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Transformation of organic matter in agricultural soils: radiocarbon concentration versus soil depth

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Abstract

Radiocarbon measured by accelerator mass spectrometry (AMS) was used as a tracer to study the origin and fate of organic materials in soils. Fractionation methods used to separate the heterogeneous organic material into functionally defined pools of different stability included acid–alkali–acid extraction and density fractionation. ^{14}C values of the humin fraction, isolated from samples of different field trials at the agricultural station Rotthalmünster (Germany), yielded ^{14}C decreases of about 30% to 54% from the surface soil to ca. 65 cm depth. These results indicate a progressive enrichment of stable organic compounds with increasing soil depth. In contrast, a minor decline in ^{14}C concentrations of the humic acid fraction, which mostly showed higher ^{14}C values than the humin, reflect the translocation of modern organic carbon towards greater depth. Low radiocarbon levels of the light occluded particulate organic matter (<1.6 g/cm^3), obtained by density separation, suggest stabilization of organic carbon in soil aggregates. A comparison of ^{14}C results for density fractions from field trials located in a rural and an industrialized region reflect their susceptibility to contamination by fossil fuel-derived carbon and their heterogeneous composition. As a consequence individual short-chain phospholipid fatty acids (PLFA), as indicators for viable soil microbial biomass, were isolated by preparative capillary gas-chromatography. Compound-specific radiocarbon analysis of the isolated PLFAs revealed the assimilation of different substrates for their synthesis.^{14}C concentrations of the monounsaturated PLFAs (*n*-C16:1, *n*-C17:1, and *n*-C18:1), which were close to the modern atmospheric ^{14}C level, suggest a high specificity to young carbon sources. The saturated PLFAs, isolated from the plough-horizon, were synthesized from sub-recent soil organic carbon (SOC) as shown by a higher contribution of bomb-^{14}C. A considerable ^{14}C decrease from the surface to 30–45 cm soil depth

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of the saturated PLFAs indicates the incorporation of more stabilized SOC particularly in subsoil *i*/*a*-C15:0, *n*-C16:0, and *n*-C17:0 PLFAs.

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1. Introduction

Soils are regarded as an important factor in the global carbon budget (Schimel et al., 2001). Therefore many studies focus on understanding the sequestration and transformation of soil organic carbon (Houghton, 1991; Trumbore et al., 1996). The present knowledge of the different processes resulting in carbon stabilization and destabilization is still incomplete (Sollins et al., 1996), due to the diversity of the soil organic matter (SOM). The analysis of carbon cycling in soils is complicated by the heterogeneous composition of SOM ranging from modern plant fragments to highly transformed, recalcitrant substances and by the continuous exchange of carbon with the atmosphere and hydrosphere (Trumbore and Druffel, 1995). Hence it is essential to separate SOM into physical or chemical organic matter pools containing compounds of different stability (Trumbore and Zheng, 1996) and, if possible, of known origin. Since the bio-availability and thus dynamics of SOM is controlled by its molecular composition and chemical structure as well as by physical protection mechanisms such as interactions with the soil matrix (Skjemstad et al., 1996; Baldock and Skjemstad, 2000), functionally defined fractions may also give information on the different stabilization processes when analyzed using ^{14}C dating (Trumbore et al., 1989). Under steady state conditions radiocarbon data of SOM fractions reflect the mean residence time for carbon in each fraction, provided that the input has a constant ^{14}C concentration. As illustrated in Fig. 1 ^{14}C concentrations in the atmosphere increased strongly from the mid-1950s up until 1963, due to the release of ^{14}C by atmospheric nuclear weapons testing. The so-called bomb-^{14}C spike, the doubling of the atmospheric $^{14}CO_2$ concentration in 1963, has been used as a time marker (Harkness et al., 1986; Trumbore et al., 1989; Trumbore, 1993). However, the determination of organic carbon transformation rates requires a precise

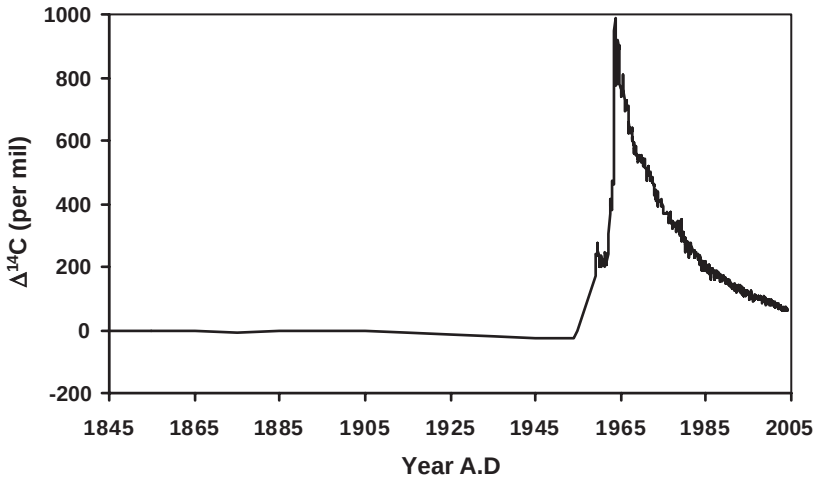

Fig. 1. Atmospheric $\Delta^{14}CO_2$ trend over Europe. Data from tree-rings (Stuiver and Quay, 1981), and air samples of the stations Vermunt (Austria), Jungfraujoch (Swizerland), and Schauinsland (Germany; Levin and Kromer, 1997; Levin et al., 2003; Levin and Kromer, submitted for publication). $\Delta^{14}C$ is the relative deviation in ^{14}C concentration from the standard atmosphere.

knowledge of carbon inputs and losses, or the availability of archived samples collected from before 1954 until the present, which directly document the incorporation of bomb-^{14}C in different SOM fractions over time.

Chemical or physical organic matter pools still consist of a heterogeneous mixture of organic compounds derived from different sources, often including organic compounds transported vertically by water (Huang et al., 1996), and variable inputs of anthropogenically derived fossil carbon (Schmidt et al., 2001; Rethemeyer et al., 2004a). As a consequence of this, turnover rates calculated from ^{14}C results for mixed pools may differ from the actual turnover of carbon in the separated reservoirs. A relatively new approach is the analysis of source-specific organic compounds (biomarkers) by ^{14}C accelerator mass spectrometry, which makes it possible to study their source and fate in soils or sediments (Bol et al., 1996; Pearson et al., 2001). The analysis of easily degradable substances, like phospholipids, excludes the use of archived pre-bomb samples. However, ^{14}C results for such compounds still give information on the contribution of different SOC sources: (i) fresh plant residues, with ^{14}C contents corresponding with the present atmospheric ^{14}C, (ii) sub-recent, degraded organic material of previous years with initial ^{14}C contents as indicated for the various years in Fig. 1, and (iii) a smaller pool of "old", biologically refractory organic components with depleted ^{14}C values ($\ll$100 pMC), which may possibly be of fossil origin (Lichtfouse et al., 1997).

In this study we determined ^{14}C concentrations of chemically and physically defined SOM pools isolated from agricultural soils down to a maximum depth of 65 cm. Fractionation methods used include chemical fractionation by standard acid–alkali–acid extraction and by density separation of the organic matter. We also isolated and dated individual phospholipid fatty acids (PLFA), which are essential components of intact cell membranes of microbes. As they are degraded within several days after cell death (Harvey et al., 1986), they can be used as indicators of living microbial biomass in soils and sediments (Baird et al., 1985; Zelles, 1999). Most of the research on PLFAs relates to the characterization of microbial communities and their reaction to changing soil conditions (Frostegård and Bååth, 1996). Petsch et al. (2001) used ^{14}C analysis of individual PLFAs to identify fossil carbon sources assimilated by soil microorganisms. Our investigations were done on soil samples from a long-term experimental site in Rotthalmünster (Germany) with field trials continuously cultivated with maize, wheat and grassland. Additionally, soil samples from a long-term trial close to the city of Halle, which is located in a heavily industrialized region of Germany, were radiocarbon dated.

2. Materials and methods

2.1. Study sites and soil sampling

Soil samples were obtained from long-term field trials at Rotthalmünster, which is located in a rural area in the south of Germany. The site is located 360 m above sea level. The mean annual precipitation is 886 mm and the mean annual temperature is 8.7 °C. The soil has been classified as a Haplic Luvisol, according to the FAO world reference base for soils (FAO, 1990), derived from loess with 10% sand, 73% silt, and 17% clay (Kleber, pers. comm.). Field trials with (i) continuous wheat (since 1969), (ii) continuous maize (since 1979), and (iii) continuous grassland (since 1961), all with mineral NPK-fertilization, were chosen for this study. The trial with maize cropping is ploughed conventionally down to about 30 cm soil depth, whereas ploughing of the wheat monoculture was stopped in 1998 and changed to grubbing. On both sites the straw is left on the field after harvest. Samples were taken from three different depths of the maize and wheat trials, prior to soil tillage, and from five different depths of the grassland trial in September 2002. To account for soil heterogeneity several kilograms of soil were taken from the ploughed surface soil (0–30 cm) with a spade from different locations on each trial and mixed to provide a representative sample. Composite subsoil samples were collected by corer from 8 to 10 locations per trial.

The long-term study site of the University of Halle is located close to the city center of Halle/Saale (Germany) in a heavily industrialized region. Mean annual temperature and mean annual precipitation are 9.2 °C and 465 mm, respectively. The soil type is a

Haplic Phaeozem (FAO, 1990), derived from sandy loess, consisting of ca. 70% sand, 20% silt and 10% clay (Merbach et al., 1999). Soil samples from fields with continuous rye since 1878 and continuous maize since 1961 were used for this study. The rye and maize straw is removed from the field after harvest, only the short stubbles are ploughed into the soil. Samples were collected at different locations on both trials from the plough-horizon (0–30 cm) and mixed to provide a composite sample. Sampling was done after harvest and soil cultivation in December 2000.

Soil samples were air-dried and sieved (<2 mm) and the fraction >2 mm was removed. For the isolation of PLFAs, fresh soil samples were transported in ice-cooled boxes and subsequently passed through a 2 mm sieve and visible plant residues were removed. The samples were stored for several weeks at −20 °C until extraction.

2.2. Isolation of phospholipid fatty acids

Phospholipid fatty acids were extracted using a method described by White et al. (1979) and subsequently individual PLFAs were isolated by preparative capillary gas-chromatography (PCGC) according to Kramer (2004) and Eglinton et al. (1996). Briefly, total lipids, extracted from fresh soil samples, were split into neutral-, glyco- and phospholipids by silica gel chromatography and eluting with chloroform, acetone, and methanol (White et al., 1979; Zelles and Bai, 1993). The phospholipid fraction was transformed to hydrolysis-resistant derivatives, fatty acid methyl esters, which were repeatedly injected into a preparative capillary gas-chromatograph (HP 6890 GC, Gerstel preparative trapping device) at the Max Planck Institute for Biogeochemistry (Jena, Germany). The most abundant PLFAs, yielding >70 μg carbon, were isolated for micro-scale AMS ^{14}C measurements.

The isolated individual PLFA methyl esters, subsequently abbreviated as PLFAs, were transferred into pre-combusted (900 °C) quartz-tubes and the solvent (methanol) was removed by evaporation and, in case of incomplete removal, under a gentle N_2 stream. Further preparation to obtain graphite targets for the AMS ^{14}C measurements was done as described below (Section 2.4). During sample isolation we repeatedly checked the procedure by preparing and measuring fatty acid methyl ester standards with ^{14}C contents of 70 pMC (*n*-C28:0) and about 110 pMC (*n*-C12:0, *n*-C18:0) in the sample size range of the isolated PLFAs, which allow the detection of modern and fossil contamination (Rethemeyer et al., 2004b). The ^{14}C data were corrected for the contribution of the methyl carbon from methanol (0.1 pMC) by isotopic mass balance (Rethemeyer et al., 2004b). Designation of PLFAs is according to: 'A:B', with 'A' indicating the number of carbon atoms, and 'B' the number of double bonds. The prefixes indicate: *n*—unbranched chain, *i*—iso- and *a*—anteiso-branching, and *cy*—cyclopropyl.

The PLFAs were isolated from a huge composite sample, several kilograms of soil collected on a field of about 90 m^2. Due to this sampling strategy and the time consuming PLFA isolation procedure no replicate samples were analyzed. For isolating 100 μg of PLFA carbon an extraction of 500 g of soil and about 50 injections of the phospholipid methyl ester fraction in the PCGC system (each PCGC run taking about 1 h) were required. The PCGC isolation procedure was previously tested by replicate preparations and ^{14}C analysis of an *n*-C18:0 fatty acid methyl ester standard dissolved in dichloromethane. This showed a reproducibility of 0.86 pMC for four isolations with a measuring uncertainty for 100-μg-sized samples of 1.0 to 1.7 pMC, indicating the isolation procedure is reproducible within the rather wide measurement uncertainty of the AMS ^{14}C analysis of small samples. The lipid extraction from fresh soil samples was tested by preparation of two sub-samples from composite soil samples of the trial at Rotthalmünster as well as of an experimental site at Halle (Rethemeyer et al., 2004b). At Rotthalmünster the difference between the duplicates was 2.50±0.38 pMC. For Halle a difference of 1.14±0.29 pMC (rye) and 1.75±0.28 pMC (maize), respectively, was determined. These differences are statistically significant (σ: 6.6, 3.9, and 6.3, respectively) and document the soil heterogeneity remaining after mixing several kilograms of soil is large compared with the instrumental reproducibility of the measurements.

The discussion of the differences of the PLFA ^{14}C-data is based on the measurement uncertainty. Since the ^{14}C values of the individual PLFAs from the maize and wheat trials at Rotthalmünster and Halle in most cases were statistically not different ($2-\sigma$ criterion,

with $1-\sigma$ values ranging from 0.6 to 3.5 pMC for PLFA-samples from 340 μg down to 40 μg), a weighted mean of the ^{14}C results for each individual PLFA from both cultures was calculated.

2.3. Physical fractionation

Soil samples were separated sequentially into four density fractions using a procedure described in detail by John et al. (this volume). The free particulate organic matter (fPOM) was obtained by gently shaking 10 g air-dried soil using 40 ml sodium polytungstate of 1.6 g/cm^3 density and subsequent centrifuging (5085×g, 1 h). The $fPOM_{<1.6}$ was recovered from the supernatant. The remaining pellet was suspended in sodium polytungstate (1.6 g/cm^3) and disaggregated by shaking with 10 quartz balls of 5 mm diameter for 16 h. After centrifuging (5085×g, 1 h) the soil suspension, the supernatant contained the occluded particulate organic matter ($oPOM_{<1.6}$) of less than 1.6 g/cm^3 density. Then, the pellet was shaken for 10 min (100 rotations/min) with sodium polytungstate of 2.0 g/cm^3 density and after centrifugation (5085×g, 1 h) the $oPOM_{1.6–2.0}$-fraction with 1.6–2.0 g/cm^3 was recovered from the supernatant by filtration. To clean the remaining pellet from the density solution, it was suspended in distilled water, centrifuged (5085×g, 20 min) and the supernatant was discarded. This procedure was repeated three times. We call the remaining pellet the 'mineral fraction' >2.0 g/cm^3.

2.4. Sample preparation and AMS ^{14}C measurements

Soil samples were inspected under a light microscope and identifiable plant fragments were removed. Bulk soil samples were chemically fractionated by standard acid–alkali–acid extraction for radiocarbon samples (Grootes et al., 2004), as described subsequently. Carbonates were removed by extraction with 1% HCl for about 8 h followed by washing with demineralized water till a pH of >4. Then the soil was extracted with 1% NaOH (4 h, 60 °C), resulting in an alkali-soluble fraction (humic acid) and a non-soluble residue (humin). The humic acid fraction was precipitated with 37% HCl from the NaOH extract, while the remaining fulvic acid fraction was not recovered. The solid residue, which was treated again with 1% HCl to remove a possible contamination by atmospheric CO_2, yields the humin fraction. Both fractions were dried at 60 °C. In view of the small sample size of the density fractions, these were only treated with 1% HCl to remove carbonates. The hydrochloric acid was evaporated under a gentle N_2 stream at 40 °C.

The samples were transferred into pre-combusted quartz tubes and, depending on sample size, 75–450 mg copper oxide and 30–450 mg silver wool were added. The tubes were evacuated and subsequently flame sealed. To avoid possible losses by volatilization of individual compounds, the PLFAs were evacuated at −80 °C (Eglinton et al., 1996; Rethemeyer et al., 2004b). Samples were combusted at 900 °C for 4 h, and the resulting CO_2 was subsequently reduced to graphite with a 10% excess of hydrogen at 600 °C over an iron catalyst (Vogel et al., 1984; Nadeau et al., 1998).

The AMS measurements were made at Leibniz-Laboratory in Kiel (Germany), with a precision of about 0.3 pMC for modern, standard sized (1 mg C) samples (Nadeau et al., 1998). For small samples (<350 μg C) the measurement uncertainty increased due to a larger contribution of the uncertainty in the blank correction (ca. 1/3 of the blank value) to the overall measurement uncertainty (Rethemeyer et al., 2004b).

^{14}C concentrations were calculated from the measured $^{14}C/^{12}C$ ratio of the sample compared to 95% of the NIST oxalic acid standard (NIST, National Institute of Standards and Technology, Gaithersburg, MD; Stuiver and Polach, 1977), both corrected for isotopic fractionation using the simultaneously measured $^{13}C/^{12}C$ ratio. Data are expressed according to Stuiver and Polach (1977) in percent modern carbon (pMC; 100 pMC=1950 AD) with $1-\sigma$ measurement uncertainty.

3. Results and discussion

3.1. Vertical distribution of ^{14}C in chemical SOM fractions

Fig. 2 shows changes in ^{14}C concentrations with depth for the humin and the humic acid fraction extracted from samples taken on the different trials at

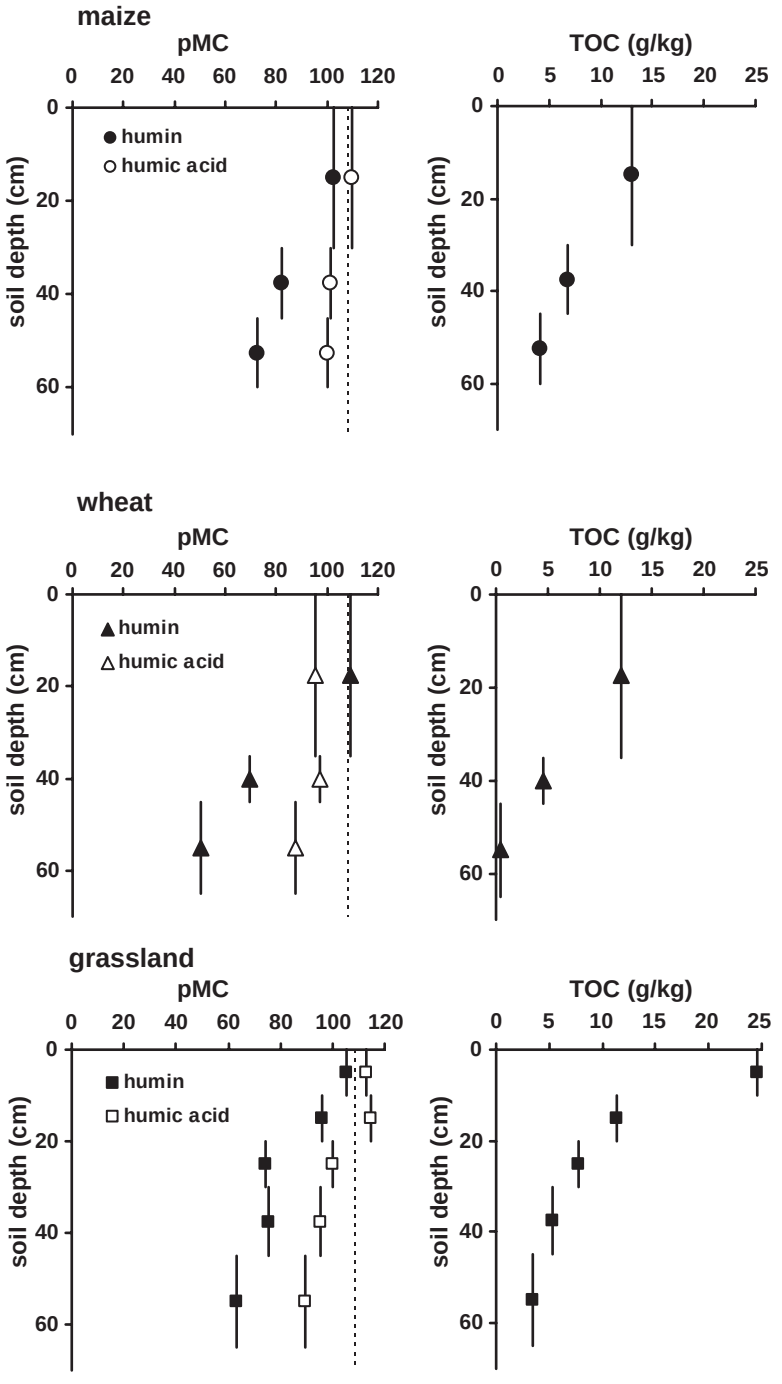

Fig. 2. ^{14}C concentration of humin (solid dots) and humic acid fractions (open dots) and total organic carbon (TOC) of bulk soil versus depth at Rotthalmünster (Germany). The vertical bars represent the depth in which mixed soil samples were collected. The ^{14}C measurement uncertainties are smaller than the dot size. The dashed line displays the atmospheric ^{14}C content in 2002 AD.

Rotthalmünster. ^{14}C contents of SOM fractions from the surface soil were close to the atmospheric $^{14}CO_2$ level of ca. 107.2±0.2 pMC in 2002 (Levin et al., 2003), the year of soil sampling, reflecting a high proportion of recent photosynthesis products. A considerable ^{14}C decrease with increasing soil depth of 30% to 54% was observed for the humin fraction in the sampled maize, wheat, and grassland trials, similar to earlier observations by Trumbore (1993) and Paul et al. (1997). This indicates a relative increase of resistant organic components in the humin fraction (Rice, 2001) with soil depth and consequently an increasing apparent residence time as demonstrated by Trumbore et al. (1989) tracing the infiltration of bomb-^{14}C in SOM fractions. We found a strong correlation (r=0.92) of ^{14}C values of this fraction with the total organic carbon (TOC) content in the sampled soil profiles of the wheat, maize, and grassland cultures, reflecting that high TOC contents derive from high portions of modern SOM (O'Brien and Stout, 1978).

On the maize field the ^{14}C concentration of the humin fraction ranged from 102.8±0.3 pMC in the plough-horizon (Ap) to 72.6±0.4 pMC in 45–60 cm depth. In the Ap-horizon under continuous wheat cropping a higher value of 109.0±0.3 pMC was obtained for this fraction. However, ^{14}C was more depleted toward 65 cm soil depth on this culture (50.6±0.4 pMC in 45–65 cm). The high concentrations of ^{14}C and TOC, measured in the topsoil, reflect the large contribution of modern, vegetation-derived SOM. Decreased values of both parameters below the plough-horizon indicate an enrichment of SOM in more resistant organic material. It also suggests a relatively low contribution of young, translocated carbon to the humin fraction at greater depth.

The humic acid fraction showed values of 109.7±0.4 pMC (Ap-horizon) and a minor decrease to 100.1±0.4 pMC in 45–60 cm depth on the maize field, indicating a high contribution of young organic carbon (Martel and Paul, 1974), which is translocated in the profile by processes such as leaching or bioturbation. In contrast, considerably lower values of 95.2±0.4 pMC (Ap-horizon) to 87.5±0.4 pMC (45–65 cm) were measured on the wheat trial. The results obtained for the wheat subsoil may reflect a lower transfer of crop-derived modern carbon, since about 60% less carbon derived from wheat straw is incorporated into the plough-horizon compared to the maize culture (John et al., this volume). This is also reflected by lower TOC levels particularly below 45 cm soil depth.

Radiocarbon concentrations of the humin fraction extracted from grassland soil decrease in the upper 30 cm from 105.2±0.3 pMC (0–10 cm) to 74.2±0.4 pMC (20–30 cm) associated with the decrease in TOC. We assume the high ^{14}C and TOC values in the upper 10 cm result from the accumulation of grass-derived carbon in the soil during the past 40 years since the site was established. At 20–30 cm soil depth the humin fraction already consists of mostly aged, refractory SOM yielding an apparent age of ca. 2400 ^{14}C years. Hence, without tillage downward transport of young SOM becoming part of the humin fraction at greater depth is apparently low. The humic acid fraction of the grassland soil showed high ^{14}C concentrations (113.3 to 114.7 pMC) in the upper 20 cm exceeding the atmospheric level in 2002. These values indicate the contribution of organic carbon from the last ca. 40 years, with increasing bomb-^{14}C levels back to 1963, to this fraction (Fig. 1).

3.2. ^{14}C concentrations of individual phospholipid fatty acids

The radiocarbon results for individual PLFAs, as proxies of living microbial biomass, are displayed as weighted averages of data from the maize and wheat cultures at Rotthalmünster (Fig. 3) where the differences in ^{14}C concentration between the single values of the two cultures were statistically not significant. The most abundant PLFAs in surface soil as well as in subsoil samples were n-C16:0, n-C17:0, n-C17:1, and n-C18:1 which can be found in many bacteria.

In the surface soil (0–30 cm) relatively high ^{14}C values of 102.9 pMC to 113.5 pMC were found. The ^{14}C contents of the monounsaturated PLFAs n-C16:1, n-C17:1, and n-C18:1, which showed no statistically significant differences, were close to the atmospheric ^{14}C level at their time of growth. This suggests that these PLFAs are almost exclusively synthesized from fresh organic substances both in the Ap-horizon and at 30–45 cm depth. Similarly high ^{14}C levels of these three PLFAs from a fossil-carbon rich Ap-horizon of the long-term trial at Halle

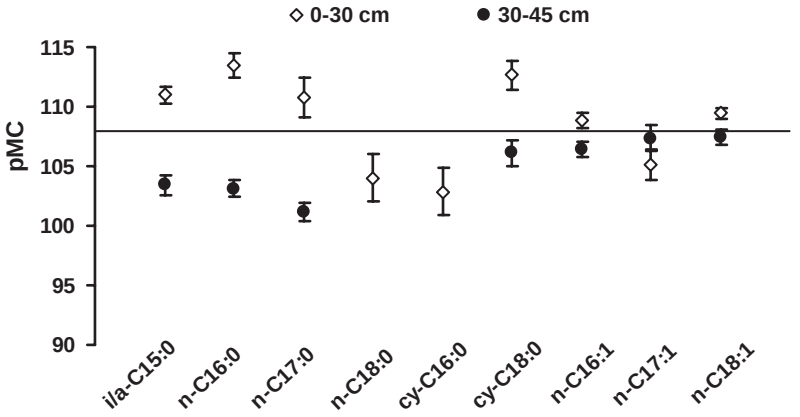

Fig. 3. Radiocarbon results for individual saturated and monounsaturated PLFAs isolated from soil samples taken in 0–30 cm and 30–45 cm depth of Rotthalmünster soil. Weighted averages for individual PLFAs from the maize and wheat trials were calculated for each depth except of single values for *n*-C17:0 (0–30 cm, wheat), *n*-C17:1 (0–30 cm, maize), and all unsaturated PLFAs from 30–45 cm depth (maize). The error bars show the $1-\sigma$ measurement uncertainties for single values and calculated uncertainties for the averages. The *x*-axis represents the atmospheric $^{14}CO_2$ in 2002 AD.

support this interpretation (Rethemeyer et al., 2004b). ^{14}C concentrations of the saturated PLFAs *i*/*a*-C15:0, *n*-C16:0, *cy*-C18:0 in 0–30 cm were significantly ($2-\sigma$ criterion) above the atmospheric $^{14}CO_2$ concentration in 2002, indicating microbial incorporation of older SOC from the last 40 years, containing elevated levels of bomb-^{14}C. The *n*-C18:0 (104.0±2.0 pMC), and *cy*-C16:0 (102.9±2.0 pMC) saturated PLFAs showed bomb-^{14}C at levels which are below the atmospheric $^{14}CO_2$ content at the time of sample collection. This most probably reflects the assimilation of older, pre-bomb SOM.

The ^{14}C results for saturated PLFAs were considerably lower at 30–45 cm depth. All ^{14}C values were below the atmospheric ^{14}C level of 2002 but still show a bomb-^{14}C contribution. The strongest depletion by about 10% was found for *n*-C16:0 (103.1±0.7 pMC), and *n*-C17:0 (101.1±0.6 pMC) PLFAs. The differences in the individual saturated PLFA ^{14}C concentrations between surface and subsoil were consistent with their ^{14}C values observed in the surface soil. For the synthesis of *i*/*a*-C15:0, *n*-C16:0 (both ca. 103±0.8 pMC) and *n*-C17:0 (101.1±0.8 pMC) organic matter was used that was somewhat older, showing higher bomb-derived ^{14}C values in the surface soil and a larger contribution of older, pre-1954 material with lower ^{14}C values in the subsoil. The monounsaturated PLFAs showed only a minor depletion in ^{14}C and, as observed in the plough-horizon, reflect a preferential incorporation of fresh SOM at 30–45 cm soil depth. This suggests downward transport of fresh, probably dissolved organic matter derived from plant litter and/or root exudates (Huang et al., 1996), which may also account for the younger humic acid fraction. Our results indicate that even individual microbial-derived PLFAs are synthesized from different organic carbon sources, which may be continuously recycled by soil microorganisms. Further studies using compound-specific ^{14}C measurements are thus needed.

3.3. Physical protection of SOM: ^{14}C results of density fractions

We determined ^{14}C concentrations of different density fractions (Fig. 4) to characterize the stability of SOC pools located between and within soil aggregates (Christensen, 2001; Six et al., 2001). Density fractions, separated from the Ap-horizon at Rotthalmünster, were inspected under a light microscope showing that the $fPOM_{<1.6}$ contained relatively large plant residues and coarse organic material. In contrast, the light $oPOM_{<1.6}$ had a much darker colour and looked more homogeneous, consisting of fine organic material without identifiable fragments. Investigations of Golchin et al. (1994a) applying ^{13}C and NMR analysis, and John et al. (this volume) using ^{13}C measurements indicate a higher degree of degradation

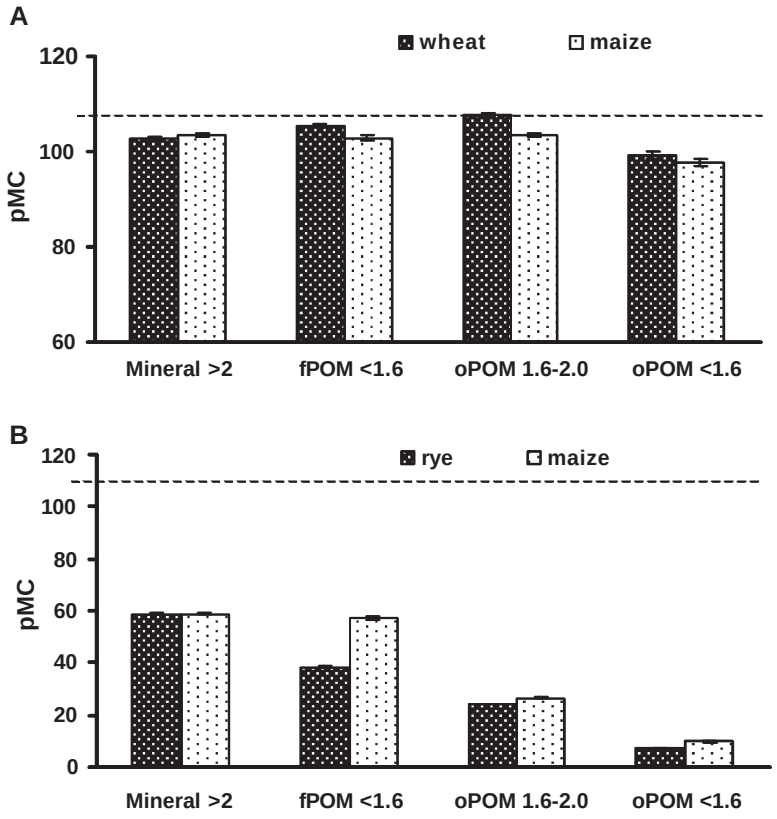

Fig. 4. ^{14}C concentrations of density fractions separated from topsoil samples (0–30 cm) of field trials at (A) Rotthalmünster and (B) Halle (Germany): free- (fPOM), and occluded particulate organic matter ($oPOM_{1.6–2.0}$, $oPOM_{<1.6}$), and a remaining mineral fraction. The atmospheric ^{14}C (A: 107.5±0.4 pMC in 2002 AD, and B: 109.2±0.4 pMC in 2000 AD; Levin et al., 2003) is represented by the dashed line.

of the occluded (light $oPOM_{<1.6>}$ heavy $oPOM_{1.6–2.0}$) compared to the free particulate organic matter.

The ^{14}C values of the mineral, $fPOM_{<1.6}$, and $oPOM_{1.6–2.0}$ fractions from surface soil sampled on the maize and wheat trials at Rotthalmünster ranged from 102.7 to 107.7 pMC (Fig. 4A). ^{14}C results for presumably mineral-free $fPOM_{<1.6}$, which is thought to be mainly composed of fresh to slightly decomposed plant residues found between aggregates (Golchin et al., 1994b), of 105.4±0.5 pMC (wheat) and 102.9±0.5 pMC (maize), indicate a contribution of pre-1954 carbon to this fraction. Substantially lower concentrations of 99.3±0.9 pMC (wheat) and 97.6±0.8 pMC (maize), obtained for the light $oPOM_{<1.6}$ fraction suggest physical protection of decomposed organic matter by occlusion in soil aggregates as also indicated by the results of ^{13}C studies by Golchin et al. (1994b). However, as pointed out by John et al. (this volume), mean apparent ages or turnover rates derived from carbon isotopes do not give information on the mechanisms responsible for physical SOC protection. Since only a small proportion of TOC is contained in the light oPOM fraction (John et al., this volume), total carbon cycling in the maize and wheat surface soils is dominated by the faster turning organic matter fractions. The $oPOM_{1.6–2.0}$ fraction, that contains organic components working as binding-agents between aggregates (Golchin et al., 1994a), showed the highest ^{14}C values on both cultures close to those of the bulk soil (humin fraction). These results agree with the higher apparent turnover rate determined for the heavy $oPOM_{1.6–2.0}$ fraction from ^{13}C data by John et al. (this volume) compared to a slower turnover of the light occluded particulate organic matter.

We compared the data obtained at Rotthalmünster with measurements of density fractions separated from rye and maize cultures at the Halle study site. The low radiocarbon values of all fractions (Fig. 4B) reflect the contribution of, most probably, lignite particles (Rethemeyer et al., 2004a) to the separated density fractions. The mineral >2.0 g/cm^3 fraction had a similar ^{14}C concentration of 58.9±0.2 pMC under maize and rye cultivation, exceeding that of the bulk topsoil (54.5 pMC, ~4880 years BP). In contrast, fPOM$_{<1.6}$ was more depleted in ^{14}C (38.5±0.4 pMC) on the rye trial than on the maize culture (57.2±0.4 pMC), indicating a high but variable contribution of fossil carbon to this predominantly plant-derived, fast cycling fraction (Baisdent et al., 2002). The lowest ^{14}C values were obtained for the two occluded particulate organic matter fractions. The heavy fraction oPOM$_{1.6–2.0}$ yielded values of 23.9±0.2 pMC (rye) and 26.3±0.2 pMC (maize), respectively, while extremely low concentrations of 7.1±0.1 pMC (~21,200 years BP; rye) and 9.6±0.2 pMC (~18,800 years BP; maize) were measured for light oPOM$_{<1.6}$. These results indicate an apparent preferential enrichment of the lignite contamination, due to the low density of the particles (Trumbore and Zheng, 1996) and moreover, document the susceptibility of physically fractionated SOM pools to contamination by fossil fuel-related carbon (Schmidt et al., 1999). The contamination can result in a drastic overestimation of carbon stability. The contribution of lignite-carbon (with a C-3 isotopic labeling) to SOM fractions will also lower the calculated turnover rates based on the natural ^{13}C abundance method. This may be the cause of the low percentage of maize-derived carbon in the oPOM$_{<1.6}$ fraction at Halle, which John (2003) calculated using natural ^{13}C labeling, and for the implied slow turnover of this fraction.

4. Conclusions

The acid–alkali–acid extraction, yielding a humic acid and a residual humin fraction, showed a high contribution of young, vegetation-derived organic carbon to the humic acid fraction and suggest its vertical translocation in soil profiles. Moreover, soil cultivation seems to promote a younger humic acid fraction below the plough-horizon. This is indicated by a slower ^{14}C decline with increasing soil depth, which was found under maize and wheat cultivation, than in the unploughed grassland soil at Rotthalmünster. ^{14}C values of the humin fraction, showing a strong decrease from the surface to 65 cm depth, suggest a depth related accumulation of slowly degradable organic compounds.

The monounsaturated PLFAs, which had ^{14}C values close to that of the atmosphere at the time of soil sampling, only showed a minor ^{14}C decrease in the subsoil. This suggests a preferential incorporation of modern carbon, derived from plant residues and/or root exudates, which may be transported to deeper parts of the soil presumably as dissolved organic matter. By contrast^{14}C concentrations of short-chain saturated PLFAs from Rotthalmünster soil reflect the incorporation of (i) organic carbon, dating back to previous decades with higher atmospheric bomb-^{14}C levels, in the surface soil, and (ii) of older organic carbon, formed prior to the bomb-period, in 30–45 cm soil depth.

The ^{14}C results for particulate organic matter fractions obtained by density separation reflect protection of humified organic components by occlusion in soil aggregates displayed by low ^{14}C concentrations of the intra-aggregate fraction (oPOM$_{<1.6}$). At the experimental site at Halle, ^{14}C concentrations indicate a variable contribution of lignite fragments to the density fractions. The data obtained at this site indicate that functionally defined SOM pools are easily contaminated by anthropogenic carbon sources. Therefore, investigations using molecular ^{14}C analysis of organic compounds are needed to help understand organic carbon transformation in soils. In view of the importance of microbial-derived carbon as binding agent for aggregate formation (Six et al., 2001), the isolation and dating of microbial compounds, such as PLFAs, is of considerable interest regarding SOM dynamics.

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