotopic mass balance calculation:

$$^{14}C_{free} = (C_n + 1) / C_n \cdot {}^{14}C_{ester} - 1/C_n \cdot {}^{14}C_{Methanol} \quad (1),$$

where $^{14}C_{free}$ is the ^{14}C concentration of the PLFA without the contribution of the methyl group, $^{14}C_{ester}$ is the measured ^{14}C concentration of its methyl ester ($^{14}C_{Methanol}$ = 0.1 pMC), and C_n represents the number of carbon atoms of the PLFA prior to derivatization. PLFA methyl ester, subsequently abbreviated as PLFA, are designated according to A:B, with *A* indicating the number of carbon atoms and *B* the number of double bounds. The prefixes indicate the following : *n*–unbranched chain; *i*–iso-; *a*–anteiso-branching; and *cy*–cyclopropyl. We calculated mean values of individual PLFAs from 2 cropping variants of each site (Rotthalmünster: maize, wheat; and Halle: maize, rye) if they did not show statistically significant differences (1-σ level).

Sample Treatment for AMS

The dried lipid compound classes were dissolved again using dichloromethane and methanol, depending on the polarity of the fraction. Lipid classes and PLFAs were pipetted in solution into precombusted (4 hr, 900 °C) quartz combustion tubes. The solvent was removed by evaporation overnight and, in case of incomplete removal, under a gentle N_2 stream. Samples with carbon contents of >500 µg were combusted with 450 mg CuO and 150 mg silver wool. For combustion of small samples containing <500 µg of carbon, reduced portions of 75 mg CuO and 30 mg silver were added. The tubes were evacuated to a pressure of about 10^{-4} mbar while immersed in dry ice/ethanol

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to avoid possible loss of highly volatile compounds, and subsequently flame-sealed. Samples were combusted at 900 °C for 4 hr. The resulting CO_2 was collected in a cold trap with liquid nitrogen, and subsequently reduced to graphite with a 10% excess of hydrogen at 600 °C over an iron catalyst (Nadeau et al. 1997, 1998). The AMS measurements were made at the Leibniz Laboratory in Kiel, Germany. ^{14}C data are reported as percent modern carbon (pMC) with 1-σ measurement uncertainty. The measurement precision lies in the range of 0.3 pMC for modern, standard-sized (about 1 mg C) samples (Nadeau et al. 1998). The precision achievable for small samples is worse, as the relative importance of the blank correction increases and its uncertainty (about 1/3 of the blank value) makes an ever larger contribution to the overall measurement uncertainty of small samples. Figure 1 shows the 1-σ uncertainties for our PLFA samples with carbon weights of 40 to 340 µg C (between 3.9 to 0.6 pMC).

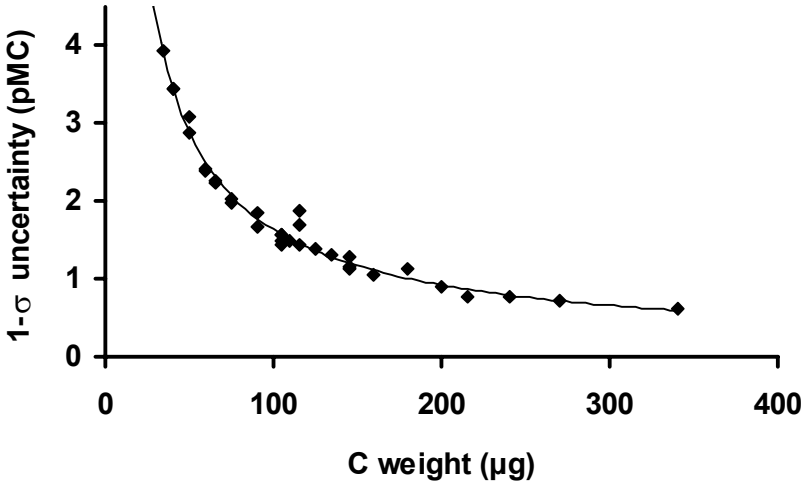

Figure 1 Uncertainty (1 σ) for PLFAs as a function of sample carbon weight. All PFLA single measurements are displayed.

RESULTS AND DISCUSSION

Lipid Compound Classes

^{14}C concentrations of the different lipid compound classes from surface soil samples of the field trials at Rotthalmünster and Halle are compared in Figure 2. ^{14}C values of compound classes at Rotthalmünster are close to the modern atmospheric ^{14}C level (Figure 2a). The total lipid extract (Ex) is 3.3 ± 0.7% depleted in ^{14}C compared to the bulk soil (S) (106.5 pMC). ^{14}C contents of the isolated compound classes range from 98.7 ± 0.3 pMC for the neutral lipids (N), with closely similar values for the acid (H) and basic fraction (Q), to 105 pMC for the high molecular fraction (W). Aliphatic (A) and aromatic hydrocarbons (B), isolated from the neutral lipids (N), have a low ^{14}C content of 44 ± 0.3 pMC (A) and 25 ± 1.7 pMC (B), which may be caused by a contribution of fossil carbon to these fractions. These ^{14}C-depleted fractions represent only a small proportion (about 0.5% [B] to 6.0% [A]) of the total extract. Main constituents (about 85%) of neutral lipids are low polar heterocompounds (C) with a relatively high ^{14}C content of 103.4 ± 0.3 pMC. Since N was subdivided into fractions A, B, and C, the weighted mean ^{14}C concentration of the 3 fractions should be equal to the ^{14}C content of N. Actually, the resulting ^{14}C value, calculated by mass balance of fractions A, B, and C, suggests a small loss of about 4.4% modern carbon compared to the higher

^{14}C content of the directly measured fraction N. This may result from volatilization of substances during solvent evaporation and/or incomplete recovery in the preparation of fractions A, B, and C.

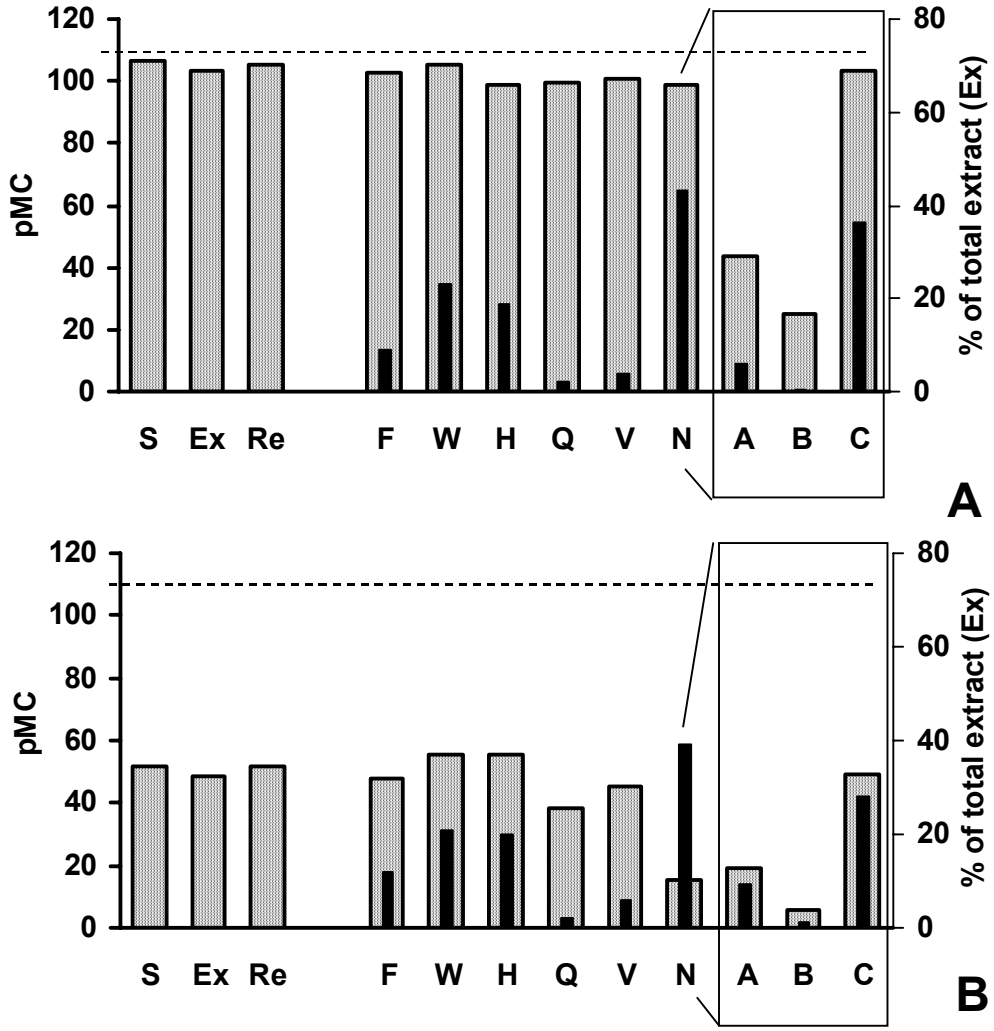

Figure 2 ^{14}C values of lipid compound classes from topsoil samples (0–30 cm) of (a) the rural site at Rotthalmünster (maize plots) and (b) the urban site at Halle (continuous rye). The black bars refer to the right y-axis. The dashed line represents the ^{14}C content of the atmosphere in 2000. Lipid compound classes are designated according to the following: F-medium polar compounds; W-high molecular lipids; H-acid fraction; Q-basic fraction; V-high polar fraction; and N-neutral fraction. Other abbreviations display: S-bulk soil; Ex-total lipid extract; and Re-residue after extraction.

In contrast to the rural, uncontaminated Rotthalmünster site, lipid compound classes from surface soil samples of the rye plots at Halle show highly depleted ^{14}C values (Figure 2b). Low ^{14}C concentrations were measured in aromatic (B: 5.7 ± 0.3 pMC) and aliphatic hydrocarbons (A: 19 ± 0.2 pMC) isolated from the neutral lipids, which can be attributed to a high contribution of old, ^{14}C-free carbon. Wiesenberg et al. (forthcoming b), using molecular markers combined with $\delta^{13}C$ analysis, showed that lignite, derived from nearby open-pit mining, and coke particles, from steam trains running close to the experimental site until the 1980s, are major sources of fossil carbon at

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Halle. Fossil carbon compounds contribute preferentially to the aromatic and aliphatic hydrocarbon fraction. Additionally, significantly higher proportions of aromatic (1.2%) and aliphatic hydrocarbons (9.4%) were found in the total extract from Halle in comparison to that from Rotthalmünster. ^{14}C concentrations of about 55 ± 0.2 pMC, measured in the acid (H) and the high molecular fraction (W), reflect a lower contamination of these more functionalized fractions which, however, still include about 50% fossil carbon.

The ^{14}C data of lipid compound classes from the rural Rotthalmünster and the urban Halle site revealed that the isolated fractions are composed of a mixture of substances originating from natural sources as well as from anthropogenic sources with quite different ^{14}C contents. Furthermore, these fractions have shown to be highly susceptible to contaminations such as fossil fuel-derived carbon. To exclude contaminations as observed in Halle, it is essential to isolate and analyze compounds on the molecular level.

Phospholipid Fatty Acids

PLFAs were obtained from the maize and wheat plots at Rotthalmünster and from maize and rye at Halle. As the sample quantities were extremely small (40 to 340 µg of carbon), the standard deviations were relatively large, and no statistically significant differences were visible between maize and wheat at Rotthalmünster and maize and rye at Halle. The weighted averages of the ^{14}C content of individual phospholipid fatty acids at Rotthalmünster and Halle are given with their (reduced) uncertainty for each site in Figure 3, displayed in relation to the atmospheric ^{14}C level of about 109 pMC in 2000 (Schauinsland, Germany; B Kromer, personal communication).

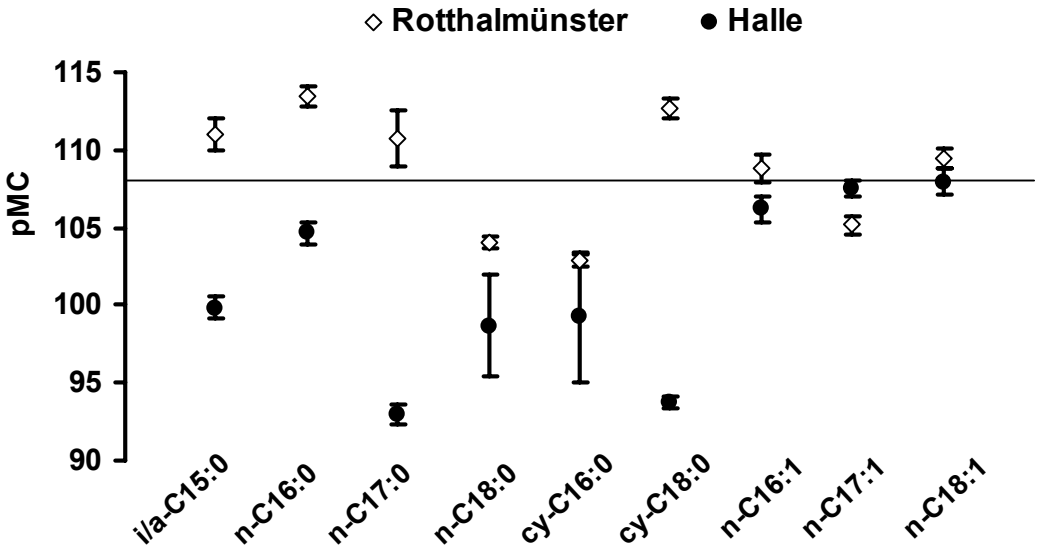

Figure 3 Comparison of calculated ^{14}C concentrations of individual PLFAs from surface soil samples (0–30 cm) collected at Rotthalmünster (average of maize, wheat values) and Halle (average of maize, rye). The error bars show the calculated uncertainties for these averages and for single values of *n*-C17:0 from Rotthalmünster (wheat) and *cy*-C16:0 and *n*-C18:0 from Halle (maize). The x-axis displays the atmospheric ^{14}C content in 2000.

In contrast to the extremely low ^{14}C content of the bulk soil (52 pMC) at Halle, the ^{14}C values of its PLFAs are surprisingly high, ranging from about 93 pMC to 108 pMC. The relatively low ^{14}C contents of monounsaturated PLFAs, particularly *n*-C17:0 (93.0 ± 0.6 pMC) and *cy*-C18:0

(93.8 ± 0.4 pMC), still suggest an assimilation of fossil, most probably lignite-derived carbon by soil microorganisms. Assuming that the input materials are fresh crop residues with atmospheric ^{14}C levels, the proportion of fossil carbon can be estimated by a mass balance calculation to amount to about 14% in *n*-C17:0 and *cy*-C18:0, and about 9% in *n*-C18:0, *cy*-C16:0, and *i/a*-C15:0 fatty acids from Halle soil. The saturated PLFAs *i/a*-C15:0, *n*-C16:0, *n*-C17:0, and *cy*-C18:0 yield statistically significant (2-σ criterion) differences in ^{14}C values in Halle and Rotthalmünster. At Rotthalmünster, their ^{14}C levels, with the exception of *n*-C17:0, are significantly above that of the present atmosphere, reflecting the contribution of organic material from the last 40 yr containing bomb ^{14}C. Relatively low ^{14}C values of these PLFAs at Halle reflect the incorporation of fossil, supposedly refractory carbon. The pMC values of the *n*-C18:0 and *cy*-C16:0 PLFAs at Halle and Rotthalmünster are not statistically different, but are both significantly below atmospheric ^{14}C levels. This suggests the contribution of pre-bomb SOM to these compounds as fossil carbon contamination is low or absent at Rotthalmünster. At both sites, the monounsaturated PLFAs show no significant difference from each other, nor from the atmospheric ^{14}C level at their time of growth. These PLFAs are apparently synthesized exclusively from fresh organic material. Thus, ^{14}C data of the different PLFAs show an interesting range of substrate use from fresh SOM (monounsaturated PLFAs) via a preference for material from the last decades to pre-bomb SOM.

CONCLUSIONS

^{14}C measurements of lipid biomarkers, such as different compound classes, obtained by medium-pressure liquid chromatography and individual phospholipid-derived fatty acids, and isolated by preparative gas chromatography, have proven valuable to identify sources of organic carbon.

^{14}C concentrations of lipid compound classes reflect their heterogeneous composition, consisting of a mixture of material ranging from plant-derived substances with modern ^{14}C concentrations, to old, ^{14}C-free compounds. At the field trial in Halle, particularly, the neutral fraction, which contains aromatic and aliphatic hydrocarbons and low polar heterocompounds, was found to be strongly influenced by fossil carbon (Wiesenberg et al., forthcoming b). The highest proportions of most probably fossil fuel-derived carbon were detected in the aromatic and aliphatic hydrocarbon fraction, resulting in extremely low ^{14}C contents of about 6 pMC (aromatic) and 19 pMC (aliphatic fraction). In contrast, these fractions are less abundant and, moreover, contain higher portions of younger, vegetation-derived substances at Rotthalmünster. Lipid classes that are supposed to consist of recent plant constituents, such as the carboxylic acid fraction and the high molecular fraction containing long-chain wax esters, yield higher values of about 55 pMC at Halle.

^{14}C data of individual PLFAs, which are not supposed to be influenced by anthropogenic contaminations, suggest a preference of soil microorganisms for the uptake of modern organic carbon. Depleted ^{14}C contents of saturated PLFA at Halle indicate an incorporation of up to 14% fossil carbon. This ability of soil microorganisms to use old and supposedly refractory material as carbon sources (Petsch et al. 2001; Rumpel et al. 2003) complicates the use of ^{14}C as a tracer for soil carbon turnover if fossil carbon is present. It also questions the concept of a pool of refractory organic compounds, used in soil carbon models, that are biologically inert (Falloon et al. 1998).

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