Organic carbon transformation in agricultural soils: Radiocarbon analysis of organic matter fractions and biomarker compounds

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Summary

Soils, containing globally about 1,200 to 1,700 gigatonnes of carbon, have been assumed to act as both, sinks and sources of atmospheric CO_2. Changes in the carbon reservoir of agricultural soils are induced by management practices but also by global warming, which both can increase decomposition rates of soil organic matter (SOM) and, in consequence, cause the loss of carbon as CO_2. The main source of SOM is plant material, i.e. CO_2 fixed by plants through photosynthesis, such as leaf residues, roots and root exudates. Its stabilisation in soils is affected by the quantity and quality of the plant substrate and its protection against microbial and oxidative degradation. However, the different processes resulting in carbon stabilisation and decomposition are not well understood because of the difficulties of analysing SOM, which is a complex mixture of fresh to highly transformed organic components with turnover times from days to thousands of years.

Measurements of radiocarbon in archived and recent soil samples, mechanically and chemically separated into SOM fractions of different stability, as well as in biomarker compounds were used to identify organic matter sources and their transformation in agricultural soils. The heterogeneous composition of SOM at the long-term study site at Halle (Germany) was reflected by ^{14}C ages of different SOM fractions in a wide range from recent (>100 pMC) to >20,000 years BP (7 pMC). The high apparent ^{14}C age of SOM in the plough horizon of 4880 years BP (54 pMC) was attributed to a contribution of ≥52 % fossil, ^{14}C-free carbon, estimated by mass balance calculation. Inspection by light and scanning electron microscopy revealed the contribution of black, coal-like and charred particles to SOM at Halle. The location of the Halle site in a region with lignite using and processing industries, as well as low O/C ratios of the black particles, determined by energy-dispersive X-ray spectroscopy, suggest lignite as the main fossil carbon source at Halle. Decreasing ^{14}C values of archived soil, collected in the topsoil at Halle in 1949 and 1961, reflect an increase in industrial activity, i.e. lignite use, and thus prevent their use for the quantification of carbon turnover rates via the tracing of (nuclear) bomb-^{14}C from pre-1954 until today. Other agricultural long-term field trials, located in less industrialised and rural areas, yielded a much lower contamination - up to 23 % at the Rothamsted Experimental Station (U.K) and less than 5 % fossil fuel-derived carbon in the surface soil at Rotthalmünster (Germany) - and thus are more favorable for the investigation of SOM dynamics.

The fossil contamination at Halle extended to all physical and chemical soil fractions, even to more specific lipid compound classes, resulting in a drastic overestimation of SOM stability. This demonstrates the difficulties associated with the investigation of soil carbon dynamics by the analysis of still heterogeneous, functionally-defined organic

matter pools and points out the requirement for investigations at the molecular level. ^{14}C-based estimates of fossil carbon in physical soil fractions from the Halle site were used to correct the proportion of maize-derived carbon which accumulated in the respective fractions after a vegetation change from rye to maize cropping about 40 years ago. Thus corrected natural ^{13}C labelling data indicate the importance of silt and clay particles (<2-20 µm) , which stored more than 50 % of the total soil organic carbon and yielded slow turnover times of 160-170 years. Furthermore, ^{14}C data and similarly corrected percentages of maize-derived carbon in density fractions suggest protection of SOM in the intra-aggregate fraction ($oPOM_{<1.6}$), which, however, contained only a minor proportion of the total organic carbon.

The effect of tillage on the amount and stability of organic carbon in aggregates was shown by ^{14}C results of water-stable aggregates obtained by wet sieving. Carbon and radiocarbon distributions in aggregates from a no-tillage grassland soil compared with results for ploughed surface soils at Rotthalmünster repeated the findings of previous studies which showed that tillage destroys macroaggregates. These store a large amount of relatively young organic carbon, which is lost upon ploughing. Carbon storage in microaggregates was not influenced by soil cultivation, but no-tillage combined with grassland cultivation seemed to promote organic carbon stabilisation in this fraction.

The relatively new technique of compound-specific radiocarbon analysis was tested and applied to microbial phospholipid fatty acids (PLFAs). ^{14}C concentrations of individual PLFAs - cell membrane components, used as proxies for living microbial biomass in soils - reflect different organic carbon sources assimilated by different soil microbes in the surface and in the subsoil at the rural Rotthalmünster site. A comparison of these data with results for the fossil carbon contaminated surface soil at Halle revealed a preferential synthesis of straight-chain monounsaturated PLFAs (*n*-C16:1, *n*-C17:1, *n*-C18:1) from recent organic substrate. A potential source of fresh organic material at 35-45 cm depth is mobile, dissolved organic matter, which is leached from the surface into the subsoil and probably is also responsible for a relatively younger humic acid fraction below the plough horizon. ^{14}C concentrations of different saturated PLFAs suggest the ability of Gram-positive microbes to metabolise old and fossil organic carbon respectively.

Zusammenfassung

Böden, die weltweit etwa 1200 bis 1700 Gigatonnen Kohlenstoff speichern, können sowohl eine Senke als auch eine Quelle von CO_2 sein. Veränderungen des in landwirtschaftlich genutzten Böden gespeicherten organischen Kohlenstoffs werden durch die Art der Bodenbewirtschaftung aber auch infolge der globalen Erwärmung hervorgerufen, weil sich diese jeweils positiv auf den Abbau der organischen Bodensubstanz (OBS) auswirken und somit einen Verlust an Kohlenstoff in Form von CO_2 bewirken. Die Hauptquelle der OBS ist pflanzliches Material, d.h. durch Photosynthese fixiertes CO_2, wie z.B. Blätterreste, Wurzeln und Wurzelexudate. Ihre Stabilisierung in Böden wird durch die Menge und die Art des Pflanzenmaterials und durch ihren Schutz vor mikrobiellem und oxidativem Abbau bestimmt. Die unterschiedlichen Prozesse, die zur Stabilisierung und zum Abbau von Kohlenstoff beitragen, sind jedoch nicht ausreichend bekannt. Ein Grund hierfür liegt in der Schwierigkeit die OBS zu untersuchen, da diese eine komplexe Mischung von frischen bis zu hochgradig umgewandelten organischen Komponenten darstellt, die durch Umsatzraten, die von wenigen Tagen bis zu mehreren tausend Jahren reichen, gekennzeichnet ist.

Zur Identifizierung der Herkunft der OBS und ihrer Umwandlung in landwirtschaftlich genutzten Böden wurden Radiokarbonmessungen von archivierten und modernen Bodenproben, die in physikalische und/oder chemische Fraktionen unterschiedlicher Stabilität sowie in einzelne Biomarker Komponenten aufgetrennt wurden, durchgeführt. Die gemessenen ^{14}C-Alter der unterschiedlichen OBS-Fraktionen, die in einer weiten Spanne von modern (> 100 pMC) bis hin zu >20.000 Jahre BP (7 pMC) variieren, spiegeln die heterogene Zusammensetzung der OBS des Langzeitfeldversuchs in Halle/Saale (Deutschland) wider. Das scheinbar hohe ^{14}C-Alter von 4880 Jahren BP (54 pMC) der OBS im Pflughorizont wurde auf einen hohen Gehalt von ≥52 % fossilen, ^{14}C-freien Kohlenstoff zurückgeführt, der mit Hilfe einer Massenbilanz abgeschätzt wurde. Untersuchungen von Bodenproben mit Hilfe von Licht- und Raster-elektronenmikroskopie wiesen auf das Vorkommen von schwarzen, kohleartigen Partikeln in der OBS in Halle hin. Die Lage des Versuchsstandortes in Halle in einer Region mit Braunkohleabbau und braunkohleverarbeitender Industrie sowie die ermittelten niedrigen O/C-Verhältnisse der selektierten schwarzen Partikel weisen auf Braunkohle als Hauptquelle des fossilen Kohlenstoffs in Halle hin. Abnehmende ^{14}C-Werte in archivierten Bodenproben, die 1949 und 1961 im Oberboden entnommen wurden, spiegeln einen Anstieg der industriellen Aktivität, d.h. der Braunkohlenutzung, wider und beeinträchtigen somit ihre Verwendbarkeit für eine Quantifizierung von Kohlenstoffumsatzraten mittels der Verfolgung des (nuklearen) Bomben-^{14}C im

Zeitraum von vor 1954 bis heute. Andere landwirtschaftliche Langzeitfeldexperimente, die in weniger industrialisierten bzw. in ländlichen Gegenden liegen, zeigten eine deutlich geringere Kontamination – bis zu 23 % im Oberboden eines Feldversuchs der Rothamsted Experimental Station (UK) und weniger als 5 % Kohlenstoff fossiler Herkunft im Oberboden eines Versuchs in Rotthalmünster (Deutschland) – und sind deshalb für die Untersuchung der OBS Dynamik besser geeignet.

Die fossile Kontamination in Halle beeinflußt alle physikalischen und chemischen Bodenfraktionen, sogar spezifischere Lipid-Komponentenklassen, was zu einer drastischen Überschätzung der OBS-Stabilität führt. Dieses Ergebnis verdeutlicht die Problematik der Untersuchung der Kohlenstoffdynamik basierend auf einer Analyse von heterogenen, methodisch-definierten Bodenfraktionen. Weiterhin unterstreicht es die Notwendigkeit von Untersuchungen auf der molekularen Ebene.

^{14}C-basierte Schätzungen des fossilen Kohlenstoffs in physikalischen Bodenfraktionen des Standortes in Halle wurden dazu genutzt, den Anteil an maisbürtigem Kohlenstoff zu korrigieren, der sich in den entsprechenden Fraktionen in Folge eines Fruchtwechsels vom Roggen zu Maisanbau, der vor ca. 40 Jahren durchgeführt wurde, angereichert hat. Die auf diese Weise korrigierten Daten des „natürlichen ^{13}C-labelling“ zeigen die Bedeutung der Schluff- und Tonfraktionen (<2-20 µm), die mehr als 50 % des gesamten organischen Kohlenstoffs speichern und langsame Umsatzzeiten von 160-170 Jahren aufweisen. Darüber hinaus weisen die ^{14}C-Daten und die auf die zuvor genannte Weise korrigierten Anteile an maisbürtigem Kohlenstoff in Dichtefraktionen auf eine Stabilisierung der OBS in Bodenaggregaten ($oPOM_{<1.6}$) hin, die allerdings nur einen geringen Anteil des gesamten organischen Kohlenstoffs enthielt.

Der Effekt des Pflügens auf die Menge und die Stabilisierung des organischen Kohlenstoffs in Aggregaten wurde mit Hilfe von ^{14}C-Gehalten in wasserstabilen Aggregaten, die durch Nasssieben gewonnen wurden, aufgezeigt. Der Vergleich von Kohlenstoff- und ^{14}C-Gehalte in Aggregaten eines ungepflügten Grünlandbodens mit Werten zweier gepflügter Oberböden in Rotthalmünster, bestätigten die Ergebnisse vorhergehender Untersuchungen, die gezeigt haben, dass Pflügen insbesondere Makroaggregate zerstört. Diese speichern große Mengen an relativ jungem organischen Kohlenstoff der durch das Pflügen verloren geht. Die Stabilisierung von Kohlenstoff in Mikroaggregaten zeigte hingegen keine Beeinflussung infolge von Bodenbearbeitungsmaßnahmen. Die Kombination von Grünlandkultivierung ohne Bodenbearbeitung scheint jedoch die Stabilisierung von organischem Kohlenstoff in dieser Fraktion zu fördern.

Weiterhin wurde die relativ neue Methode der komponentenspezifischen ^{14}C-Analyse getestet und auf mikrobielle Phospholipid-Fettsäuren (PLFAs) angewendet. Die ^{14}C-Gehalte einzelner PLFAs - die Bestandteile von Zellmembranen sind und als Indikatoren für lebende mikrobielle Biomasse im Boden genutzt werden können - spiegeln unterschiedliche Kohlenstoffquellen wider, die von verschiedenen Bodenmikroorganismen genutzt wurden. Der Vergleich von Daten, die im Ober- und im Unterboden des Standortes in Rotthalmünster erhoben wurden, mit Ergebnissen des durch fossilen Kohlenstoff kontaminierten Oberbodens in Halle weisen auf eine bevorzugte Synthese kurzkettiger, einfach ungesättigter PLFAs (*n*-C16:1, *n*-C17:1, *n*-C18:1) aus rezentem, organischem Substrat hin. Eine mögliche Quelle des frischen organischen Materials in 35-45 cm Bodentiefe ist mobile, gelöste organische Substanz, die vom Oberboden in den Unterboden verlagert worden ist und vermutlich auch für die relativ junge Huminsäurefraktion unterhalb des Pflughorizontes verantwortlich ist. Die ^{14}C-Gehalte verschiedener gesättigter PLFAs weisen auf die Fähigkeit von Gram-positiven Bakterien zur Metabolisierung von altem bis hin zu fossilem, organischem Kohlenstoff hin.

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1 Introduction

Radiocarbon dating by accelerator mass spectrometry (AMS) is used to investigate the composition, transformation and stabilisation of organic matter in agricultural soils. The aim of this study is to improve the understanding of the mechanisms which influence soil carbon dynamics. Methodological approaches include the analysis of functionally-defined organic matter fractions as well as the testing and the application of compound-specific radiocarbon analysis (CSRA) of biomarker substances, as a relatively new technique which has mainly been used in marine geochemical studies.

The motivation for this study is the continuous increase in atmospheric carbon dioxide (see chapter 2.1), which is a major 'greenhouse gas' supposed to have an impact on global warming through the 'greenhouse effect'. The observed rise in CO_2 concentration from the pre-industrial time until today, mainly caused by fossil fuel burning but also by changes in land-use, is about 30 % within a period of only about 200 years (Schlesinger, 1997). The balance of the emitted CO_2 with the amount of carbon sequestered by the oceans and by terrestrial ecosystems, revealed an unknown additional sink of annually about 2.3 ± 1.3 gigatonnes (Gt) of carbon (1989-1998) in the global carbon budget (Prentice, 2001). Recent investigations indicate that currently (in the 1990's) the 'missing' carbon is taken up by the terrestrial biosphere (e.g. Houghton, 1995; Tans and White, 1997; Schimel et al., 2001). The potential of soils for mitigating atmospheric CO_2 levels is still under controversial scientific (e.g. Post, 1993; Houghton, 2001) and political (United Nations, 1997; IGBP, 1998) discussion. Soils are regarded as a medium-term sink for atmospheric carbon-dioxide, since they are the most stable carbon reservoir of terrestrial ecosystems (Aiken et al., 1985; Schlesinger, 1997), exchanging large amounts of carbon with the atmosphere within years to several hundreds of years. Moreover, soil organic matter (SOM) contains about twice the amount of carbon stored in the atmosphere and three times that contained in the biosphere (Lal et al., 1997).

The sequestration of carbon in soils, and thus its withdrawal from an exchange with the atmosphere, is the result of humification of fresh plant detritus and its subsequent transformation to more stable forms of organic matter (see chapter 2.3). Stabilised organic matter can persist in soils for periods from centuries to millennia (Anderson and Paul, 1984; Jenkinson et al., 1992). It is widely assumed that physical processes, such as soil aggregation, as well as chemical factors, like chemical composition and structure, influence the stability of the organic material and thus its resistance against

bio-chemical degradation (Oades, 1995; Sollins et al., 1996). However, these processes are still incompletely understood, so that soil organic carbon fluxes cannot be calculated precisely.

This project is part of the Priority Program 1090 ‘Soils as source and sinks for CO_2’, which was established by the *Deutsche Forschungsgemeinschaft* (DFG; German Science Foundation) in 2000, to improve the understanding of the mechanisms resulting in the release or fixation of the greenhouse gas CO_2 in soils. The program provided the opportunity to combine the expertise of different working groups by using new analytical techniques to describe structure and turnover of SOM. The objective of this study was to use the bomb-^{14}C spike, the doubling of the atmospheric ^{14}C concentration in 1963 caused by the release of ^{14}C due to the atmospheric nuclear weapons tests in the early 1960s, to trace organic carbon turnover in soils under natural conditions on time scales of decades to hundreds of years (O'Brien, 1984; Harrison, 1996). This method requires the availability of archived ‘pre-bomb’ soil samples collected before 1954. Since all experimental and analytical work within the Priority Program should concentrate on a few study sites to allow a comparison of data obtained with different techniques, an agricultural site was chosen, which could provide both, archived pre-bomb soil samples as well as trials with a vegetation change for working groups using the natural ^{13}C labelling method (see chapter 3.1). The chosen long-term experimental site, established in 1878 with a soil archive started in 1949, is located close to the City of Halle/Saale in a highly industrialised region of Germany. This site turned out to have been influenced by the contribution of fossil-fuel derived carbon. As a consequence an additional agricultural long-term site in a rural area in the south of Germany at Rotthalmünster was investigated, which unfortunately could not provide archived soil samples thus excluding the use of the bomb-^{14}C tracing method for the quantification of carbon turnover rates.

Radiocarbon dating of SOM has already been used since the 1960s (Scharpenseel, 1968; Campbell et al., 1967). The results of these investigations indicate that under steady state conditions ^{14}C concentrations of bulk SOM reflect the 'apparent mean residence time' or the 'apparent mean age' of carbon in this complex mixture of organic constituents in various stages of decomposition or transformation. The analysis of SOM composition and its dynamics therefore requires a separation of the organic matter into organic matter pools of different stability (Trumbore et al., 1989, 1990; Trumbore and

Zheng, 1996; Scharpenseel et al., 1989; Skjemstad et al., 1996). ^{14}C results for physically or chemically-defined SOM fractions give information on organic carbon sources and transformation processes as well as on carbon turnover rates, when tracing the contribution of bomb-^{14}C over a certain period of time (Trumbore et al., 1989).

As functionally defined physical and chemical organic matter fractions still consist of a complex mixture of organic molecules with different origin and decomposability, a relatively new approach is the isolation of source-specific organic compounds (biomarkers) which are derived from certain sources or synthesised only within specific organisms. Biomarkers, which can be separated by chromatographic techniques and analysed by AMS ^{14}C (Eglinton et al., 1996; Pearson et al. 1998), provide information on carbon sources and their transformation, as shown by recent investigations in marine (Eglinton et al., 1997; Pearson et al., 2001) as well as in terrestrial geosciences (Petsch et al., 2001).

The examination of SOM composition and transformation was done in close collaboration with other working groups within the Priority Program. The objectives of the different analyses are listed below:

- To assess the quality of the different field trials for the analysis of soil organic carbon dynamics and particularly that of the archived soil samples from the Halle site, which may be contaminated by fossil fuel-derived carbon due to the nearby industries, pre-bomb and recent soil samples from Halle and from other study sites were radiocarbon dated. Furthermore, SOM heterogeneity at Halle was investigated by radiocarbon analysis of various organic matter fractionations (see chapters 5.1).

- The contribution of fossil fuel-derived carbon from industrial activities to SOM at Halle was examined by qualitative analyses of black, charred fragments via scanning electron microscopy coupled to energy-dispersive X-ray spectroscopy. The amount of this most probably ^{14}C-free material in different physical soil fractions was quantified based on their ^{14}C data (see chapter 5.2).

- Radiocarbon analysis was applied to different lipid compound classes, separated by automated organic geochemical isolation methods to identify different carbon sources. Compound-specific ^{14}C data of individual phospholipid fatty acids, isolated by preparative capillary gas-chromatography, are supposed to reflect the

assimilation of different organic substrates by soil microorganisms, playing a key role in the transformation and mineralisation of organic carbon in soils (see chapter 5.3).

- To assess the transformation of organic carbon with increasing soil depth, ^{14}C concentrations of chemically isolated SOM fractions from different depth intervals were determined. The influence of quantitative and qualitative changes of the organic matter at greater depth on soil microbes is examined by the comparison of surface with subsoil PLFA ^{14}C data (see chapter 5.4).

- The effect of physical organic carbon protection was studied by ^{14}C measurements applied to size and density fractions. Moreover, ^{14}C results of particle-size fractions were compared with the proportion of maize-derived carbon, calculated from $\delta^{13}C$ data on trials where C_3 vegetation has been changed into C_4 crops (see chapter 5.5).

2 Significance of soils as source and sink for CO_2

2.1 Global aspects of soil organic carbon

Carbon dioxide is the most important of the 'greenhouse gases', which are expected to promote global warming in the coming decades, as its concentration increases rapidly caused by human activities (Houghton et al., 2001). Since the pre-industrial period the CO_2 concentration in the atmosphere has risen from about 280 parts per million (ppm) in 1800 at first slowly and then progressively faster to a value of 367 ppm in 1999 (Prentice, 2001). This is known from direct atmospheric CO_2 measurements since 1959 (Keeling et al., 1989; Keeling, 1993) and analyses of the CO_2 of air bubbles trapped in Antarctic ice-cores during the last ice age (Leuenberger et al., 1992). The main sources responsible for an increase in atmospheric CO_2 concentration of 3.2 ± 0.1 Gt C per year are relatively well documented (Prentice, 2001). About 6.3 ± 0.4 Gt C per year arise from fossil fuel burning, and a smaller portion of 1.6 ± 0.8 Gt C per year is attributed to changes in land-use including CO_2 release from soils (Houghton, 2000; Prentice, 2001).

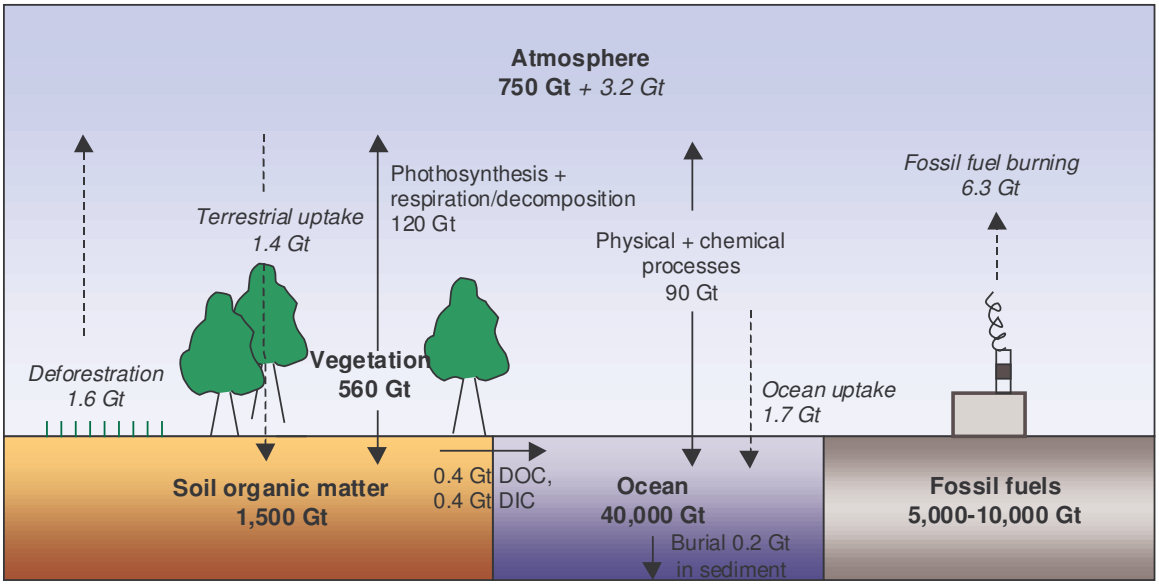

Figure 2.1: Global carbon reservoirs and major CO_2 exchanges between them in gigatonnes (Gt) of carbon per year. The dashed arrows show annual CO_2 fluxes caused by human activities (data from Schlesinger, 1997; Houghton et al., 2001; Prentice, 2001; Schimel et al., 2001). DOC (dissolved organic carbon) and DIC (dissolved inorganic carbon) are exported by rivers and derive from weathering of $CaCO_3$ respectively.

The balance of these emissions with the carbon uptake by the oceans (about 1.7 ± 0.5 Gt C yr^{-1}) and by the terrestrial biosphere (1.4 ± 0.7 Gt C yr^{-1}) revealed an annual deficit of about 1.6 Gt of carbon in the global carbon budget. Estimates of high uncertainty by Schimel et al. (2001) range between 2 to 4 Gt C per year for the 1990's, which means an increase of this residual sink since the 1980's (-3.8 to 0.3 Gt C, Prentice, 2001). Compared to the great land and ocean carbon reservoirs and the carbon exchanges between them as illustrated in figure 2.1 this amount seems relatively low. However, turning the unknown carbon sink into a source of CO_2 would further enhance the CO_2 concentration in the atmosphere. Thus it is crucial to know where the missing carbon is taken up and for how long it is stored.

The fate of this missing carbon is discussed controversial until now. Data of Broecker and Tsung-Hung (1992), who assumed that this carbon fraction is transported by ocean circulation, and Schlesinger (1990), who calculated low accumulation rates of organic matter in soils, did not give evidence for carbon sequestration in the terrestrial biosphere. However, new results for the 1990s based on indirect estimates obtained from atmospheric and oceanic data, isotopic analyses as well as ecosystem models indicate a net uptake of carbon in terrestrial ecosystems of the northern hemisphere (Tans et al., 1990; Houghton, 1991; Ciais et al., 1995; Keeling et al., 1996; McGuire et al., 2001). The contribution of different potential processes to an enhanced uptake is still uncertain. A major mechanism to explain the biospheric carbon sink is the so-called 'CO_2-fertilisation effect' an increased net primary production as a positive response of the vegetation to elevated atmospheric CO_2 levels (Friedlingstein et al., 1995; Houghton et al., 1998; Schimel et al., 2000; Cao and Prince, 2002; Melillo et al., 2002; Janssens et al., 2003).

A greater biomass production provides a larger organic carbon input into soils, which are the second largest carbon reservoir in the terrestrial carbon cycle after geological reservoirs storing 5,000 to 10,000 Gt of carbon as fossil organic carbon and rock carbonates. Depending on the rate at which the biomass input decays, the added organic material may enhance the soil organic carbon pool. Recent estimates of globally sequestered carbon in the upper 1 m soil depth are in the range of 1,200 to 1,700 Gt C (Bolin and Fung,1992; Eswaran et al., 1993, 1995; Oades, 1995). This is nearly three times more than is stored in vegetation (about 560 Gt C) and about twice the amount of carbon contained in the atmosphere as CO_2 (about 750 Gt C) (Lal et al., 1997; Schlesinger, 1997). Hence, soils are the largest 'active' terrestrial carbon pool,

which exchange large amounts of carbon on time scales from less than years to millennia, compared to 'passive' terrestrial geological carbon reservoirs, such as fossil fuels (Lal et al., 1995; Post 1993; Schlesinger, 1997; Houghton, 2000).

The mitigation potential of soils for atmospheric CO_2 depends on the transformation of organic materials into forms of higher resistance to biological decomposition (Swift, 2001). Processes increasing carbon sequestration are strongly influenced by land-use and management practices (Bruce et al., 1999; Lal, 2004) and hence could be regulated by human activity (Watson et al., 1996), which is a major subject in the framework of the Kyoto Protocol (United Nations, 1997) aiming at the mitigation of the greenhouse effect. Particularly the conversion of natural forests into cropland can cause significant carbon losses of 20-50 % from the soil into the atmosphere in the first 20 to 50 years (Scholes and Scholes, 1995; Houghton, 2000) and therefore should be avoided by political regulation. Cropland soils can lose carbon as a consequence of soil disturbance e.g. by tillage (Six et al., 1999; Kristensen et al., 2003). Moreover, investigations of Jenkinson et al. (1991) and Trumbore et al. (1996) indicate that global warming stimulates the decomposition and so the loss of soil organic carbon. Atmospheric CO_2 levels and the average surface temperature are expected to rise in the coming decades, as demonstrated in most scenarios for global climate change (Houghton et al., 2001), which may turn soils into sources of CO_2 in the future.

2.2 Composition and function of SOM

Soil organic matter comprises the living and dead organic matter in soils while the term 'humus' designates only the non-living organic matter (Stevenson,1994; Baldock and Nelson, 1999). SOM includes an infinite number of different organic compounds, ranging from relatively easily mineralisable plant residues to complex and recalcitrant products from biotic and abiotic transformation processes, as well as the microbial biomass (Stevenson, 1994; Hayes and Clapp, 2001; Kögel-Knabner, 2002).

The non-living organic matter can be divided into two major groups: non-humic and humic substances. Non-humic substances are composed of different, well characterised compound classes, including carbohydrates, fats, waxes, and proteins (Stevenson, 1994). The highly transformed humic substances are colloidal, high molecular weight organic compounds classified by their chemical properties (Kononova, 1966). They are traditionally divided into alkali-soluble humic acids, fulvic acids (figure 4.4), which remain in the acid solution after precipitation of humic acids,

and a humin fraction, which is not extractable with both acid and alkali (Stevenson, 1994). The structural properties of the extremely diverse humic substances and its chemical extraction are still unclear and an objective for further research (Schnitzer and Schulten, 1992; Stevenson, 1994). The microbial biomass, which represents about 1 % to 3 % of the soil organic carbon, utilises any of the organic residues including humic substances and supposedly recalcitrant compounds as sources for nutrients and energy (Stevenson, 1994). Microbes thus are key agents as they decompose organic residues, and currently release nutrients as well as the greenhouse gas CO_2.

SOM influences physical and chemical soil properties such as soil structure, pH, redox system, as well as the microbial activity and the retention and release of nutrients (Stevenson, 1994). Hence, it plays an important role in the maintenance of soil fertility which is of considerable importance for plant growth (Stevenson, 1994; Swift, 2001). Soils vary greatly in their organic matter content depending on the so-called factors of soil formation such as time, climate, vegetation, parent material, topography, and cultivation (Jenny, 1941) which influence the accumulation and decomposition of SOM. In agricultural soils the SOM content is generally lower compared to undisturbed soils, due to mostly lower organic inputs, since a large portion of plant material is removed as harvest. A second cause is the tilling of soils which increases the mineralisation of organic carbon, caused by the disruption of soil aggregates and changed soil conditions, such as temperature and aeration (Golchin et al., 1994a; Six et al., 1998, 1999). Management practices like reduced tillage and the return of crop residues to the soil, can help decrease carbon losses as CO_2 and increase the amount of more stable forms of organic matter.

2.3 Transformation and stabilisation of SOM

The cycling of organic matter in soils is mainly caused by selective microbial decomposition of organic compounds to either more resistant humic substances and CO_2 and CH_4, but also by abiotic polymerisation of humic monomers to complex humic substances (Stevenson, 1994). Easily decomposable, low molecular weight compounds, such as simple monomers of sugars, amino acids, and fats are preferentially degraded by microorganisms and thus have short lifetimes of month to years (Schnitzer, 1978), while more complex polymers, protein, cellulose, lignin, hydrocarbons, and complex re-synthesised humic compounds are decomposed much slower within decades to millennia (Oades, 1988; Stevenson, 1994). Consequently,

SOM is often partitioned into pools of different stability based on physical properties, such as particle- or aggregate-size, or chemical characteristics.

Since the processes of organic matter transformation and stabilisation in soil are still incompletely understood, quantitative information on SOM turnover can only be obtained by modelling. Different conceptual models are available that simulate carbon cycling particularly in agricultural soils and can be used to predict the effect of different management practices or, on the global scale, of environmental change on SOM. However, most models only simulate carbon dynamics in surface soils (in about 0 to 30 cm). The models usually differentiate between three or more pools ranging from labile, 'active' SOM, turning over within a few weeks, to stable, 'passive' fractions persisting in soils for years or decades (e.g. Jenkinson et al., 1991, 1992; Coleman and Jenkinson, 1999; Parton, 1987). However, these pools are operationally defined by their origin and turnover rate and not measurable as no methods are available to separate them. Recently, different attempts were made to derive parameters for a model with measurable SOM pools (e.g. Sohi et al., 2001). Most models include a pool of refractory or inert carbon whose turnover-time is in the range of thousands to ten-thousands of years (Parton et al., 1988; Falloon et al., 1998, 2000). Falloon and Smith (2000) defined this conceptual organic matter fraction as biologically not decomposable organic matter. However, they admitted that part of the inert material may not truly be refractory but has long turnover times (Falloon et al., 1998).

The majority of soil carbon-models describe carbon transformation and stabilisation as carbon transfer between the different pools and therefore do not account for physico-chemical processes which are regarded as major factors controlling organic matter stabilisation. Although the knowledge of the stabilisation mechanisms is still incomplete, different investigations indicate that chemical, physical, and microbial processes are involved (Oades, 1988; Skjemstad et al., 1996; Sollins et al., 1996). Three major factors are regarded to be responsible for the protection of organic carbon in soils, (i) the molecular composition and chemical structure of the organic matter components (Gregorich et al., 1996; Martins, 2000; Kögel-Knabner, 2002), (ii) their association with the inorganic soil matrix (Golchin et al., 1994b; Oades, 1995), and (iii) the location of organic substances within the soil structure which determine the accessibility of organic compounds to microbes and oxygen (Tisdall and Oades, 1982; Six et al, 2001). However, protection of organic matter from decomposition is not considered to be permanent, but seen as a result of a reduced decomposition rate in comparison to unprotected material (Baldock and Skjemstad, 2000).

2.4 Refractory organic carbon in soils

SOM is thought to comprise a small pool of refractory, biologically highly resistant organic compounds with turnover rates up to millennia (Balesdent and Mariotti, 1996) which is considered to be important for long-term carbon sequestration. This assumption originated from a number of previous studies in which different drastic chemical oxidations as well as physical separation methods were applied, yielding much higher radiocarbon ages for non-hydrolysable and physically protected organic matter respectively than for bulk soil (Campbell et al., 1967; Anderson and Paul, 1984; Trumbore et al., 1989; Skjemstad et al., 1993; Poirier et al., 2002). Moreover, analyses using ^{13}C nuclear magnetic resonance spectroscopy (NMR) and pyrolysis gas-chromatography coupled to a mass spectrometer indicate that this stable carbon pool contains resistant organic compounds with complex alkyl-aromatic structures (Schulten et al., 1992) and a greater proportion of aliphatic compounds (Lichtfouse et al., 1998a).

Recent investigations have shown that the resistant carbon pool may contain variable quantities of black carbon (Skjemstad et al., 1996; Poirier et al., 2002; Schmidt et al., 2002). The term ‘black carbon’ designates the carbonaceous residues produced by incomplete combustion of biomass and fossil fuel burning (Kuhlbusch, 1998; Masiello and Druffel, 1998). It is a chemically heterogeneous class of carbon compounds which is assumed to be highly resistant to biological degradation due to the highly aromatic structure of this material (Goldberg, 1985), and therefore of considerable importance as a carbon sink in the global carbon cycle (Kuhlbusch et al., 1995; Schmidt et al., 2002).

Schmidt et al. (1996, 2002) estimated the proportion of black carbon in soils at 15-45 % of total soil organic carbon and thus demonstrate the importance of this long living carbon fraction. As this material yields high radiocarbon ages between 1000 to 5000 years (isolated by photo-oxidation; Schmidt et al., 2002), its presence may lead to an overestimation of the stability of ‘natural’, non-black carbon soil organic matter. It may also hinder the identification of physical or chemical stabilisation mechanisms when functionally defined SOM fractions are analysed. Hence it is crucial to differentiate between stabilised ‘natural’ organic matter and anthropogenic-derived, old and fossil carbon from vegetation fires and fossil fuel combustion respectively, by quantifying the portion of black carbon in soils. However, until now no ultimate method of directly identifying and quantifying the products of biomass or fossil fuel combustion in soils is available (Skjemstad et al., 1996; Schmidt and Noack, 2000; Schmidt et al., 2001).

3 Principles of AMS radiocarbon analysis

3.1 Natural ^{13}C and ^{14}C abundance as tracers for SOM formation and degradation

Carbon has three naturally occurring isotopes, that have different numbers of neutrons and thus different masses. The most abundant isotope is ^{12}C making up approximately 98.89 % of all carbon in nature. The abundance of the stable isotope ^{13}C is about 1.11 %, whereas the radioactive ^{14}C is present in only very small quantities of 1.176×10^{-12} atoms (Karlén et al., 1968). By studying the ratios of the two stable isotopes as well as of the radioactive and a stable carbon isotope in soil organic matter it is possible to decipher processes and rates of organic carbon production, transformation, and degradation in soils as described in the following chapters.

The stable isotope abundance is reported as the deviation in the $^{13}C/^{12}C$ ratio of a sample from that of an international standard, expressed as $\delta^{13}C$ in ‰ PDB:

$$\delta^{13}C\ (‰\ PDB) = [(^{13}C/^{12}C_{sample}) / (^{13}C/^{12}C_{reference}) - 1] \times 1000 \quad (1)$$

The reference material is the carbonate fossil *Bellemnitella americana* from the Pee Dee formation in South Carolina (PDB, $\delta^{13}C$ = 0 ‰). Radiocarbon data are also reported relative to the $^{14}C/^{12}C$ or $^{14}C/^{13}C$ ratio of a standard, but corrected for the effect of isotopic fractionation as explained in chapter 3.3.2.

3.1.1 Natural ^{13}C labelling

Natural ^{13}C labelling, resulting from the isotopic difference between C_3 and C_4 vegetation, makes it possible to trace organic matter transformation in soils (Balesdent and Mariotti, 1996). Due to different biochemical properties of CO_2-fixation (primary carboxylation) during photosynthesis C_3 and C_4 plants discriminate differently against $^{13}CO_2$ resulting in different stable carbon isotope ratios (Boutton, 1996). C_3 plants, which reduce the CO_2 to a carbohydrate containing three carbon atoms (phosphoglycerate) have $\delta^{13}C$ values of -32 ‰ to -22 ‰ PDB. Plants which use the C_4 pathway, producing aspartic or malic acid with four carbon atoms, have higher $\delta^{13}C$ values of -17 ‰ to -9 ‰ PDB (Boutton, 1991). On sites where vegetation has changed from the predominance of one photosynthetic pathways to the other the $\delta^{13}C$ value of soil organic carbon is progressively replaced by the $\delta^{13}C$ signature of carbon derived from the new vegetation. The proportion of carbon derived from the new vegetation can

be determined via the $\delta^{13}C$ contents of SOM and of plant material by the mass balance calculation as explained in chapter 4.5. This technique makes it possible to calculate turnover times of organic carbon in SOM occurring on decadal and shorter time scales (Balesdent et al., 1987; Huggins et al., 1998).

3.1.2 Radiocarbon analysis of soil organic matter

Radiocarbon analysis has been used since the early days of this method for the investigation of soil organic matter. In the beginning one major objective was to determine the absolute age of soils (Scharpenseel, 1968; Campbell et al., 1967; Gerasimov, 1974). However, SOM is composed of a heterogeneous mixture of organic components accumulating and decaying at different rates, which is reflected by ^{14}C ages of organic components from recent to more than 20,000 years (Scharpenseel and Becker-Heidmann, 1992). Furthermore, soils are an open system which continuously receive organic carbon as plant residues and loose gaseous and dissolved carbon via mineralisation and leaching respectively. Hence radiocarbon data of SOM do not represent the soil age but reflect the 'apparent mean age', the average age of the different organic components in SOM (Scharpenseel and Becker-Heidmann, 1992; Trumbore, 1996). As a consequence of this numerous physical and chemical fractionation methods have been used with the objective to isolate a 'passive' SOM fraction that turns over on time scales of millennia and thus is least influenced by recently introduced organic matter and can be used as an indicator for the soil age (Scharpenseel, 1972, 1977; Huang et al., 1996; Pessenda et al., 2001).

The ^{14}C method proved to be helpful for identifying organic matter sources, their transformation and stabilisation in soils. This approach requires the separation of physically and/or chemically defined SOM fractions which provide information on their origin or can be associated with transformation processes respectively (Jenkinson and Rayner, 1977; Trumbore and Zheng, 1996). For example Scharpenseel and Becker-Heidmann (1989) examined the effect of bioturbation by the analysis of the bulk soil ^{14}C distribution in soil profiles. Trumbore et al. (1989, 1990) determined ^{14}C concentration of different physical and chemical fractions in soil profiles to determine carbon sources at different depth and identify transport processes. Moreover, ^{14}C results can be used to verify physically or chemically defined SOM pools of alleged different stabilities (Anderson and Paul, 1984; Trumbore et al., 1989).

Furthermore, ^{14}C analysis has repeatedly been used to calculate carbon turnover times in bulk soil as well as in different chemically or physically defined SOM pools. This can be done on two different time-scales: (1) natural ^{14}C, produced in the upper atmosphere, reflects the turnover of organic matter stabilised by physical interactions with the soil matrix or by chemical processes, which thus resides in soils long enough for significant radioactive decay to occur, and (2) the so-called 'bomb-^{14}C', derived from atmospheric testing of nuclear weapons, mainly in the late 1950s until the early 1960s (figure 3.1), provides information on carbon exchanges that occur within several years to decades:

(1) The ^{14}C age, determined from the AMS ^{14}C data as in equation 10 (chapter 3.3.2), has been used equivalent to a the 'apparent mean residence time' of organic carbon in SOM under steady state conditions (Trumbore and Druffel, 1995). Because this approach assumes SOM to be a homogeneous pool with one decay rate, apparent mean residence times can drastically underestimate the turnover of carbon in bulk SOM as well as in separated organic matter fractions (Trumbore and Druffel, 1995).

(2) The bomb-^{14}C method is a sophisticated tool to calculate turnover rates of fast cycling organic carbon on times-scales of years to decades, comparable to the natural ^{13}C labelling method (chapter 3.1.1). The bomb-^{14}C is progressively incorporated in SOM by the addition of plant residues that are in equilibrium with the atmospheric $^{14}CO_2$ via photosynthesis. However, the calculation of carbon turnover times requires the availability of archived soil samples collected from before the release of bomb-^{14}C into the atmosphere (1954), which directly document the incorporation in SOM over a certain period of time. In numerous studies bomb-^{14}C has been used to trace organic carbon cycling in bulk SOM (O'Brien and Stout, 1978; O'Brien, 1984, 1986; Harkness et al., 1986; Harrison, 1996, 1998). Other researchers, for example Goh et al. (1984), Trumbore et al. (1989, 1990), Trumbore (1993), Paul et al. (1997), and Römkens and Hassink (1998), took advantage of the possibilities offered by the AMS technique (chapter 3.3) and analysed bomb-^{14}C concentrations of physical and chemical organic matter fractions with often very low carbon contents. Their data allow a differentiation of 'active' and 'passive' organic matter pools, where the latter one is, due to its slow turnover rate, expected not to reflect the increase in atmospheric ^{14}C during the time since the release of bomb-^{14}C. 'Active', labile organic matter pools, will be influence to variable degrees by bomb-^{14}C depending on the turnover time of carbon in the separated fraction. Since labile SOM is hypothesised to respond quickly to changed

environmental or climatic conditions, it may be valuable as short-term indicator for changes in total SOM (Gregorich and Ellert, 1993; Wang et al., 1999).

3.1.3 Compound-specific radiocarbon analysis

Operationally-defined organic matter fractions still consist of a mixture of organic materials 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., 1999a). Hence, carbon dynamics of mixed pools may differ from the actual turnover of carbon in the separated reservoirs. To evaluate changes in soil carbon dynamics correctly, it is necessary to eliminate unknown or contaminating carbon sources which can be achieved by ^{14}C analyses at the molecular-level, where differences between individual compounds of specific sources (biomarkers) are fully expressed. However, a prerequisite of this approach is that the analysed biomarkers can unequivocal be linked to their sources, which is still a matter of discussion (Lichtfouse, 1998).

Compound-specific radiocarbon analysis (CSRA) became practicable with the development of preparative capillary gas-chromatography, that allows the isolation of individual molecules from complex mixtures, and the reduction of the necessary sample size for ^{14}C measured by AMS to <100 µg of carbon (Eglinton et al., 1996; von Reden et al., 1998). Until now CSRA has mainly been used in marine geosciences, e.g. to distinguish if sediment material originates from terrestrial or marine environments (Pearson et al., 2000, 2001; Uchida et al., 2000). In soil science, radiocarbon analyses at the molecular level are still scarce awaiting methodological improvements to be used as an alternative or additional dating tool. However, CSRA has been applied to identify the addition of ancient hydrocarbons to modern soils (Lichtfouse et. al., 1997) as well as to examine the incorporation of fossil carbon derived from weathered shale by soil microorganisms (Petsch et al., 2001).

3.2 Principles of radiocarbon dating

The existence of ^{14}C and its use for dating was first demonstrated by Willard Libby (1946, 1955) who received the Nobel Prize in chemistry for his investigations. The radiocarbon method is generally suitable for age determination up to 50,000 years, but has been extended via isotopic enrichment to about 75,000 years for conventional decay counting methods (Grootes, 1978). An extension to 60,000 years for AMS can

be achieved under special circumstances, as shown by Völker et al. (2000) who correlated climate records and ^{14}C ages of the planktonic foraminifera of a deep-sea sediment core from the Iceland Sea.

^{14}C is mainly produced in the lower stratosphere by collision of low-energy cosmic ray neutrons (n) with nitrogen atoms (p denotes a proton):

$$^{14}N + n \rightarrow {}^{14}C + p \qquad (2)$$

The produced ^{14}C is oxidised rapidly to ^{14}CO and then, within months, to $^{14}CO_2$, which is distributed in the total atmosphere. ^{14}C enters the plant biomass by assimilation of CO_2 for photosynthesis, and subsequently the heterotrophic organisms via the food chain. Various exchange processes between the atmosphere, the biosphere, and the hydrosphere result in a dynamic equilibrium between ^{14}C production and decay, displayed by a constant $^{14}C/^{12}C$ ratio. Exchange rates between the different reservoirs differ greatly depending on the respective carbon dynamics (Taylor, 1987; see chapter 3.2.1).

When the exchange between an organism and the atmosphere is stopped by death, the radiocarbon concentration begins to decrease through radioactive decay (equation 3) with a half-life of 5730 ± 40 years (Godwin, 1962).

$$^{14}C \rightarrow {}^{14}N + \beta^- + \bar{\nu} \qquad (3)$$

with : β^- : electron, $\bar{\nu}$: antineutrino

The residual ^{14}C concentration of a sample at a certain time is defined by the radioactive decay equation (4):

$$N(t) = N_0 \cdot e^{-\lambda t} \qquad (4)$$

with: N: the number of atoms at time t
N_0: the initial number of atoms
λ: the decay constant of ^{14}C

The age of a sample at a certain time can be derived according to equation 5 when the initial number of atoms (N_0) is known. Since this is impossible, N_0 is estimated via a modern standard as described in chapter 3.3.2.

$$t = -\frac{1}{\lambda} \cdot \ln \frac{N(t)}{N_O} \tag{5}$$

with: $\frac{1}{\lambda} = \frac{t_{1/2}}{\ln 2}$, being the mean-life and $t_{1/2}$ the half-live of ^{14}C.

The conventional ^{14}C age is calculated with the not precise half-life $t_{1/2}$ of 5568 years determined by Libby instead of the more precise value of 5730 years determined by Godwin (1962; see chapter 3.3.2).

3.2.1 Basic assumptions underlying the ^{14}C method

The determination of absolute or calendar ages requires several general assumptions underlying the ^{14}C method which are listed below:

1. The production of ^{14}C has been constant for more than 10^5 years.
2. The carbon exchange between the reservoirs (atmosphere, biosphere, and ocean) is rapid compared to the half-live of ^{14}C, so that the reservoirs are well mixed.
3. Carbon isotope ratios in samples are in equilibrium with their surroundings before the exchange ceases.
4. After the death of the organisms or the deposition of carbonates no further exchange takes place (*closed system*-assumption).
5. ^{14}C decays at a constant rate and is not influenced by its chemical or physical surroundings.

These assumptions are only partly fulfilled. In radiocarbon dating of soil organic matter particularly assumptions 1 and 4 have to be considered, since they complicate or even prevent the determination of absolute soil ages.

In fact, (1.) the atmospheric ^{14}C concentration has varied with time due to changes in the ^{14}C production (de Vries, 1958). Since recent plant material, the main source of SOM, is in equilibrium with the atmospheric CO_2, the amount of ^{14}C added to SOM changed over time. Changes in the ^{14}C production are caused by variations of the

earth's magnetic field (Stuiver et al., 1991; Sternberg, 1992), the sunspot cycle (Stuiver and Quay, 1980), and cosmic ray fluxes (Suess, 1986; Stuiver et al., 1991; Kocharov et al., 1992). More recently, since about 1870, the consumption of fossil fuel has led to a dilution of ^{14}C in the atmosphere by release of $^{12}CO_2$, *Suess-* or *Industrial-effect* (Suess, 1955). A contrasting effect was caused by the atmospheric testing of nuclear weapons in the late 1950s until the early 1960s which released large quantities of ^{14}C into the atmosphere. In August 1963 the atmospheric radiocarbon concentration reached a maximum (Northern Hemisphere) and was nearly twice as high (about 200 percent modern carbon; chapter 3.3.2) as in 1950 (Nydal and Lövset, 1983; Levin and Kromer, 1997). The so-called *bomb-effect*, displayed in figure 3.1, dominates the natural and fossil fuel effects. The bomb-^{14}C spike, which subsequently entered the vegetation and the organic matter in soils, can be used as a tracer to follow the transformation of organic carbon in SOM under natural conditions on a timescale of decades (Trumbore, 1996; Levin and Hesshaimer, 2000; chapter 5.1).

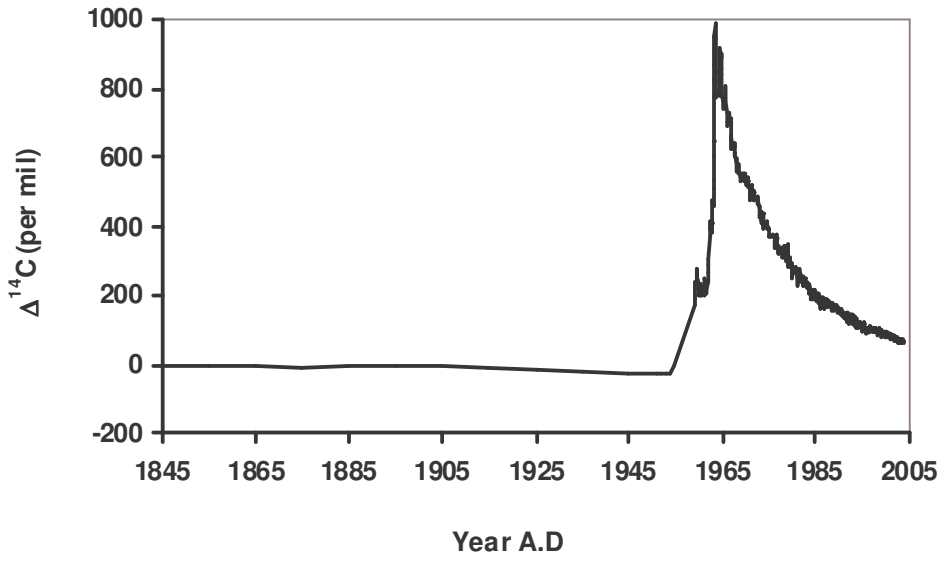

Figure 3.1: Atmospheric $^{14}CO_2$ between 1870 to 2003. Proxy data estimated from tree-rings (Stuiver and Becker, 1993), and directly measured air samples of the stations Vermunt (Austria), Jungfraujoch (Switzerland), and Schauinsland (Germany; Levin and Kromer, 1997; Levin et al., 2003; Levin and Kromer, 2004). $\Delta^{14}C$ values were calculated as defined in equation 9, chapter 3.3.2.

When analysing SOM the *closed system*-assumption (4.) is another crucial condition which is not fulfilled because soils continuously exchange carbon with the atmosphere and with the hydrosphere. On the one hand recently fixed CO_2 in plant tissues is

continuously added to the SOM pool and on the other hand carbon is lost from this pool by mineralisation of organic matter as well as via losses of dissolved organic carbon leached into the ground water.

Furthermore, different organic materials have slightly different initial ^{14}C concentrations caused by isotopic fractionation, e.g. during photosynthesis plants discriminate against heavier carbon isotopes. This effect has to be corrected by determining the stable isotope ratio ^{13}C:^{12}C of the sample material (chapter 3.3.2).

3.3 Principles of ^{14}C measurement by accelerator mass spectrometry (AMS)

Since the end of the 1970s tandem accelerators have been used to measure natural ^{14}C levels mass spectrometrically (Nelson et al., 1977; Bennett et al.,1977). The main advantage of AMS over the conventional decay counting methods is its about 10,000-times higher sensitivity. This is achieved by the direct detection of all ^{14}C atoms instead of only those which decay, as it is done in conventional decay counting methods such as liquid scintillation or proportional gas counters which detect β^--particles emitted during radioactive decay of ^{14}C. Radiocarbon analysis by AMS makes it possible to reduce the necessary sample size from over 1 gram to less than 1 milligram of carbon and to complete a measurement within 1 hour instead of in days.

The AMS technique is an extension of conventional isotope ratio mass spectrometry (IR-MS) in which a magnetic field is used to separate the ionised carbon isotopes by their different masses so that they can be quantified separately. The lower abundance limit of IR-MS however, is set by the presence of mass interfering isotopes and molecular fragments (isobars). Thereby carbon isotope ratios of as low as about $1:10^{-9}$ can be measured and thus conventional IR-MS cannot be used to detect ^{14}C. The measurement of rare natural isotopes with relative abundances of 10^{-9} to 10^{-16} requires to discriminate against unwanted isobars, which are in the case of radiocarbon the mass-14 interfering molecular fragments ^{13}CH and $^{12}CH_2$, and the nitrogen isotope ^{14}N. This is possible by starting with negative ions and thus discriminating against ^{14}N, which does not form a stable negative ion. Furthermore, the use of a tandem electrostatic accelerator equipped with a terminal stripper for charge exchange to positive ions makes it possible to destroy isobaric molecular ions (Vogel and Nelson, 1995; Tuniz et al., 1998). The mass spectrometric determination of isotope ratios are relative measurements made against an international standard of known isotope ratio.

The contamination introduced during sample preparation and during AMS analysis is determined and corrected for by background measurements.

3.3.1 The AMS system of the Leibniz-Laboratory at Kiel

The ^{14}C measurements of this study were made at the Leibniz-Laboratory (Christian-Albrechts-University of Kiel) with a 3 million volt (MV) Tandetron 4130 AMS system from High Voltage Engineering (HVE), which is schematically presented in figure 3.2. The different stages of the ^{14}C measurement process and the characteristics of the AMS system at Kiel are summarised below. A detailed description of the system can be found in Nadeau et al. (1997).

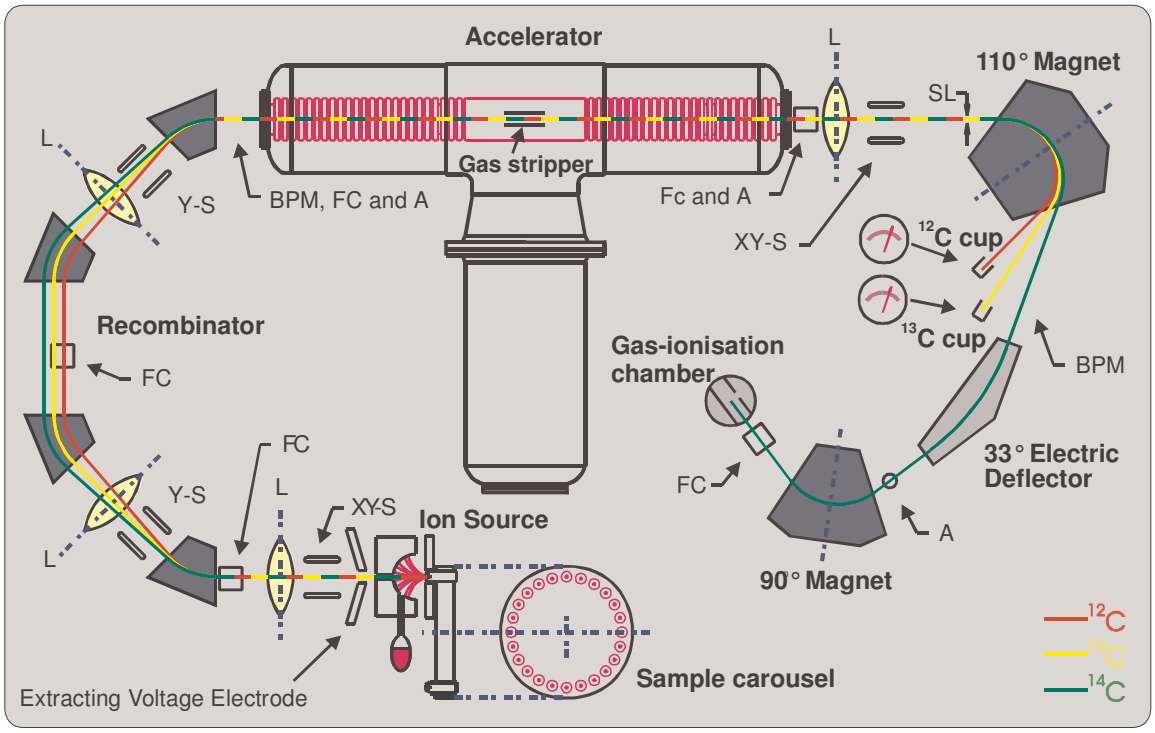

Figure 3.2: The HVE AMS system of the Leibniz-Laboratory (Kiel) with a separator-recombinator unit for simultaneous acceleration of the three carbon isotopes (schematic modified from Nadeau et al., 1997). The symbols indicate L a lens, Y-S one pair, and XY-S two pairs of steerers (horizontal and vertical), BPM a beam profile monitor, FC faraday cups, SL vertical slits, and A aperture. See text for further descriptions. Some instruments are only diagnostic tools not operating during measurement.

The sample graphite is pressed into aluminium target holders which are placed in a sample carousel for up to 59 targets. The target holders are automatically pushed into

and removed from the ion source, which remains under high vacuum. In the ion source the sample graphite is sputtered with caesium (Cs^+) ions producing a negative ion beam which consists of $^{12}C^-$, $^{13}C^-$, $^{14}C^-$ and other elemental and molecular negative ions. This procedure discriminates against ^{14}N as this isobar does not form stable negative ions. In order to simultaneously accelerate the three carbon masses, the AMS system of the Leibniz-Laboratory uses a separator - recombinator unit (Nadeau et al., 1997): The ions emerging with low-energies of 35 kilo electron volt (keV) from the source pass through an analyser where they are split into mass 12, 13, and 14 and other, unwanted masses are eliminated. Then the $^{12}C^-$ beam is reduced to about 1 % of its intensity by a mechanical chopper, to prevent overloading of the accelerator, and after that the three masses are recombined. Subsequently, the negative ion beam is accelerated toward the terminal in the centre of the accelerator which is at +2.5 MV thus gaining 2.5 million electron volts (MeV) energy. There the beam passes through a low-density gas stripper (argon). The stripper removes several electrons from the ions thereby, multiply positive ions are produced, and molecular isobars are destroyed. The positively charged ions, mainly +3, are then further accelerated from 2.5 MV to earth potential gaining another 7.5 MeV. A 110° magnet separates the ion beam into the three masses at charge +3 and eliminates other ions. The relative abundances of the stable, abundant isotopes ^{12}C and ^{13}C are measured as charge deposited in Faraday cups. The rare ^{14}C isotopes pass an electrostatic analyser (33° deflection) and a 90° magnet to remove residual products of the molecular ions and scattering before they are counted in a gas-ionisation chamber.

Particle identification is possible by determination of their energy loss rate and total energy. The particle count rate and the Faraday cup currents of the AMS measurement are used to calculate a ratio of $^{14}C/^{12}C$ and $^{13}C/^{12}C$ for samples as well as for the reference standard.

3.3.2 Calculation of ^{14}C data

Radiocarbon contents are calculated from the measured $^{14}C/^{12}C$ ratio of the sample compared to that of an international standard and commonly expressed in percent modern carbon (pMC; equation 6).

According to Stuiver and Polach (1977) the internationally accepted radiocarbon dating reference standard is 95 % of the activity of the oxalic acid standard (Ox I; measured in 1950, corrected to $\delta^{13}C$ of -19 ‰ PDB) provided by NIST (National Institute of

Standards and Technology, Washington, DC, USA). This correction adjusts the isotope ratio of Ox I (105.26 pMC), measured in 1950, to that of pre-industrial wood from 1850 AD, containing no fossil fuel-derived carbon. Thus 'modern' carbon is defined as the $^{14}C/^{12}C$ ratio in 1950 AD which is by convention 100 pMC. Since the original material no longer exists, it has been replaced by other standards such as Ox II (134.07 pMC) provided by IAEA (International Atomic Energy Agency, Vienna, Austria). Thus the isotope ratio of OX II has to be corrected to that of the primary Ox I standard (conversion factor: 1.2736; Mann, 1983) and adjusted to pre-industrial wood (= 0.95/1.2736).

$$pMC = (R_{sample^*}) / (R_{Ox^*}) \cdot 100 \quad (6)$$

$$\text{with:} \quad R_{sample^*} = R_{sample} \cdot [(1 - 25/1000) / (1 + \delta^{13}C/1000)]^2 \quad (7)$$

$$R_{Ox^*} = 0.95 \cdot R_{Ox\,I} \; [(1 - 19/1000) / (1 + \delta^{13}C/1000)]^2 \quad (8)$$

$$= 0.7459 \cdot R_{Ox\,II} \; [(1 - 25/1000) / (1 + \delta^{13}C/1000)]^2$$

where R_{sample} is the $^{14}C/^{12}C$ ratio of the sample, R_{Ox} those of the oxalic acid standard, and R_{sample^*} and R_{Ox^*} are the respective values corrected for isotopic fractionation as described below.

Correction for isotopic fractionation

To account for mass-dependent isotopic fractionation arising from natural processes, ^{14}C results have to be corrected (normalised) to constant $\delta^{13}C$ values. The fractionation is for ^{14}C roughly twice as large as for ^{13}C, due to the mass differences. By convention ^{14}C results of the samples (R_{sample^*}; equation 7) are normalised to $\delta^{13}C$ of -25 ‰ relative to PDB and results of the oxalic acid standard (R_{Ox^*}; equation 8) are corrected to $\delta^{13}C$ of -19 ‰ PDB (Ox I) and to $\delta^{13}C$ of -25 ‰ PDB (Ox II) respectively (Mook and van der Plicht, 1999; Mann, 1983). The correction is done via the $^{13}C/^{12}C$ ratio of the sample and of the oxalic acid standard which is measured by the AMS system simultaneously with the $^{14}C/^{12}C$ ratio. $\delta^{13}C$ of sample and standard respectively, are calculated according to equation 1 (chapter 3.1) relative to the reference standard (PDB).

Correction for background

The contamination introduced during chemical sample processing, the process blank, is assessed and corrected by preparing and measuring ^{14}C-free anthracite. The blank value correction, depending on the effect of the process blank on both the sample and

the oxalic acid, is subtracted from the $\delta^{13}C$ corrected ^{14}C data. The effect of the sample contamination on the ^{14}C result depends on the amount of carbon in the sample and on the age difference between the contaminant and the sample. Further information on the laboratory blank and the machine background of the AMS system at the Leibniz-Laboratory can be found in chapter 4.4.3.

Measurement uncertainty

^{14}C results are reported with ± 1-σ measurement uncertainty, yielding the confidence interval in which the true value is to be expected with 68.3 % probability (Stuiver and Polach, 1977). At the Leibniz-Laboratory the larger value of (i) the reproducibility of the 8 to 9 measurements of each sample or (ii) the Poisson counting statistics, the total number of ^{14}C counts for each sample, is taken as measurement uncertainty for ^{14}C results (Nadeau et al., 1998).

Calculation of $\Delta^{14}C$ values and of the conventional radiocarbon age

In geochemical studies radiocarbon data are usually expressed as $\Delta^{14}C$, the deviation in parts per thousand (‰) of the sample $^{14}C/^{12}C$ ratio from that of the absolute standard (equation 9). These values are corrected for isotopic fractionation and radioactive decay from the year of sample origin to the pre-1950 atmosphere ($\Delta^{14}C$ = 0 ‰). In the present study, positive values of $\Delta^{14}C$ indicate the presence of bomb-produced ^{14}C and negative values correspond to atmospheric ^{14}C levels before 1950.

$$\Delta^{14}C\ (‰) = (fMC \cdot e^{(-\,(y\,-\,1950)\,/\,8267)} - 1) \cdot 1000 \qquad (9)$$

where fMC is the fraction modern carbon (pMC / 100), y is the year of sample collection, and 8267 represents the mean-life of ^{14}C calculated with the precise half-life of 5730 years.

The conventional radiocarbon age (t) in years before present (BP) is calculated using the radiocarbon decay equation (10). It comprises the (incorrect) half-life of 5568 years determined by Libby and is corrected to 1950 AD as 0 years BP.

$$t\ (\text{years BP}) = -\,1/\,\lambda \cdot \ln\,(R_{sample^*} / R_{Ox^*}) \qquad (10)$$

where 8033 represents the mean lifetime (1/λ) of ^{14}C.

To obtain a historical age (cal AD, cal BP) the conventional radiocarbon age needs to be calibrated with the help of an international tree ring calibration data set (Stuiver et al., 1998) which is included in different calibration programs (e.g. CALIB, Stuiver et al., 2003). Calendar ages can only be determined for the time previous to the release of ^{14}C-free CO_2 by fossil fuel combustion and to the production of bomb-^{14}C by aboveground nuclear weapon testing.

4 Study sites and methods

4.1 Study sites and soil sampling

The investigations on SOM stabilisation were mainly done on soil samples from two agricultural long-term field trials located in Germany (figure 4.1). The characteristics of both sites are listed in table 4.1. The site at Halle, located in a highly industrialised region, could provide archived soil samples from trials with crop rotation and with continuous rye. The second experimental site is located at Rotthalmünster, in a rural area. In addition, a few samples from a long-term experiment at the Rothamsted Experimental Station in Harpenden, UK, located about 40 km to the south of London were analysed. Most trials used for this study are cultivated in monoculture, and thus provided the advantage of nearly constant, and well documented conditions.

Figure 4.1: Location of the German long-term study sites at Halle and at Rotthalmünster.

Table 4.1: Soil type and cultivation of the study sites at Halle and Rotthalmünster.

	Halle	**Rotthalmünster**
Cultures (starting year)	rye (since 1878) maize (since 1961) crop rotation (since 1949)	wheat (since 1969) maize (since 1979) grassland (since 1961)
Archived samples	rye (since 1961) crop rotation (since 1949)	not available
Soil type	Haplic Phaeozem (FAO)	Haplic Luvisol (FAO)
Soil texture	70% sand, 20% silt, 10% clay [1]	11% sand, 72% silt, 17% clay [2]

[1] Merbach et al., 1999, 2000;
[2] Personal communications Kleber, University of Halle, 2003

4.1.1 Halle

The field trials of the University of Halle are located close to the city centre of Halle in the State Sachsen-Anhalt in Germany (N 55°30.8'; E 11°59.9'). Geographically it is located to the east of the Harz mountains (*Östliches Harzvorland*) on a plateau which is about 110 m above sea level. The Leipzig – Halle region is strongly influenced by about 350 years of open cast lignite mining and lignite using and processing industries (Eissmann, 1994). Industrial emissions were not controlled or reduced in the German Democratic Republic until the German Unification, in 1989. As a consequence of this, large quantities of lignite particles and fly ash were deposited (Enders and Bambauer, 1994) particularly close to lignite mines, power plants, and coal using industries (Schmidt et al., 1996). After the German Unification coal mining and the use of lignite was reduced or shut down. The experimental site is located adjacent to a railway track (figure 4.2) on which lignite was transported during the 1990s (Wiesenberg et al., 2004a).

The climate at Halle, belonging to the 'Central German dry region', is characterised by a low mean annual precipitation of 465 mm and a mean annual temperature of 9.2°C. The soil of the field trial is a degraded Chernozem (*Parabraunerde-Tschernosem*) developed on Pleistocene sandy loess, mainly derived from the Saale cold stage, which has been classified according to FAO (1990) as a Haplic Phaeozem (figure 4.3). The soil texture in the plough horizon consists of about 70 % sand, 20 % silt and 10 % clay (Merbach et al., 1999, 2000).

Figure 4.2: Photo of the long-term experimental site 'Eternal rye' with (harvested) rye and maize cultures (photography by Ute Hamer).

The investigations were done on soil samples from the field trial 'Eternal rye', a rye monoculture, which was established in 1878 and partly converted into continuous maize cropping in 1961, and additionally on soil from a field trial with crop rotation (potatoes, oats, maize, barley, sugar beets, and wheat), started in 1949. The straw of the rye and maize culture is removed from the field after harvest. The maize is used for silage-making so that only short maize stubbles are left on the field and ploughed into the surface soil. Soil cultivation changed over time. Until 1969, both trials were cultivated by a horse pulled plough to 20 cm depth. Then the horse was replaced by a tractor plough and the depth of the plough horizon was increased to 25 cm. Since the mid 1990s the plough depth was increased further to about 30 cm depth.

Soil samples were taken from the continuous rye and continuous maize trials from plots (i) without fertilisation, (ii) with mineral fertilisation (nitrogen, phosphate, potassium: NPK), and (iii) with farmyard manure. The minerally fertilised plots receive about 40 kg N ha^{-1} a^{-1} until 1991 and 60 kg N ha^{-1} a^{-1} in the following years. Soil sampling in September 2000 was done after harvest and after cultivation of the rye culture but before cultivation of the trial with maize. Samples were taken from the three experiments at different dates as described below and in table 4.2.

- The unfertilised rye plot was sampled at three depths (0-5 cm, 30-35 cm, 60-68 cm) by a 6 cm diameter corer in December 2000. Six replicates from the same depth were mixed to provide a representative sample of each depth.
- A topsoil sample from 0-25 cm was taken from a soil pit in September 2000.
- A young rye plant, sown in September 2000, was sampled in December 2000.
- Particle-size and density fractions were obtained from a composite sample (five replicates) of the unfertilised rye and maize plots and collected in September 2000.
- Lipid compound classes were extracted from soil samples of the minerally fertilised rye plot taken in March 2001 from 0-25 cm soil depth.
- Total lipids and phospholipids were extracted from fresh soil samples collected in September 2001 on the rye and maize plots (unfertilised, mineral fertiliser, manure) in 0-35 cm and 35-45 cm depth. Individual phospholipid fatty acids (PLFAs) were isolated from the phospholipid fraction of the unfertilised rye and the maize cultures at Halle.
- The experiment with crop rotation was sampled in March 2001 with intervals of 20 cm down to 60 cm depth, using a 2.5 cm diameter auger. Soil samples taken from three different locations of the unfertilised plots were mixed per depth interval.

Table 4.2: Soil sampling on the field trials at Halle and applied fractionation methods.

Trial (fertilisation)	Soil depth	Sampling	Date	Fractions analysed
Rye (unfertilised)	0-5, 30-35, 60-68 cm	Corer	Dec. 2000	Humin, Humic acids
Rye (unfertilised)	0-25 cm	Soil pit	Sept. 2000	Humin, Humic acids, Particle-size-, Density-, Aggregate fractions, Total-, Phospholipids
Rye (NPK)	0-25 cm	Corer	Sept. 2000	Lipid classes, Total-, Phospholipids
Rye (manure)	0-25 cm	Corer	Sept. 2000	Total-, Phospholipids
Maize (unfertilised)	0-10, 0-25 cm	Corer	Sept. 2000	Particle size-, Density-, Aggregate fractions, Total-, Phospholipids[1], Individual PLFAs[1]
Maize (NPK)	0-25 cm	Corer	March 2001	Lipid classes, Total-, Phospholipids, Individual PLFAs
Maize (manure)	0-25 cm	Corer	Sept. 2000	Total-, Phospholipids
Crop rotation (unfertilised)	0-20, 20-40, 40-60 cm	Corer	March 2001	Humin, Humic acids

[1] From 0-25 cm soil depth

4.1.2 Rotthalmünster

The long-term field trials at Rotthalmünster, which are run by a school for agriculture (*Höhere Landbauschule Rotthalmünster*), are located in a rural area in the south of Germany in the State Bavaria (N 48°21'47", E 13°11'46") with low pollution from anthropogenic sources. Rotthalmünster is located in the 'Tertiary Hilly Country'. which was formed in the Pleistocene. The study site is at 362 m above sea level. The mean annual temperature in this region is 8.2°C and the mean annual precipitation is 890 mm. The soil was classified as Stagnogleyic argillic brown earth (*Pseudogley-Parabraunerde*) derived from loess (pers. comm. Markus Kleber, University of Halle, 2003) which is according to FAO (1990) a Haplic Luvisol. The texture of the soil is a silty loam consisting of 11 % sand, 72 % silt, 17 % clay (pers. comm. Markus Kleber). Figure 4.3 shows a soil profile adjacent to the maize plot.

For the investigations three field trials were chosen: (i) continuously cropped maize since 1979, on former grassland (until 1970), followed by wheat cultivation, (ii) continuous wheat since 1969, established on former grassland, and (iii) grassland established in 1961 (table 4.3). The minerally fertilised plots (NPK) of the three trials were chosen for this study. The mean annual nitrogen application between 1971 and 2001 was about 180 kg nitrogen (N) per hectare (ha) on the maize culture

(Schnellhammer and Sirch, 2002), and about 171 kg N ha^{-1} on the wheat culture. The trial with grassland received about 160 kg N ha^{-1} (pers. comm. Obermeier, Höhere Landbauschule Rotthalmünster, 2002). On both cropped sites the straw was left on the field after harvest. The grassland was cut four times a year. The soil of the maize and wheat culture was ploughed to about 30 cm depth. While the trial with maize cropping was ploughed conventionally, the ploughing of the wheat monoculture has been changed to grubbing in 1998 (pers. comm. Obermeier, Höher Landbauschule Rotthalmünster, 2002). Depending on the pH of the soil, lime was applied every few years, the last time in 2001. The trials are located on a moderate slope (about 3°) with the grassland trial at the upper part, followed by the wheat culture, and the maize trial.

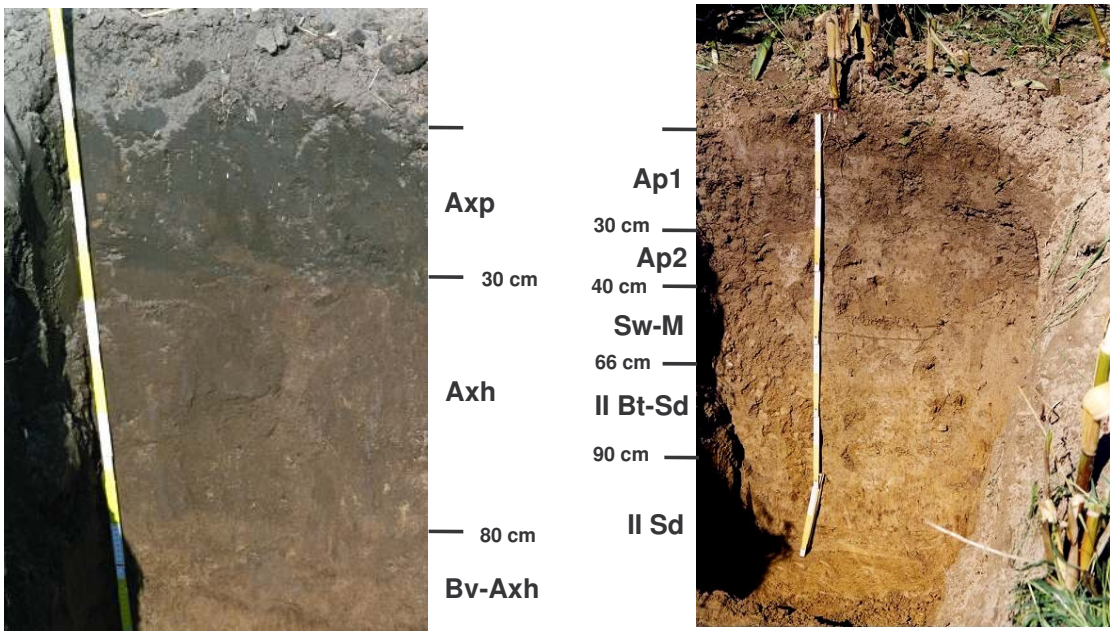

Figure 4.3: Soil profiles close to the rye trial at Halle (Haplic Phaeozem, left) and adjacent to the maize trial at Rotthalmünster (Haplic Luvisol, right). Nomenclature of soil horizons according to AG Boden (1995).

All soil samples were collected in September 2002 after harvest on the wheat trial but about 4 to 6 weeks before harvest on the maize culture both prior to tillage of the soil. Several kilograms of soil were taken from 8 to 10 different locations per plot and the soil was mixed to obtain a representative sample. The ploughed surface soil was sampled with a spade whereas subsoil samples were collected by a 10 cm diameter corer:

- Soil samples of the maize and wheat cultures were taken from three different depths (maize: 0-35, 35-45, 45-60 cm depth; wheat: 0-35, 35-45, 45-65 cm depth).

- The upper 30 cm depth of the grassland trial was sampled in 10 cm intervals and the subsoil in 30-45 cm and 45-65 cm soil depth.

Table 4.3: Soil sampling and characteristics of the field trials at Rotthalmünster.

Trial (fertilisation)	Soil depth	Sampling	Date	Fractions analysed
Maize (NPK)	0-35 cm, 35-45, 45-60 cm	Spade Corer	Sept. 2002	Humin, Humic acids, Density fractions[1], Aggregate fractions[1], Lipid classes[1], Total-, Phospholipids, Individual PLFAs[1,2]
Wheat (NPK)	0-35 cm, 35-45, 45-65 cm	Spade Corer	Sept. 2000	Humin, Humic acids, Density fractions[1], Aggregate fractions[1], Total-, Phospholipids, Individual PLFAs[1,2]
Grassland (NPK)	0-10, 10-20, 20-30, 30-45, 45-65 cm	Corer	March 2001	Humin, Humic acids, Aggregate fractions[3]

Soil samples taken in: [1] 0-35 cm depth; [2] 35-45 cm depth; [3] 0-10 cm depth

4.1.3 Rothamsted

The long-term experiment 'Broadbalk Continuous Wheat' at the Rothamsted Experimental Station, Hertfordshire, UK, was established in 1843. Rothamsted is located about 40 km to the south of London (N 51°49', W 0°21') and has a mean annual precipitation of 717 mm and a mean annual temperature of 9.1°C (Rothamsted, 1991). The continuous wheat trial is about 100 m above sea level. The soil has been described as Stagnogleyic paleoargillic brown earth derived from flinty, silty clay loam over clay-with-flints. According to FAO (1990), it has been classified as a Chromic Luvisol with about 21 % sand, 53 % silt and 26 % clay (Jenkinson et al., 1977,1992).

A topsoil sample, taken in 0-20 cm depth from a minerally fertilised plot (phosphate, potassium, manganese) with winter wheat, was provided by Ruth Ellerbrock (Müncheberg, Germany). This sample was taken with a spade in 1997. The wheat trial is cultivated by conventional ploughing and the harvest is removed from the field.

4.2 Separation of functionally-defined organic matter fractions

Different physical as well as chemical separation methods were used in collaboration with other working groups within the DFG Priority Program 1090 to obtain SOM fractions of different stability with the objective to identify protection mechanisms of organic carbon in soils as described in detail in chapter 5.5.

Prior to fractionation most soil samples, except fresh samples for lipid extraction, were air-dried and, as a common practice in soil science, sieved through 2 mm mesh size to homogenize the material, and then the fraction >2 mm was removed. The soil samples were then inspected under a microscope (Olympus SZX 9) with a magnification of 12x to 45x and not soil-derived particles as well as identifiable plant residues were removed. To study SOM heterogeneity (chapter 5.1) the bulk soil (<2 mm) was split into identifiable components such as plant residues and black particles which were selected under the microscope and treated separately. For the soil that was left after separating these components the term 'selected soil' is used. Only black particles of 200 µm to 2 mm grain-size could be separated from bulk soil samples by hand-picking. These particles as well as the plant residues were cleaned with distilled water using sonification and then air-dried.

4.2.1 Particle-size fractionation

Particle-size fractions from surface soil samples of the Halle site (maize, and rye plots) were obtained by wet sieving and centrifugation. The disaggregation was done by a two-step ultrasonication procedure according to Amelung et al. (1998) to avoid redistribution of coarse organic matter among fine-sized fractions. The fractionation was done by Bettina John (Institute of Soil Science and Forest Nutrition, University of Göttingen) as part of her Ph.D. thesis (John, 2003). 30 g air-dried soil were suspended in 150 ml distilled water and sonicated at 60 Joule ml^{-1}. The calibration of the sonitrode was done according to Schmidt et al. (1999b). Coarse (630-2000 µm) and medium grained sand (200-630 µm) were obtained by wet sieving. The remaining fraction was sonicated a second time at 440 Joule ml^{-1} and then wet sieved to obtain the fine sand (63-200 µm) and the coarse silt (20-63 µm) fractions. The clay fraction (<2 µm) as well as the middle and fine silt fraction (2-20 µm) were separated by centrifugation (John, 2003). The centrifugation time was calculated according to Stokes' Law, assuming no diffusion and a spherical shape of the particles. After centrifugation of the material, the

clay fraction was precipitated using 0.5 molar aluminium chloride and the supernatant was discarded. All size fractions were oven-dried at 50 °C.

4.2.2 Density fractionation

Surface soil samples were separated sequentially into four density fractions according to a procedure described by Golchin et al. (1994c). The fractionation was done by Bettina John (Institute of Soil Science and Forest Nutrition, University of Göttingen). The free particulate organic matter ($fPOM_{<1.6}$) was obtained by gently shaking air-dried soil (<2 mm) suspended in sodium polytungstate of 1.6 g/cm³ density, followed by centrifuging for 1 hour with 4700 rotations per minute (rpm), equivalent to 5085 g, and filtration of the supernatant. The $fPOM_{<1.6}$ fraction was recovered from the filter. The remaining pellet was re-suspended in sodium polytungstate (1.6 g/cm³) and disaggregated by shaking with 10 quartz balls of 5 mm diameter for 16 hours. After centrifuging (4700 rpm, 1 h) the soil suspension, the supernatant contained the occluded particulate organic matter ($oPOM_{<1.6}$) of less than 1.6 g/cm³ density. The pellet was shaken for 10 minutes at 100 rpm with sodium polytungstate of 2.0 g/cm³ density and after centrifugation (4700 rpm, 1 h) the oPOM fraction of 1.6-2.0 g/cm³ ($oPOM_{1.6-2.0}$) was recovered from the supernatant by filtration. To clean the remaining pellet from the density solution, it was suspended in distilled water, centrifuged (4700 rpm, 20 min), and the supernatant was discarded. This procedure was repeated three times. The remaining pellet of >2.0 g/cm³ was called the mineral fraction ($mineral_{>2.0}$). To account for soil heterogeneity four soil samples were fractionated and subsequently mixed to provide a representative sample.

4.2.3 Fractionation of water-stable aggregates

The fractionation of water-stable aggregates was done by Bettina John (Institute of Soil Science and Forest Nutrition, University of Göttingen) combining the fractionation procedures of Puget et al. (2000) and Six et al. (1998). Details can be found in John (2003). 100 g air-dried soil, which were not sieved, were placed on a 2000 µm sieve and immersed in distilled water for 10 minutes. Subsequently, the sieve was moved up and down (about 3 cm) about 50 times and the aggregates of >2000 µm size were collected. The sieving procedure was repeated as described previously using 1000 µm mesh size, followed by 250 µm and 53 µm sieve and the corresponding aggregate fractions were collected. Aluminium chloride (0.5 *M*) was added to the water used for fractionation to precipitate the fraction <53 µm. All fractions were air-dried at 25 °C.

4.2.4 Acid-Alkali-Acid extraction

Most soil samples, as well as SOM components (e.g. plant residues, black particles) were chemically fractionated by acid-alkali-acid extraction (AAA; figure 4.4), which is a standard method in ^{14}C dating to remove contaminating carbon from organic samples prior to radiocarbon measurement (Grootes et al., 2004). The procedure is similar to the classical humus fractionation procedure (Stevenson, 1994) and results in three fractions: the fulvic acid and humic acid fraction, and the humin.

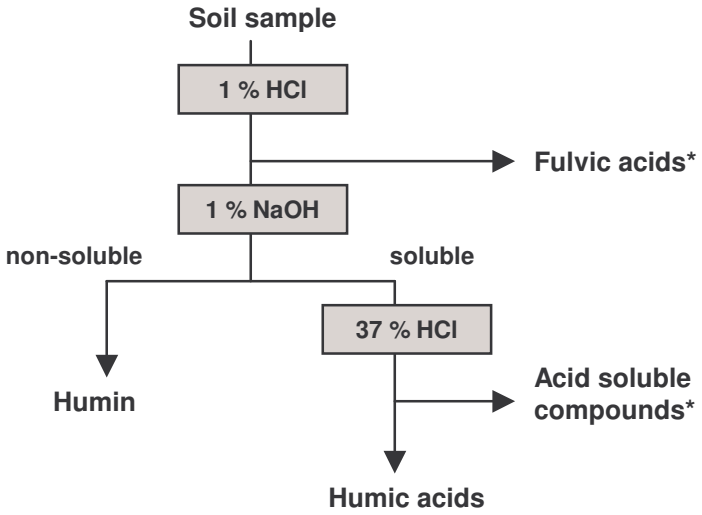

Figure 4.4: Acid-Alkali-Acid extraction of soil organic matter. The marked fractions (*) were not recovered.

First, dried soil or plant remains are treated with diluted (1 %) HCl for >4 hours (in this study about 10 h) to remove carbonates. The soil suspension is centrifuged for 5 to 10 minutes with 1500-4000 rpm and the supernatant containing acid soluble compounds is discarded. The hydrochloric acid is removed by washing the pellet repeatedly with ultra pure water and centrifuging until a pH of >4. Most samples, depending on sample size, are subsequently extracted with 1 % NaOH for approximately 4 hours at 60°C yielding an alkali-soluble fraction and a non-soluble residue (humin). Then humic acids are precipitated from the soluble fraction by acidification with 37 % HCl to pH <1. The acid soluble fulvic acids are not recovered. The precipitate is subsequently rinsed with ultra pure water to a pH of about 2. The solid, NaOH insoluble residue, the humin fraction, is also washed with ultra pure water until a pH of about 10 is reached. Following this, the humin is again treated with 1 % HCl for about 10 hours to remove any atmospheric CO_2 which might have been introduced during the alkali treatment, and rinsed with

water to pH >4 as described above. Both, the humic acid and humin fraction, are dried at 60 °C.

4.3 Isolation of biomarker substances

To obtain information on the origin and the transformation of organic carbon in soils, different biomarkers, which can be attributed to specific sources, were extracted and analysed by compound-specific radiocarbon analysis. The isolation methods for the lipid compound classes and for the individual phospholipid fatty acids as well as their preparation for AMS ^{14}C analysis have previously been tested as described in the following chapters 4.3.2 and 4.3.4 to identify possible contamination by background.

4.3.1 Extraction and separation of lipid compound classes

The isolation of different lipid compound classes was done by Guido Wiesenberg (Geological Institute, University of Cologne) as part of his Ph.D. thesis (Wiesenberg, 2004). An automated accelerated solvent extraction followed by a hetero-compound medium pressure liquid chromatography (MPLC; Willsch et al., 1997; Wiesenberg et al., 2004b), adopted from organic geochemical studies, was used, which is capable of separating complex mixtures such as SOM into pure compound classes. These substances can be analysed by gas chromatography-mass spectrometry (GC/MS) or prepared for AMS ^{14}C measurement (Wiesenberg et al., 2004b).

Total lipids were extracted from bulk soil samples by accelerated solvent extraction (ASE) (Dionex ASE 200): A dried soil sample of 30 g was divided in 6 g portions which were filled in five extraction vessel. The soil was extracted two times for 20 minutes at 50 bar and a temperature of 1.) 75°C, and subsequently 2.) 140°C with a mixture of dichloromethane (DCM) and methanol (MeOH; DCM:MeOH: 93:7 by vol.). The five extracts of both isolation steps were combined resulting in the total lipid extract (Ex) (Wiesenberg et al., 2004b). The total lipids were then separated by sequential hetero-compound medium pressure liquid chromatography (MPLC) into six fractions of different polarity (Willsch et al., 1997) as displayed in figure 4.5: a high polarity and/or high molecular weight fraction including long-chain wax esters (W), an acid fraction (H), a fraction of organic bases (Q), a high polar fraction of undefined content (V), an intermediate polarity fraction comprising straight chain and branched chain alcohols and sterols (F), and a low polarity fraction containing aliphatic and aromatic hydrocarbons as well as acyclic ketones (N).

The low polarity fraction (N) was further separated by a second MPLC as described by Radke et al. (1980), which produced three additional fractions: aliphatic hydrocarbons mainly consisting of *n*-alkanes (A), aromatic hydrocarbons (B), and low polar hetero-compounds mainly ketones (C). The extracts were evaporated to dryness by rotary evaporation. Further preparation of the lipid compound classes for AMS ^{14}C analysis is described in chapter 4.4.2.

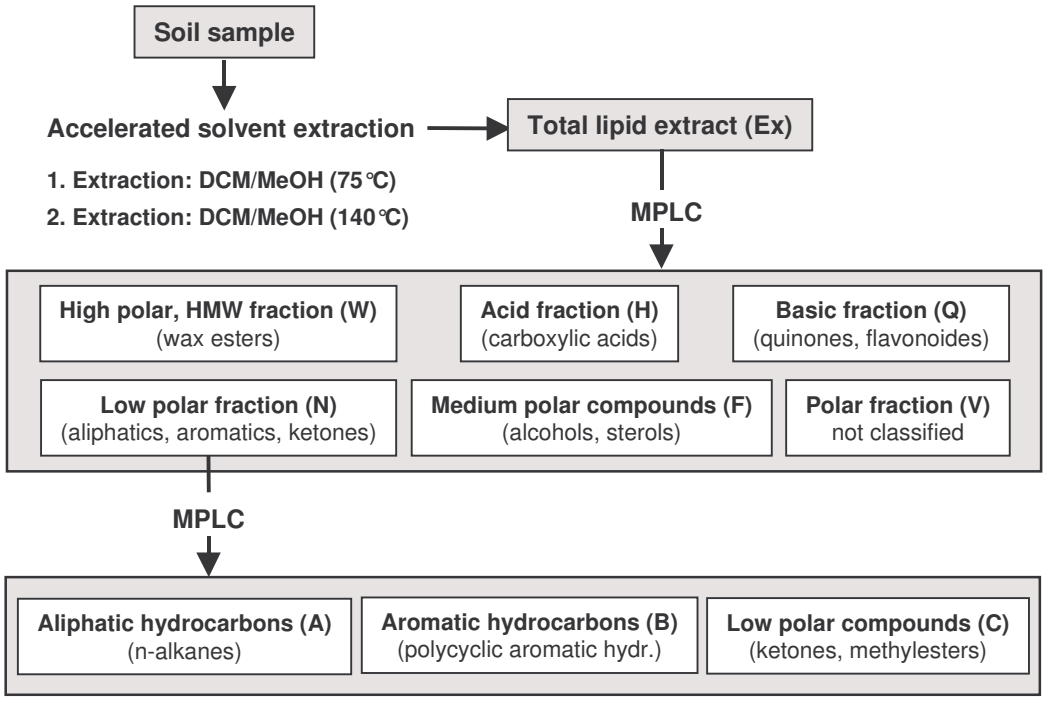

Figure 4.5: Isolation procedure of lipid compound classes by automated accelerated solvent extraction and medium pressure liquid chromatography (MPLC; figure modified from Wiesenberg, 2004). Main constituents of each compound class are given in brackets. The letters refer to descriptions in the text, HMW denotes high molecular weight.

4.3.2 Testing of the lipid isolation procedure

The isolation method was tested with different materials (shale, sediment of known age, samples) and found to be reproducible by (a) mass recoveries of individual fractions, (b) compound distribution patterns were reproducible within individual fractions, and (c) fractions were clean compound classes, free of interfering substances detectable by gas chromatography-mass spectrometry analysis. Further details can be found in Wiesenberg et al. (2004b). A small portion of <15 % of the total extract (Ex) remained insoluble in DCM or could not be recovered from the MPLC columns.

A possible contamination of the lipid compounds by modern or old carbon was assessed by isolation and ^{14}C analysis of (i) a lake sediment of known age (Steisslinger Lake, Germany) as well as of (ii) a ^{14}C-free lacustrine shale (Enspel, Germany) from the Oligocene about 24.7 million years ago (Storch et al., 1996; table 4.4).

Table 4.4: Testing of the separation procedure for lipid compound classes: ^{14}C values of compound classes from a ~5000 year old lake sediment and a oligocenian, ^{14}C-free shale both from Germany.

Fraction		^{14}C (pMC)	±	^{14}C age (years BP)	±
Steisslinger Lake sediment (KIA 20179)					
Bulk material		48.97	0.26	5740	40
Total extract	Ex	48.08	0.17	5880	30
Extraction residue	Re	48.35	0.16	5840	30
High polar, HMW lipids	W	47.59	0.17	5965	30
Acid fraction	H	44.07	0.19	6585	35
Basic fraction	Q	47.13	0.16	6045	30
Polar fraction	V	46.74	0.16	6110	30
Medium polar fraction	F	47.59	0.16	5965	30
Low polar fraction	N	47.10	0.16	6050	30
Aliphatic hydrocarbons	A	47.69	0.25	5950	45
Aromatic hydrocarbons	B	50.13	1.26	5550	205
Low polar compounds	C	48.14	0.16	5870	30
Lacustrine shale from Enspel (KIA 17927)					
Bulk material*		0.07	0.02	56010	2405
Total extract*	Ex	0.40	0.04	44170	970
Extraction residue	Re	0.29	0.07		
High polar, HMW lipids	W	0.41	0.07	44170	1480
Acid fraction	H	0.73	0.07	39540	870
Basic fraction	Q	1.44	0.24	34060	1490
Polar fraction	V	0.96	0.13	37300	1200
Medium polar fraction	F	0.62	0.06	40800	880
Low polar fraction	N	1.22	0.48	35420	3990
Aliphatic hydrocarbon	A	0.95	0.61	>30780	
Aromatic hydrocarbon	B	1.99	1.45	>24230	
Low polar compounds	C	1.28	0.07	35010	450

Fractions are designated as in figure 4.5.
* Average of two separate targets from one extract.

^{14}C results for the Steisslinger Lake sediment (bulk material: 49.0 ± 0.3 pMC, 5736 years BP) indicate that the total lipid extract (Ex) contains less young carbon (48.1 ± 0.2 pMC). A similar result was obtained for Ex extracted from soil samples of the study sites at Halle and Rotthalmünster (figure 5.9, chapter 5.3.1). All isolated lipid compound classes of the lake sediment were slightly depleted in ^{14}C. These values indicate a lower contribution of young carbon to these extracted fractions and no significant

contamination by modern or old background. In contrast, the higher ^{14}C values of the total lipid extract (Ex: 0.4 ± 0.04 pMC) as well as of the isolated compound classes from the Enspel shale (Germany) most probably show a contamination of this ^{14}C-free material (bulk shale: 0.07 ± 0.02 pMC, 56,010 years BP) by modern carbon. This may be introduced during lipid extraction as indicated by differing ^{14}C value of a second total lipid extract separated from the same composite soil sample (KIA 17926-Ex: 0.87 ± 0.07 pMC). To assess the variability of ^{14}C results due to the performance of the AMS measurement for several compounds the large quantity of recovered material was split into two separate graphite targets.

4.3.3 Isolation of phospholipid fatty acids

The extraction of total- and phospholipids as well as the isolation of individual PLFAs was done by Christiane Kramer as part of her Ph.D. thesis (Max Planck Institute for Biogeochemistry, Jena, Germany; Kramer, 2004). The method described by White et al. (1979a) was used to extract a phospholipid fraction (figure 4.6).

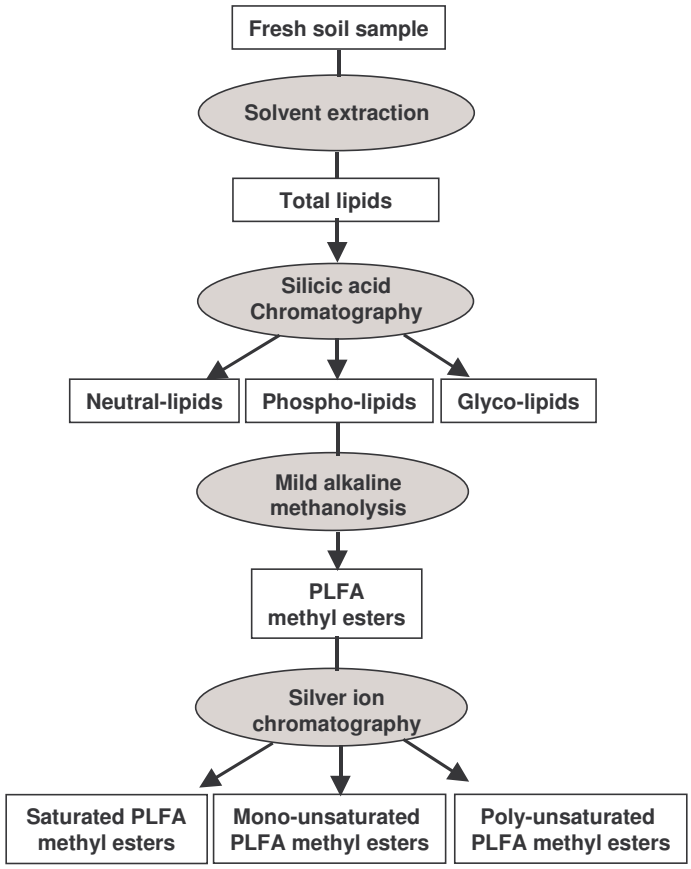

Figure 4.6: Extraction and separation of phospholipid fatty acid methyl esters (PLFAs) from fresh soil samples.

Fresh soil samples, transported in ice-cooled boxes and stored several weeks at -20°C, were extracted by shaking with chloroform, methanol, and a buffer (potassium phosphate, pH 7.4; 2.5:5:2.5 by vol.) for 3 hours. The resulting total lipid extract was then separated into three fractions by solid phase chromatography using silica gel columns conditioned with chloroform. According to White et al. (1979a) and Zelles and Bai (1993) neutral-, glyco- and phospholipids were eluted with chloroform, acetone, and methanol. The phospholipid fraction was derivatised to hydrolysis-resistant fatty acid methyl esters using a mild, alkaline methanolysis as described by White et al. (1979a) and then split into saturated, mono-unsaturated and poly-unsaturated PLFA methyl esters via solid phase silver ion chromatography according to Zelles and Bai (1993). PLFA methyl esters were identified mass spectrometrically.

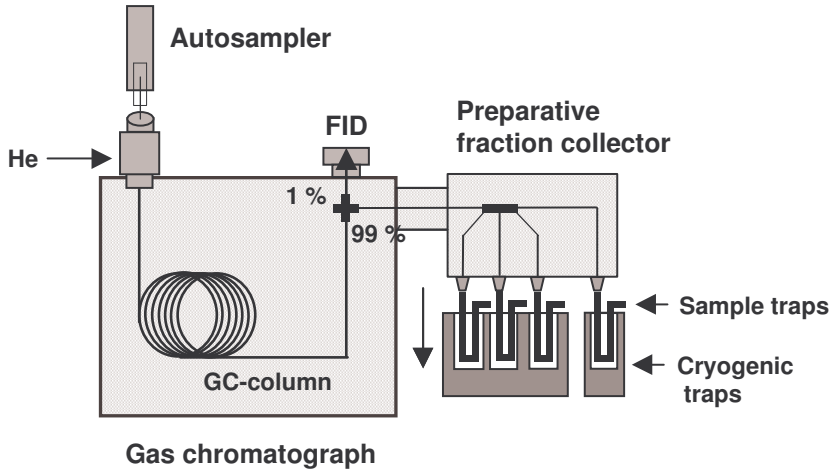

Figure 4.7: Preparative capillary gas-chromatography system for the isolation of individual PLFAs (modified from Kramer, 2004). 1 % of the effluent pass to a flame ionisation detector (FID) and 99 % are collected in seven U-tubes, where one of them receives the residue of the mixture.

The saturated-, and monounsaturated PLFA methyl esters were repeatedly injected into a preparative-capillary gas-chromatograph (figure 4.7; HP 6890 GC, Gerstel preparative trapping device) and the most abundant peaks, yielding >70 µg carbon after about 50 injections, were collected in cryogenically cooled traps for micro-scale AMS ^{14}C measurements. These were 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. The designation of PLFAs is according to 'A:B', with 'A' indicating the number of carbon atoms, and 'B' the number of double bounds. The prefixes indicate: *n* - unbranched chain, *i* - iso- and *a* - anteiso-branching, and *cy* – cyclopropyl. Identification and isolation of the PLFA methyl esters was done

according to Eglinton et al. (1996). The isolated individual PLFA methyl esters, subsequently abbreviated as PLFAs, were transferred into combustion tubes for AMS measurement as described in chapter 4.4.2.

The mild alkaline methanolysis introduced a methyl group into each PLFA. Thus, ^{14}C concentrations of individual PLFAs were corrected by subtracting the contribution of methyl carbon from methanol by isotopic mass balance calculation:

$$^{14}C_{free} = (C_n + 1)/ C_n \cdot {}^{14}C_{ester} - 1/C_n \cdot {}^{14}C_{Methanol} \quad (11)$$

where $^{14}C_{free}$ is the ^{14}C concentration of the PLFA without the contribution of the carbon atom from the methyl group and $^{14}C_{ester}$ the measured ^{14}C concentration for its methyl ester. C_n represents the number of carbon atoms of the underivatised PLFAs. The ^{14}C value for methanol ($^{14}C_{Methanol}$) was 0.1 pMC.

4.3.4 Assessment of possible contaminations during PLFA isolation and preparation for AMS

The identities and purities of the individual compounds were checked by GC/MS analysis (Kramer, 2004). The stable carbon isotopic composition of (i) single compound in the saturated and mono-unsaturated fractions as well as of (ii) individual compounds isolated by preparative capillary gas-chromatography (PCGC) was determined by IR-GC/MS to assess isotopic fractionation effects during PCGC separation (Kramer, 2004).

The preparative isolation of the PLFAs as well as their conversion into AMS graphite-cathodes has previously been checked with the help of modern and old fatty acid methyl-ester (FAME) standards. Since the standards have ^{14}C contents of about 70 pMC (n-C28:0) and about 110 pMC (*n*-C12:0, *n*-C18:0) a contamination of both modern and fossil origin can be detected. The standards used were in the sample-size range of the PLFAs as shown in table 4.5. Potential sources of contamination are 'column bleed', thermal degradation of the chromatographic stationary phase, during the isolation by PCGC and incomplete removal of the solvent previous to combusting of the compounds (Eglinton et al., 1996). Since the solvent as well as the GC columns most probably derive from petroleum products, the PLFAs may have been contaminated by old, ^{14}C-free carbon. The solvent (dichloromethane) was evaporated over night. However, in case of larger sample volumes or difficulties of complete removal a gentle

stream of nitrogen was applied while the sample was heated at about 40°C to overcome cold due to evaporation. This may have resulted in the sample loss and/or isotopic fractionation.

The results of these tests summarised in table 4.5 showed:

- Carbon yields after combustion of C-18 and C-28 FAMEs to CO_2 in most cases indicated only minor losses for all standards analysed, which may also be due to the less precise weighing (five significant digits) of sample sizes smaller than 100 µg.
- In most cases the ^{14}C results for the FAME standards yield no statistically significant (2-σ measurement uncertainty) contribution of old carbon due to incomplete solvent removal, except for KIA 18432c.
- ^{14}C concentrations for PCGC isolated *n*-C12:0 FAME standard showed no statistically significant (2-σ criterion) addition or loss of carbon.
- The isolated *n*-C18:0 FAMEs however, yielded statistically not significant but slightly depleted ^{14}C values of 106.7 to 108.7 ± ≤1.7 pMC compared to those of the untreated standard (111.05 ± 0.45 pMC).
- ^{14}C results for the isolated *n*-C28:0 FAME standard of 52.45 ± 1.0 pMC and 67.27 ± 1.3 pMC were significantly lower than the directly measured standard which yielded 73.2 ± 0.45 pMC. Since the tests of solvent removal do not indicate an addition of fossil carbon, possible explanations are the loss of modern carbon or the addition of old carbon during the preparative isolation of this standard. This problem is considered to be insignificant, since PLFAs investigated in this study have chain-lengths of 15 to 18 carbon atoms, which are assumed to give reliable data as shown by the results of the C-18 FAME.
- Moreover, all FAME data suggest a depletion in ^{14}C concentration, which increases with increasing number of carbon atoms.

During the PCGC separation of the individual PLFAs the procedure and their further processing for ^{14}C AMS analysis was repeatedly checked by the *n*-C12:0, *n*-C18:0, and *n*-C28:0 FAME standards. Moreover, the variability of the AMS ^{14}C results was determined by splitting several samples into two graphite-cathodes which were measured separately as shown in the tables 4.5.

Table 4.5: Testing of solvent removal and PCGC isolation with FAME standards of different chain-length.

KIA-Nr.		FAME	Treatment	Carbon weight (mg)	Carbon yield (mg)	^{14}C (pMC)	±
18432	G	C-12	untreated standard	0.34	0.42	110.11	0.49
18432	D	C-12	dissolved in DCM	0.09	0.15	107.30	1.14
18798*	A	C-12	dissolved in DCM	0.06	0.07	113.27	2.27
18798*	Aa	C-12	dissolved in DCM	0.09	0.09	112.91	1.74
20819	B1	C-12	isolated with PCGC	0.17	0.20	108.75	0.94
20819	B2	C-12	isolated with PCGC	0.22	0.22	109.29	0.75
21585		C-12	isolated with PCGC	0.22	0.20	106.34	0.83
21089		C-12	isolated with PCGC	0.10	0.21	109.26	0.80
18432	H	C-18	untreated standard	0.30	0.48	111.05	0.45
18432	B	C-18	dissolved in DCM	0.17	0.13	111.57	1.32
18432	E	C-18	dissolved in DCM	0.24	0.24	106.71	0.88
18799	A	C-18	dissolved in DCM	0.26	0.24	111.37	0.76
19877		C-18	isolated with PCGC	0.11	0.11	106.66	1.74
20819	C1	C-18	isolated with PCGC	0.13	0.15	107.00	1.09
21586	e	C-18	isolated with PCGC	0.16	0.18	108.71	0.96
21090	f	C-18	isolated with PCGC	0.14	0.17	108.07	1.00
18432	I	C-28	untreated standard	0.31	0.38	73.20	0.45
18432	F	C-28	dissolved in DCM	0.25	0.23	73.92	0.71
18800*	A	C-28	dissolved in DCM	0.10	0.14	73.80	1.13
18800*	Aa	C-28	dissolved in DCM	0.25	0.27	73.95	0.70
20819	D1	C-28	isolated with PCGC	0.17	0.12	67.27	1.27
20819	D2	C-28	isolated with PCGC	0.14	0.15	52.45	1.01

Carbon weights of FAMEs after solvent removal were calculated with C-12: 72.89 % C, C-18: 75.52 % C, C-28: 78.87 % C, and carbon yields were determined as CO_2 pressure after combustion.

* Samples split in two separate graphite targets.

4.4 Preparation of graphite targets for AMS ^{14}C measurements

As a standard method of the Leibniz-Laboratory, all samples analysed in this study as well as the necessary glass equipment were checked under a light microscope for any kind of dirt, dust, and other contaminants prior to any chemical treatment. If necessary these were removed by hand-picking.

4.4.1 Preparation of normal sized soil samples and SOM fractions

The dried soil samples or SOM fractions were transferred into small quartz combustion tubes of 0.4 cm inner diameter and ~7.0 cm length. For combustion about 450 mg of wire-form copper oxide, supplying oxygen, and, depending on sample size, 150 mg to 450 mg of silver wool, for removal of sulfur and halogens, were added. The combustion tubes were transferred into larger outside quartz tubes of 0.7 cm inner diameter, and

~26 cm and ~35 cm length respectively. The small inner as well as the outside tubes were cleaned prior to use with diluted 1 % phosphoric acid (>10 hours), to remove any contaminating carbonate particles, and subsequently rinsed several times with ultra-pure water. After drying, the tubes as well as the copper oxide and the silver wool were pre-combusted at 900 °C for 4 hours to remove any contaminating carbon.

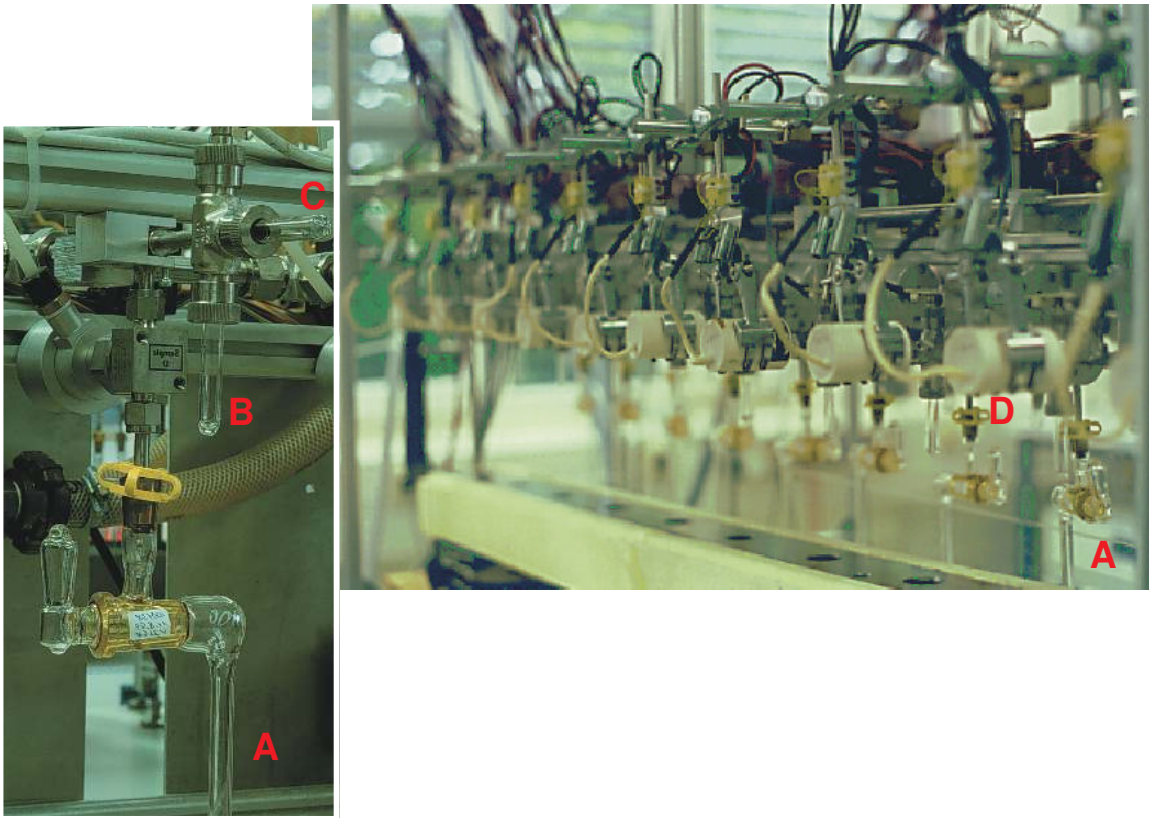

Figure 4.8: Multi port reduction system for graphitisation of sample CO_2 at the Leibniz-Laboratory, Kiel (right side). Detail photography (left side) of: bottle with sample CO_2 (A), cryogenically cooled water trap (B), reaction tube containing iron powder (C), and 600 °C oven (D), see text for further descriptions.

The tubes were evacuated on a vacuum manifold for about 10 hours to a pressure of about 10^{-4} mbar and subsequently flame sealed. All samples were combusted in a muffle furnace at 900 °C for 4 hours. The resulting CO_2 was purified cryogenically in a vacuum manifold and collected in glass bottles which were placed in a cold trap with liquid nitrogen (-196 °C). The bottles (figure 4.8: A) were then transferred to a separate vacuum reaction manifold, described in detail by Nadeau et al. (1998): An about 10 % excess of hydrogen was added to the sample CO_2. The reaction tubes, which contained 2 mg cleaned (0.7 bar O_2 at 400 °C for 15 min., 0.7 bar H_2 at 400 °C for 30

min.) iron powder as catalyst (C), were then heated at 600°C (D) for about 2 to 3 hours until the reaction was completed. (Vogel, et al., 1984; 1987, Nadeau et al., 1998). The water produced during the reaction ($CO_2 + 2H_2 \rightarrow C + 2H_2O$) was removed cryogenically (B).

The resulting graphite-iron powder was pressed into 1.5 mm diameter cavities in aluminium target holders using a pneumatic press (Nadeau et al., 1998). The graphite targets were stored in small glass bottles filled with argon before they were placed in a target wheel for AMS measurement.

The contribution of contaminating carbon during sample preparation was determined by preparing and measuring ^{14}C-free anthracite of comparable sample-size parallel to normal samples. This anthracite has previously been cleaned by standard AAA-extraction.

4.4.2 Preparation of AMS targets from specific compounds of sub-milligram samples

The dried lipid extracts were solved again in dichloromethane, and additionally methanol, depending on the polarity of the fraction. Lipid fractions as well as individual PLFAs, which were dissolved in dichloromethane, were pipetted in solution into quartz tubes, which were cleaned as described in the previous chapter. The solvent was removed by evaporation over night, and, in case of incompletely removal, a gentle N_2 stream was applied. In this case, the combustion tubes were placed in an aluminium block that was heated at about 40°C to overcome cold due to evaporation. For combustion of small samples containing <500 µg of carbon, reduced portions of about 75 mg copper oxide and about 30 mg silver wool were added. The tubes as well as copper oxide and silver wool were pre-combusted at 900°C for 4 hours.

To avoid possible losses of highly volatile compounds, the tubes containing the isolated lipids and PLFAs were evacuated for about 30 minutes while immersed in dry-ice/ethanol (about -80°C). The dry-ice/ethanol bath was then replaced by liquid nitrogen and the tubes were evacuated for additional 40-60 minutes to about 10^{-4} mbar and followed by flame sealing. The combustion and graphitisation of the sample-CO_2 was done as described before, but only 1 mg of iron was used as catalyst during graphitisation. The graphite powder was placed on an iron-bed before being pressed into 1.5 diameter target holders. The processing of sub-milligram samples, which are

more easily contaminated by background, was checked via preparation and measurement of oxalic acid standards as well as of anthracite both in the size range of the samples.

4.4.3 AMS ^{14}C measurement and reporting of ^{14}C results

The performance of the HVE 3 MV tandetron based AMS system at the Leibniz-Laboratory is described in chapter 3.3.1. Normal-sized samples were measured in a sample carousel for 59 targets containing usually 42 'unknown' sample targets, 8 oxalic acid standard (IAEA Ox II) targets, 2 IAEA standards of known ^{14}C content, 4 background targets, 1 graphite blank, and 1 target used as 'caesium beam dump' (Nadeau et al., 1998). Sub-milligram samples in most cases were measured in a separate carousel together with small oxalic acid standards in order to decrease isotopic fractionation effects in the AMS system due to lower ion currents (Pearson et al., 1998; von Reden et al., 1998). Each sample was measured 8 or 9 times during a 3-days measurement (Nadeau et al., 1998).

The ^{14}C results were checked for natural isotopic fractionation via the $^{13}C/^{12}C$ ratio of the sample compared to a standard (Nadeau et al., 1998). This ratio, however, cannot be compared with results obtained by IR-MS, since the ^{13}C beam is not analysed through the same path and is not subject to the last ion optical elements of the spectrometer. The long-term stability of the AMS measurements was evaluated via the IAEA standards with known ^{14}C contents which are included in each sample carousel.

^{14}C results are expressed according to Stuiver and Polach (1977) in percent modern carbon (pMC) with $\pm$ 1-σ measurement uncertainty as described in chapter 3.3.2. Additionally, $\Delta^{14}C$ values are given in the tables in the appendix. If two graphite targets were prepared of one composite soil sample, the weighted average is given in the text and in the figure, while the single data are given in the appendix.

The precision of the ^{14}C results is limited by the process blank, the contamination of the samples with modern CO_2 during chemical preparation and graphitisation, as well as by the stability of the AMS system. Details of the laboratory blank and the machine background at the Leibniz-Laboratory have been described in detail by Schleicher et al. (1998) and Nadeau et al. (1998). Briefly, the machine background, which was assessed by measuring pure graphite, is 0.01 to 0.03 pMC. The contamination introduced during sample combustion is about 0.3 pMC for organic samples and by

reduction and graphitisation equally about 0.05 to 0.06 pMC. The overall background currently is about 4 µg modern carbon which is equivalent to about 0.3 pMC for normal-sized samples containing 1.5-2.0 mg of carbon.

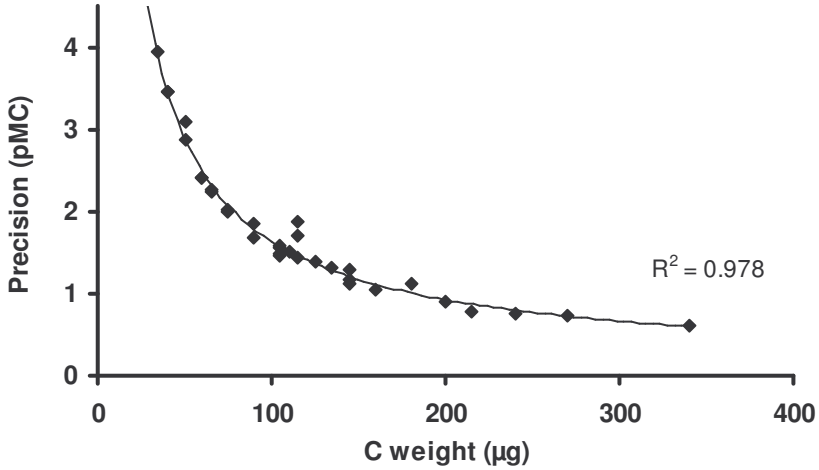

Figure 4.9: Measurement uncertainty (1-σ) for PLFAs (n = 36) as a function of sample carbon weight. All PFLA single measurements of topsoil samples from Halle and Rotthalmünster are displayed. A potential fit was used.

The precision achievable for small samples decreases, as the relative importance of the blank correction increases and its uncertainty (determined empirically: about 1/3 of the blank value) makes a larger contribution to the overall measurement uncertainty of small samples. Figure 4.9 shows the 1-σ measurement uncertainties for the PLFA samples with carbon weights of 40 µg to 340 µg C (between 3.9 to 0.6 pMC).

4.5 ^{13}C measurements and calculation of maize-derived carbon

The ^{13}C measurements were done at the Institute for Soil Science and Forest Nutrition, Universität Göttingen with a continuous flow IR-MS (DELTA^{plus}, Finnigan Mat, Bremen, Germany) coupled to an elemental analyser (EA 1108 and NA 2500, from Fisons, Milan, Italy). Sample preparation and measurements are described in John (2003). The accuracy of the measurements was about 0.1 ‰ PDB. The δ^{13}C results are given relative to standard PDB carbonate as described in chapter 3.3.2. In this thesis the term PDB is used instead of Vienna-PDB (V-PDB). V-PDB is the new standard, which has replaced the original PDB standard, since this is no longer available.

The proportion of maize-derived carbon (f) in a soil sample was calculated from the difference in $\delta^{13}C$ between the soil collected on the maize trial ($\delta_{C4\text{-}soil}$) and the reference soil kept under the initial C_3 vegetation ($\delta_{C3\text{-}soil}$) in relation to the $\delta^{13}C$ values of the C_4 (maize) and a C_3 plants (rye or wheat). According to Balesdent and Mariotti (1996) the proportion of maize-derived carbon is:

$$f = (\delta_{C4\text{-}soil} - \delta_{C3\text{-}soil}) / (\delta_{C4\text{-}plant} - \delta_{C3\text{-}plant}) \quad (12)$$

The $\delta^{13}C$ values of plant residues (stubble and roots) collected on the different cultures at Halle and Rotthalmünster are given in table 4.6. The standard deviation (s_f) of maize-derived carbon was calculated from the single values determined for $\delta_{C4\text{-}soil}$ and $\delta_{C3\text{-}soil}$ as in equation 13.

$$s_f = [(\delta_{C3\text{-}soil} / (\delta_{C4\text{-}plant} - \delta_{C3\text{-}plant}))^2 + (\delta_{C4\text{-}soil} / (\delta_{C4\text{-}plant} - \delta_{C3\text{-}plant}))^2]^{0.5} \quad (13)$$

Table 4.6: $\delta^{13}C$ values of plant residues from Halle and Rotthalmünster.

Study site	C_4–plant $\delta^{13}C$ (‰ PDB)	C_3-plant $\delta^{13}C$ (‰ PDB)
Halle	-11.6 ± 0.1 (maize)	-28.4 ± 0.1 (rye)
Rotthalmünster	-12.7 ± 0.2 (maize)	-26.8 ± 0.1 (wheat)

Data from John (2003)

Assuming steady state conditions the turnover rate (T) of soil organic carbon in physical soil fractions from the maize trial was calculated as (Balsdent et al., 1990):

$$T = (t - t_0) / \ln (C_t/C_{t0}) \quad (14)$$

with:

t = time of soil sampling (year)

t_0 = time of vegetation change (year)

C_t = amount of remaining carbon labelled from C_3 crops in the soil at the time of sampling (100- f %)

C_{t0} = initial amount of carbon labelled from C_3 crops at the time t_0 (= 100 %)

4.6 SEM/EDX measurements

The morphology of different charred, black particles selected from the soil of the field trials at Halle was examined by scanning electron microscopy (SEM; LEO 1530, Oxford Instruments GmbH, Germany) at the Institute of Soil Science and Soil Geography, University of Bayreuth, Germany by Sonja Brodowski. The microscope is equipped with a field emission cathode and coupled to an energy-dispersive X-ray spectroscope (EDX; INCA 400, Oxford Instruments GmbH, Germany). Particles were coated with a 1.5 nm layer of platinum to avoid charging of the organic material during SEM analysis. Details of the analyses can be found in Brodowski (2004) and Brodowski et al. (2004).

To characterise the organic material the elemental composition of several selected black fragments was determined by EDX analysis. The percentages of elements found in different sections on each particle were used to calculate atomic O/C ratios. Because this ratio changes during structural modifications from fresh organic matter to carbonised material and during diagenesis respectively, this method makes it possible to determine the degree of carbonisation of the black, organic material. The oxygen concentration was corrected (O_C), considering other elements besides carbon which can bind oxygen, by multiplying the ion concentration of each element with a factor calculated from their common valency within the main oxides of the earth crust according to equation 15 (Brodowski et al., 2004).

$$O_C\ (\%) = 100\ \% - \sum [\text{all elements found by EDX } (\%)] - [\text{Na } (\%) \times 0.5 + \text{Mg } (\%) \times 1 + \text{Al } (\%) \times 1.5 + \text{Si } (\%) \times 2 + \text{K } (\%) \times 0.5 + \text{Ca } (\%) \times 1 + \text{Fe } (\%) \times 1.176 + \text{P } (\%) \times 2.5] \quad (15)$$

This correction was only applied to EDX spectrograms with carbon contents of >40 % to avoid an incorrect calculation of low O_C/C ratios.

5 Results and Discussion

5.1 Radiocarbon information on soil organic matter dynamics

The experimental site at Halle (Germany) has a soil archive with samples collected since 1949. Such an archive makes it possible to determine the incorporation of bomb-^{14}C, produced by the atmospheric testing of nuclear weapons, in SOM at different times before and after the atmospheric bomb-^{14}C spike (figure 3.1) and thereby to calculate turnover times of soil organic carbon (chapter 3.1.2). The long-term trial with continuous rye cultivation at this site was already established in 1878 and thus provides the advantage of almost constant and well documented conditions over many decades. However, Halle is located in a highly industrialised area of Germany (chapter 4.1.1) and thus SOM is possibly influenced by high amounts of fossil fuel-derived carbon as reported by Schmidt et al. (1996). Therefore, it is necessary to determine a possible fossil contamination of recent as well as archived soil samples which may hinder the investigation of 'natural', plant-derived SOM cycling. This can be achieved by radiocarbon analysis of SOM (Rumpel et al., 2003).

The main objectives of the analyses described in this chapter are:

- To assess the quality of the archived soil samples available at the experimental site at Halle, which should be used for the quantification of soil organic carbon dynamics by tracing the contribution of bomb-^{14}C to SOM in the time interval from 1949 until present. Therefore ^{14}C contents of a pre-bomb samples, containing no bomb-^{14}C were analysed.

- To examine the heterogeneous composition of SOM by ^{14}C analysis of different organic matter components selected mechanically from bulk soil. Furthermore the standard acid-alkali-acid (AAA) separation, used by most radiocarbon laboratories for analysis of soils and sediments, was applied to bulk soil and to the separated organic components to test its ability in separating organic carbon pools of different stability, and to identify carbon turnover and transport processes. Samples collected from the ploughed topsoil as well as subsoil samples down to about 65 cm depth are investigated.

- To compare ^{14}C concentrations of soil samples from the Halle site, located in the highly industrialised Leipzig-Halle region, with those of two other long-term experimental sites in South Germany and in the United Kingdom, located in greater distance to industries. The aim is to evaluate a possible contribution of fossil fuel-derived carbon to SOM depending on the site location.

- To test the ability of the different fractionation methods in separating labile and stable carbon pools by radiocarbon analysis of various physically and chemically defined organic matter fractions from the Halle soil. Moreover, the susceptibility of these fractions to a contamination by anthropogenically-derived, fossil carbon is examined.

5.1.1 Quality of the archived soil samples from the long-term site at Halle

The oldest archived surface soil samples (0-20 cm depth) available at the Halle site, which were taken on the crop rotation trial in 1949 prior to the atmospheric bomb-testing period, and on the rye monoculture in 1961, were analysed. The archived soil samples were air-dried and subsequently crushed. The samples were stored in glass bottles. An inspection of these samples by light microscopy showed the presence of small paper fragments indicating that the archived samples were dried on paper. These fragments were removed by hand-picking. Furthermore, black particles were identified (figure 5.6), which could not be removed completely by mechanical selection nor by density separation due to their small particle-size and their intimate association with organic and mineral soil particles (Kuhlbusch, 1998).

^{14}C data for the pre-bomb samples (table 5.1), collected in 1949 on the unfertilised plots of the crop rotation experiment (0-20 cm depth), showed low values of 51.1 ± 0.2 pMC for the humin (~5,395 years BP) and 66.7 ± 0.3 pMC for the humic acid fraction (~3,250 years BP). However, these concentrations were unexpectedly higher than ^{14}C levels of a sample taken on the same plot in 2000, which were assumed to contain bomb-^{14}C (table 8.1) containing bomb-derived ^{14}C (soil humin: 45.5 ± 0.2 pMC, and soil humic acids: 55.3 ± 0.3 pMC). Radiocarbon concentrations of a surface soil sample (0-20 cm depth) collected in 1961 on the rye monoculture were even lower with 26.9 ± 0.1 pMC (~10,540 years BP) in the humin fraction. The humic acid fraction, which is assumed to turn over more rapidly (Martel and Paul, 1974; Trumbore et al., 1989, 1990) and thus is expected to contain higher levels of bomb-^{14}C, yielded only 47.4 ±

0.2 pMC (~5,985 years BP), even though the atmospheric $^{14}CO_2$ level in 1961 already increased to 122.3 pMC ($\Delta^{14}C$ ~221.3 ‰; Levin and Kromer, 1997). These ^{14}C values are extremely low compared to common surface soil concentrations (Scharpenseel et al., 1996) and indicate a variable contamination of both, archived as well as recent samples by fossil carbon. As shown by Schmidt et al. (1996) ^{14}C concentrations in the A horizon of soils heavily contaminated with fossil fuel-derived carbon can be as low as 14.1 pMC (~15,750 years BP).

A small portion of the black particles, identifiable by light microscopy, was separated from the topsoil samples collected in 1949 and yielded a ^{14}C content of 7.0 ± 0.1 pMC (humin) equivalent to a ^{14}C age of 21,360 years BP. The humic acid fraction of these fragments had a similar low value of 7.7 ± 0.5 pMC indicating that this fraction is almost exclusively derived from the old material and has not absorbed young, soluble carbon. Humic acids originating from the old, black particles moreover, seem to be responsible for the low ^{14}C concentration of the soil humic acid fraction. As shown by Rumpel and Kögel-Knabner (2002) high proportions of lignite-derived carbon contribute to humic acids extracted from mine soils. Since pure lignite contains no ^{14}C (most probably Tertiary origin), the ^{14}C results indicate that the black particles are not only of fossil origin but possibly also derive from burned vegetation residues or other products of incomplete combustion (Goldberg, 1985; Schmidt et al., 2001).

Table 5.1: Radiocarbon concentrations of archived topsoil samples (0-20 cm depth) from unfertilised plots of field trials with crop rotation and continuous rye at Halle.

Sample	Fraction	^{14}C (pMC)	±	Conventional ^{14}C age (years BP)	±
Crop rotation: Sampled in 1949 (KIA 14368)					
Soil	Humin	51.08	0.21	5395	35
Soil	Humic acids	66.73	0.25	3250	30
Black particles	Humin	7.00	0.11	21360	125
Black particles	Humic acids	7.70	0.47	20590	500
Rye: Sampled in 1961 (KIA 18420)					
Soil	Humin	26.94	0.14	10540	45
Soil	Humic acids	47.41	0.18	5985	30
Plant residues	Humin	117.68	0.62	modern	
Black particles	Humin	41.68	0.24	7030	50

The presence of variable quantities of old carbon in the soil at the Halle site complicates the investigation of organic carbon dynamics, as ^{14}C-based apparent mean residence times drastically underestimate the turnover of natural organic carbon

in SOM. It even hinders the intended use of bomb-^{14}C as quantification tool for soil organic carbon turnover. Turnover times based on natural ^{13}C labelling (chapter 3.1.1 and 4.5) may also be misleading, since the C_3-like isotopic label of the old, black fragments (table 5.3) will lower the increase in $\delta^{13}C$ due to maize cultivation (with a C_4 isotopic signature, table 4.6) on the former rye culture at Halle (see further discussion in chapter 5.5). Results of John (2003), who found a relatively small increase in $\delta^{13}C$ of 1.5 ‰ PDB about 40 years after a vegetation change from C_3 to C_4 plants, support this hypothesis.

The higher ^{14}C concentration (lower ^{14}C age) of the pre-bomb sample from 1949 compared to that from 2001 may reflect a lower industrial activity and in consequence a lower input of fossil fuel products into the soil. An increase in fossil fuel consumption/emission is suggested by the extremely low soil ^{14}C concentrations in 1961. This hypothesis is supported by a depleted ^{14}C value of plant residues, selected from the archive sample from 1961, which was 117.7 ± 0.6 pMC compared to a significantly higher atmospheric level (122.3 pMC) at that time. This most probably reflects a local *Suess*-effect (chapter 3.2.1), i.e. the dilution of the atmospheric $^{14}CO_2$ by fossil fuel-derived, ^{14}C-free CO_2 emitted by industries and power plants. Results of Schmidt et al. (2000) confirm this assumption. They found a gradual increase in carbon content in the plough horizon (0-20 cm) of the continuous rye trial since 1949 which was ascribed to an enhanced carbon input from coal and fossil fuel combustion products. Further results of quantitative and qualitative analysis of the black, supposedly lignite particles are described in chapter 5.2.

5.1.2 Soil organic matter heterogeneity in depth profiles at Halle

The AAA-extraction method (chapter 4.2.4) was applied to bulk soil as well as to mechanically selected organic matter components from soil samples of the unfertilised plots on the rye monoculture and on the crop rotation experiment at Halle. The samples were taken from the plough horizon as well as from different depth intervals down to 68 cm soil depth.

The chemical extractions result in a humin and a humic acid fraction and, if recovered, in fulvic acids (de Vries, 1958; Taylor, 1987; Grootes et al., 2004). The humic acid fraction is thought to be composed of the sample material itself as well as of soluble organic components that may derive from different parts of a soil profile (Grootes et al., 2004; Head and Zhou, 2000). The transport of humic acid compounds by water is

influenced by high pH values at which this fraction becomes soluble (MacCarthy, 2001). Due to its supposed higher mobility, the humic acid fraction in most cases is used as additional source of information in radiocarbon analysis of soils with respect to their genesis. The humin fraction, in contrast, is assumed to be the most stable (Lichtfouse et al., 1998b; Rice, 2001) and less influenced by vertically translocated young carbon and thus may provide a reliable ^{14}C age of soil organic matter (Balesdent, 1987; Becker-Heidmann et al., 1988; Pessenda et al., 2001). Since labile compounds such as carbohydrates, amino acids and sugars are removed by acid hydrolysis leaving a residue depleted in ^{14}C (Scharpenseel, 1968; Trumbore et al., 1989, 1990), this fraction is supposed to consist of resistant, highly condensed humic substances but also humic acids which are intimately bound to the mineral phase (Stevenson and Cole, 1999).

In radiocarbon dating of buried soils, organic sediments as well as of macrofossils (charcoal, wood) the aim of the AAA-extraction procedure, which is used by most radiocarbon laboratories, is to remove contaminating carbon that may have been infiltrated into the organic material. For the study of SOM dynamics acid and alkali extractions have shown, on the one hand to be successful in separating pools of different stabilities (Leavitt et al., 1996; Trumbore et al., 1990), but on the other ^{14}C results of such chemically-defined fractions were inconsistent for different soil types and land uses (Trumbore, 1993). Although the extracted fractions consist of diverse organic substances in various stages of polymerisation (Kononova, 1966; Stevenson, 1994), organic matter fractions extracted with acid and alkali have shown to yield information on carbon turnover and transport processes (Martel and Paul, 1974; Trumbore et al., 1989, 1990; Kristensen et al., 2003).

Analysis of the surface soil at Halle

Large variations in the radiocarbon content of different SOM fractions from topsoil samples reflect a strong age heterogeneity of the organic matter at the Halle study site. Figure 5.1 shows the ^{14}C concentrations of bulk soil, plant residues, and black particles selected by hand-picking under a microscope as well as of the ‘selected soil’ left after this mechanical separation. These fractions were chemically separated into humin and humic acids. The ^{14}C contents of separated plant residues ranged from 107.5 to 109.2 pMC and were therefore in good agreement with the $^{14}CO_2$ content of the recent atmosphere (108.7 ± 0.6 pMC in 2000; table 5.2). A ^{14}C value of 106.4 ± 0.4 pMC was measured for a young rye plant sampled in December 2000. This result shows that a

local *Suess*-effect (chapter 3.2.1), due to a higher concentration of fossil-fuel derived CO_2 in the atmosphere during winter, is presently of minor importance at Halle. All other soil fractions yielded surprisingly low ^{14}C contents for the near-surface SOM, e.g. 54.5 ± 0.5 pMC (~4880 years BP) of the untreated bulk soil in 0-25 cm depth. The contribution of fossil, ^{14}C-free carbon to the total SOM of the sampled plough horizon has to be more than 50 % to explain the observed values based on the assumptions described in chapter 5.2.3.

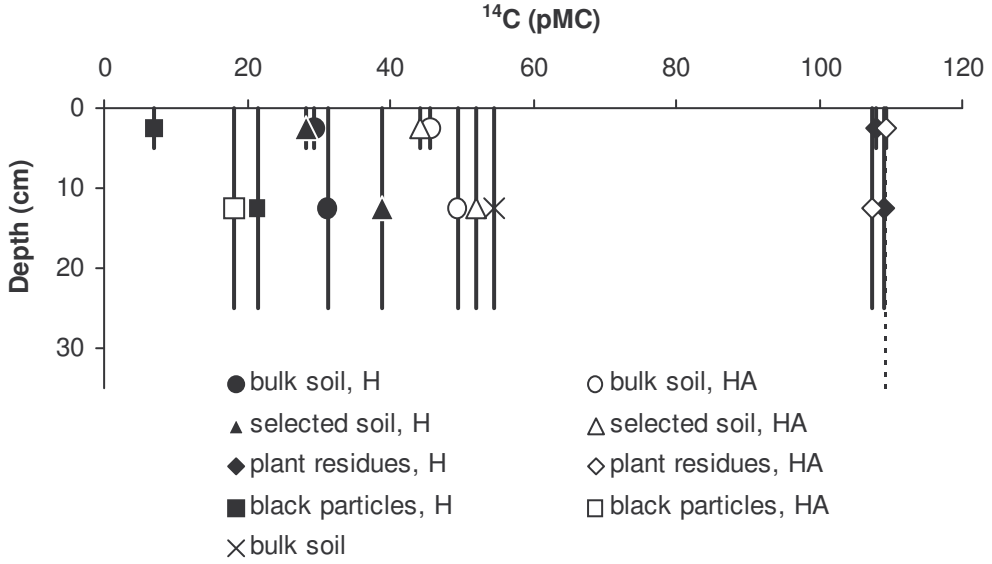

Figure 5.1: ^{14}C contents of soil fractions and separated organic matter components from the topsoil of the continuous rye trial at Halle. Samples were taken in 0-25 cm (KIA 12773) and in 0-5 cm soil depth (KIA 13262). Solid symbols represent the humin fraction (H) and open symbols the humic acids (HA). The symbols represent the mid-depth and the bars the sampling interval. The dashed line reflects the atmospheric ^{14}C content at the time of soil collection (2000; Levin et al., 2003). The measurement uncertainty (1-σ) is within the size of the symbols.

The humic acid fractions extracted from bulk and selected soils had higher ^{14}C concentrations than the humin, the residues of the alkali extraction. These findings are in agreement with results of Trumbore et al. (1989), Trumbore (1993) and Wang and Hsieh (2002) who obtained lower ^{14}C contents, i.e. higher radiocarbon ages, for organic matter left after acid hydrolysis. The acid treatment hydrolyses compounds such as carbohydrates, sugars, amino acids, and polysaccharides and in addition may lead to the loss or exchange of functional, high molecular weight groups from SOM (Stevenson, 1994). As a consequence of the removal of presumably young, easily degradable organic carbon a fraction depleted in ^{14}C is left (Trumbore,1993; Hayes and

Clapp, 2001). In contrast, Scharpenseel and Becker-Heidmann (1992) reported lower concentrations, i.e. higher ages, for the humic acid fraction and higher ^{14}C concentrations for the humin fraction from Chinese Mollisols (0-105 cm depth). The variable results are probably caused by differences in soil type and climatic conditions as supposed by Trumbore (1993).

Table 5.2: Annual mean values of atmospheric $^{14}CO_2$ contents measured at Schauinsland station (Black forest, Germany; Levin et al., 2003).

Year	^{14}C (pMC)	$\Delta^{14}C$ (‰)
2000	108.7 ± 0.3	86.2 ± 3.0
2001	108.0 ± 0.2	79.2 ± 2.0
2002	107.2 ± 0.2	72.0 ± 2.0

Both the humic acid and the humin fraction of the bulk soil showed lower ^{14}C values than the untreated bulk soil. This apparent inconsistency indicates that the acid-soluble component, the fulvic acid fraction of SOM, which was not recovered by the procedure used in this study, must have a ^{14}C content higher than the bulk soil. Lower ^{14}C contents of bulk soil and selected soil sampled in 0-5 cm compared to 0-25 cm sampling depth can be seen as the result of the different sampling intervals as well as of the different time of soil collection. The latter sample was obtained directly after tillage, in September 2000 and apparent ^{14}C ages are influenced by the input of recent rye straw that was probably partly decomposed three months later, in the soil collected in 0-5 cm depth. ^{14}C concentrations of the black particles, isolated from the plough horizon, ranged from ca. 21.5 pMC (~12,350 years BP) to 7.0 pMC (~21,360 years BP) reflecting a heterogeneous composition of this material (further results in chapter 5.2).

^{14}C distribution in soil profiles

The change of SOM ^{14}C concentration with increasing soil depth in samples from trials with continuous rye and with crop rotation of the study site at Halle was investigated (figure 5.2). In general, radiocarbon ages of bulk SOM are expected to increase with soil depth (Scharpenseel et al., 1989; Wang et al., 1999), since in deeper soil layers the input of fresh organic material, reflecting recent atmospheric ^{14}C concentrations, and the influence of bomb-^{14}C are limited. Moreover, the stability of the organic matter should increase with soil depth and ^{14}C concentrations of SOM should be more strongly dominated by older carbon (Scharpenseel et al., 1989; Harrison, 1996; Römkens and Hassink, 1998). However, at Halle, located in a heavily industrialised

area, a decrease in ^{14}C age with increasing soil depth was determined for the humin as well as for the humic acid fraction of bulk and selected soil and of the black particles.

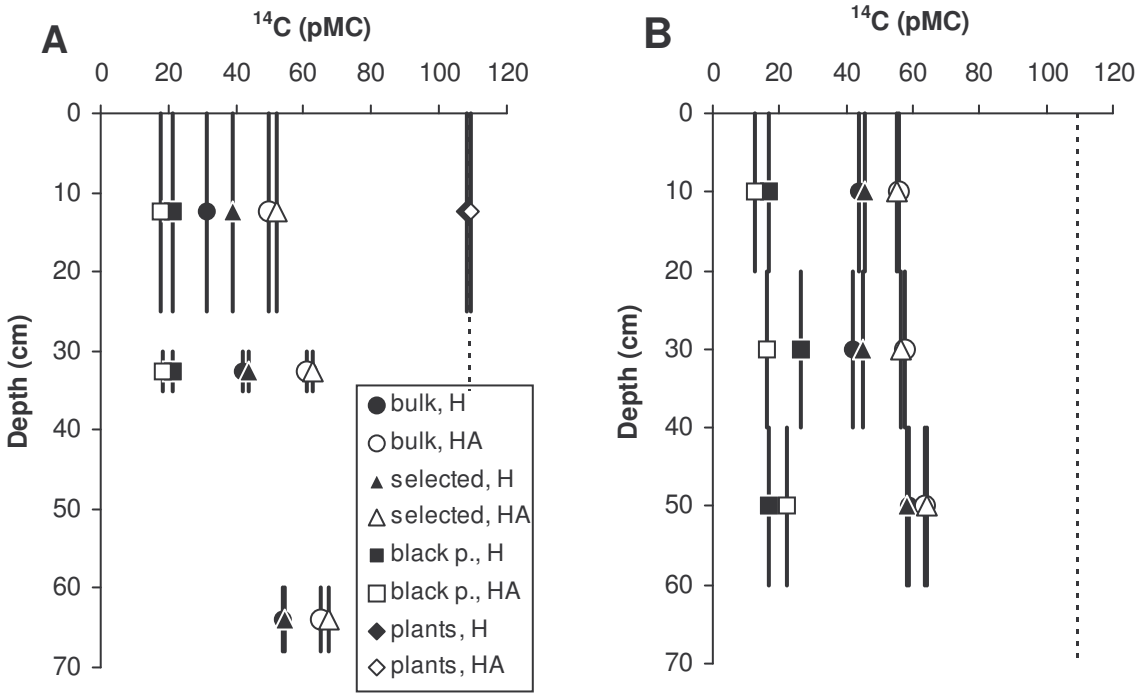

Figure 5.2: ^{14}C concentration of soil fractions and selected components in soil profiles of A: the rye monoculture (KIA 13262, 13723, and 13724), and B: the crop rotation experiment (KIA 14365 - 14367) at Halle. Soil samples were fractionated into humin (H) and humic acids (HA). The sampling intervals are represented by the bar length and the atmospheric ^{14}C level in 2000 (Levin et al., 2003) by the dashed line. The measurement uncertainty (1-σ) is within the size of the symbols.

The relatively high ^{14}C ages of the bulk soil fractions at 0-25 cm depth are most probably due to a substantial input of black particles that could not completely be eliminated by hand-picking, as shown by the still high ^{14}C age of the selected soil, or by density fractionation. The higher ^{14}C content of most of the humic acid fractions may reflect that compounds derived from the black particles contribute less to the humic acid fraction. A considerable increase in ^{14}C concentration of the humin and the humic acid fraction at 60 to 68cm (A) and 40 to 60 cm (B) soil depth suggest on the one hand a decreasing input of fossil carbon with increasing soil depth. On the other hand, still high ^{14}C values of 53.7 pMC and 65.3 pMC (A: humin and humic acids) indicate vertical transport of particulate as well as soluble fossil carbon by bioturbation, root growth and leaching. A high contribution of lignite carbon (>70 %) to dissolved organic carbon, examined in microcosm experiments, was also found by Chabbi et al. (2004) who

investigated mine sediments. The very similar radiocarbon results for both humic acids and humin of bulk and selected soil from the rye culture in 60 to 68 cm (figure 5.2-A) can be explained by incomplete removal of the black, coal-like fragments which at this depth were very small. Particle-size thus is a practical limit for the mechanical separation of SOM components.

The trial with crop rotation yielded higher ^{14}C values in 0-20 cm soil depth than the rye culture (figure 5.2-B), presumably as a consequence of a higher input of straw that is ploughed in, whereas on the monoculture experiment the rye straw is removed from the field before soil tillage. Below the plough horizon and in 40 to 60 cm depth the ^{14}C concentrations of the soil fractions approached those of the rye culture.

The separated black particles again showed significantly lower ^{14}C concentrations than the bulk and the selected soil fractions and minor variations with increasing soil depth. The lower ^{14}C concentrations of the humic acids compared to the humin of the black material in 0 to 40 cm soil depth suggests a higher contribution of old, recalcitrant compounds to this fraction.

The results obtained on the experimental site at Halle, summarised in figures 5.1 and 5.2 (tables 8.1, 8.2, and 8.3), demonstrate a strong and variable admixture of old, supposedly refractory carbon particularly in the topsoil of the Halle site. A major portion of the black material is thought to derive from lignite, since the Halle region is dominated by industries using and processing lignite (Schmidt et al., 2000) which release particulate residues of incomplete fossil fuel combustion. Moreover, a railway track (figure 4.2) used by trains transporting lignite from a nearby mine runs parallel to the field trials which most probably resulted in the addition of non-combusted lignite fragments to the soil at Halle. The higher ^{14}C concentrations towards greater soil depth may reflect a lower influence of industrial activity and lignite use but also the transport of considerable amounts of the fossil material down to 40-60 cm soil depth (table 5.4).

5.1.3 Comparative analysis of soil organic matter from agricultural sites in rural and industrialised areas

In search of a site that is less contaminated by old carbon and therefore more suitable for the study of organic carbon dynamics, surface soil samples from agricultural field trials at the Rothamsted Experimental Station and at Rotthalmünster, located at greater distance to industrial areas, were analysed (figure 5.3, tables 8.4, 8.5 and 8.6).

The ^{14}C values of soil fractions from the long-term trial 'Broadbalk Continuous Wheat' (Rothamsted) showed a range from 105.7 ± 0.3 pMC (soil humic acid) to 88.6 ± 0.5 pMC (soil humin) indicating an admixture of old material that is relatively small compared to Halle (table 5.4). The field trial in Rotthalmünster seems very little affected by material derived from fossil fuel because radiocarbon results for the untreated bulk soil of 107.6 ± 0.3 pMC and for the humin fraction of the selected soil, containing no plant residues, of 106.1 ± 0.3 pMC, were close to the ^{14}C content of the atmosphere at the time of soil sampling in 2002 (table 5.2). The humic acid faction had a ^{14}C value of 111.8 ± 0.5 pMC which is above that of the atmosphere at the time of soil collection. This relatively high amount of bomb-^{14}C indicates that the organic carbon in this fraction was fixed several years ago, during a time with higher atmospheric ^{14}C levels (figure 3.1).

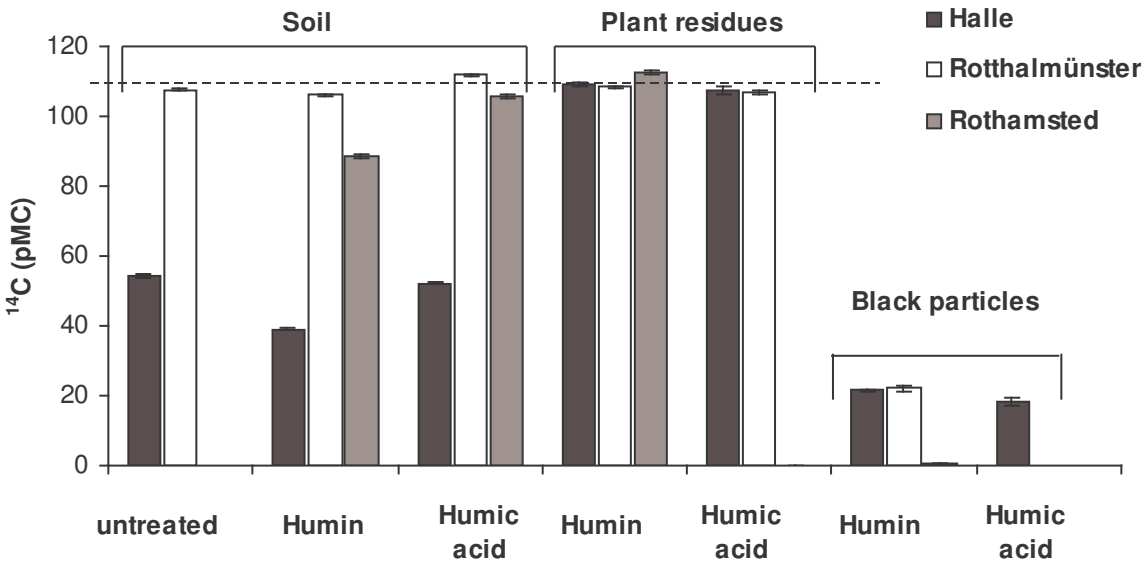

Figure 5.3: Comparison of ^{14}C concentrations in soil organic matter fractions from long-term field trials in industrialised, Halle (0-25 cm depth; KIA 12773) and Rothamsted (0-20 cm depth, KIA 15533) and rural areas, Rotthalmünster (0-20 cm depth; KIA 15532). The dashed line represents the atmospheric ^{14}C concentration in 2000 (Levin et al., 2003). The error bars show the 1-σ measurement uncertainty.

The ^{14}C results for SOM fractions from the different study sites indicate that fossil carbon is less important at Rothamsted and may be negligible at Rotthalmünster, which thus is the most suitable field trial for analysis of natural SOM dynamics. However, since this site could not provide archived soil samples, preventing the calculation of

carbon turnover times based on the bomb-^{14}C method, the most suitable long-term trial is that of the Rothamsted Experimental Station.

5.1.4 Susceptibility of physical and chemical organic matter fractions to fossil carbon contribution at Halle

Operationally-defined organic matter fractions are investigated to test the ability of the different physical and chemical separation techniques to isolate SOM pools of different stability as well as to obtain more information about the sources and potential stabilisation mechanisms of the fossil carbon in the soil at Halle. A further objective of this analysis is to overcome the contamination problem by finding a fraction which is less or not contaminated by fossil carbon and can be used to investigate the turnover of natural soil organic carbon.

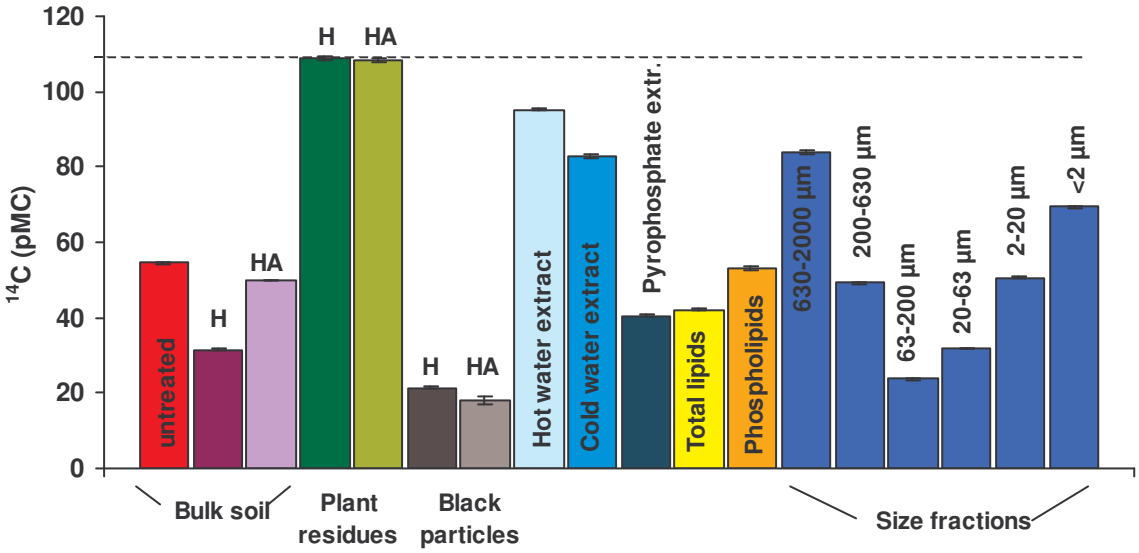

Figure 5.4: ^{14}C content of bulk soil and organic matter fractions from topsoil samples of the rye monoculture at Halle. Humin (H) and humic acids (HA) were extracted from bulk soil, plant residues, and black particles (KIA 12773). Labile SOM was extracted with hot- and cold water, and stable SOM with pyrophosphate (KIA 15369-15371). Total- and phospholipids (KIA 15918) were obtained by solvent extraction followed by solid phase chromatography (chapter 4.3.3). Particle-size fractions (KIA 19634) were separated by wet sieving and centrifugation (chapter 4.2.1). The error bars display the 1-σ measurement uncertainty and the dashed line the atmospheric ^{14}C level (2000; Levin et al., 2003).

^{14}C concentrations of the different SOM fractions separated from topsoil samples of the unfertilised plot on the rye monoculture at Halle are shown in figure 5.4 (tables 5.5, 8.1, and 8.8). The large variations in ^{14}C concentration of the numerous fractions illustrate the failure of fractionation procedures based on physical or chemical properties in consistently isolating organic matter pools characterised by different mean residence times as previously reported e.g. by Scharpenseel and Becker-Heidmann (1992) and Trumbore et al. (1989). Moreover, the susceptibility of the different fractions or compound classes to a contamination by anthropogenically-added, fossil carbon is demonstrated by these data. Particularly the humin fraction of the bulk soil seems to be enriched in fossil carbon. An even higher proportion is accumulated in the fine sand fraction (63-200 µm) as shown by an extremely low ^{14}C concentration of 23.7 ± 0.2 pMC (~11,560 years BP). This result may indicate either a predominance of black particles in this size class (Schmidt et al., 1996) or physical stabilisation of the refractory material (Glaser et al., 2000; see further discussion in chapter 5.2). The pyrophosphate extractable organic matter was separated by Michael Kaiser (ZALF, Müncheberg, Germany) according to the method described by Hayes (1985). This fraction, which is thought to contain organic compounds of high stability, yielded a ^{14}C concentration of 40.5 ± 0.2 pMC which is considerably higher than those of the soil humin fraction. A possible explanation is, that the stronger alkali extraction removes a higher portion of young components (Stevenson, 1994) compared to the less effective, mild pyrophosphate extraction. The highest ^{14}C value of a soil fraction was obtained for the hot water extractable (90°C, 9 hours) organic matter having 95.2 ± 0.3 pMC. In contrast, the organic matter extracted with cold water (24 hours) was more depleted in ^{14}C (82.9 ± 0.4 pMC) indicating a higher contribution of fossil carbon. The water-soluble organic matter thus is the least contaminated and supposedly the most labile, i.e. easily degradable soil fraction.

The extraction of total lipids from fresh surface soil and the subsequent isolation of a fraction that mainly contains phospholipids by liquid chromatography via solid phase (silicic acid) columns (figure 4.6) was intended to derive ^{14}C information for compounds that can be attributed to a specific source. Phospholipids are assumed to be indicators of microbial biomass in soils, as they are essential membrane components of living cells and short-lived in the soil (Zelles, 1999). The ^{14}C concentration of the total lipid fraction extracted from fresh topsoil samples was 41.4 ± 0.2 pMC, which is even lower than the ^{14}C content of the bulk soil (54.5 pMC). The radiocarbon content of the phospholipid fraction, which yielded 53.1 ± 0.4 pMC, was higher than that of the total

lipids and close to that of the bulk soil. However, the apparent high age of the phospholipid fraction (~5090 years BP) is inconsistent with its alleged lability and consequent modern origin of this compound class. It suggests (i) that fossil carbon was incorporated in the soil microbial biomass and/or (ii) a high proportion of old, non-microbial polar lipid is contained in this fraction. Investigations of Petsch et al. (2001), using ^{14}C analysis of individual phospholipid fatty acids, and Rumpel et al. (2001), who applied the chloroform fumigation extraction method (Vance et al., 1987) combined with radiocarbon dating, indicate a possible uptake of fossil carbon by soil microorganisms. However, the extremely low ^{14}C values suggest that most probably compounds derived from the old, black particles contribute to the analysed fraction. This is also evident from increasing ^{14}C concentrations of the total lipid fraction with soil depth (figure 5.5) corresponding to the ^{14}C distribution of the soil humin and humic acid fractions in the profile at Halle. Moreover, the analysed phospholipid fraction was not subject to a mild alkaline hydrolysis and thus can contain other polar lipids which may be derived from both living and dead cells. Analyses of fatty acid methyl-esters obtained after the hydrolysis of the phospholipid fraction showed that interference of non-microbial polar compounds, e.g. fatty acids derived from humic substances, can be about 5 to 10 % of the total phospholipid methyl-esters (Nielsen and Petersen, 2000; Aries et al., 2001).

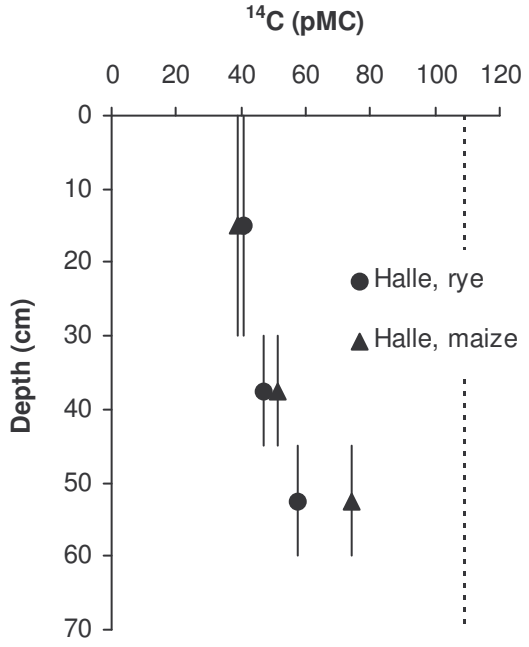

Figure 5.5: ^{14}C distribution of the total lipid fraction in soil profiles of the rye and maize cultures at Halle (KIA 20172, 20173). The sampling intervals are represented by the bar length and the atmospheric $^{14}CO_2$ level (Levin et al., 2003) by the dashed line.

The phospholipid fraction was further separated into individual phospholipid fatty acids by preparative capillary gas-chromatography with the objective to exclude the fossil material. The results of compound-specific radiocarbon analyses of these biomarker substances which were isolated from the surface and the subsoil of different field trials are presented in chapters 5.3.2 and 5.4.2.

5.2 Characterisation and quantification of fossil carbon at Halle

The low ^{14}C concentration of bulk soil and SOM fractions from the field trial at Halle suggests a high input of fossil carbon particularly to topsoil organic matter. It was assumed that a large proportion of the black material is derived from airborne lignite fragments and fossil-fuel combustion products as the study site at Halle is located in the East German industrial region where lignite has been used for centuries mainly as energy source. For the area around Bitterfeld, located about 30 km to the north-east of Halle, Neumeister et al. (1991) reported a variable deposition of lignite particles, soot and dust which has reached an average thickness of about 70 cm in an 850 km² area during the last 100 years. Stumpe (1967) estimated the monthly input of soot and coal dust to amount to 20 g/m² at the Halle site. This large portion of the old, presumably recalcitrant material may thus have a significant influence on organic matter dynamics in soils in industrialised areas (Schmidt et al., 1996). Furthermore, because of its assumed extremely slow degradability it is thought to be a significant carbon sink in the global carbon cycle (Kuhlbusch and Crutzen, 1995; Schmidt et al., 2002). Thus, a quantification of the material is important when investigating the dynamics of natural, vegetation-derived organic carbon in soils.

Various methods have been used to chemically characterise and to quantify black carbon, the residue of incomplete fossil fuel and biomass combustion (chapter 2.4), and lignite respectively, in soils or sediments. This is complicated by the wide range of products and their variable, predominantly small particle-size. Many of these methods are based on indirect estimates such as oxidation techniques combined with structural analysis, but also molecular markers are used (Skjemstad, 1993; Glaser et al., 1998; Schmidt et al., 2002; Dickens et al., 2004). Until now no method has been found giving consistent results. In this study black particles, supposedly derived from fossil fuels, in the soil of the Halle long-term study site are examined:

- Black fragments, selected from the surface and subsoil of the study site at Halle, were inspected by light microscopy to separate optically different particles. Their organic carbon contents, ^{14}C as well as $\delta^{13}C$ concentrations were determined with the aim to identify the origin of the material.

- To be able to differentiate between different sources, optically different black particles, selected by hand-picking, were analysed by scanning electron microscopy (SEM) coupled to energy-dispersive X-ray spectroscopy (EDX). This technique

makes it possible to analyse the morphology of the fragments and furthermore, determine the chemical composition of selected sections on each particle. Atomic O/C ratios of different sections were used as an additional parameter to distinguish charred plant residues from coal or highly graphitised fragments (Stoffyn-Egli et al., 1997; Brodowski et al., 2004).

- The proportion of fossil carbon in topsoil organic matter at Halle was quantified from bulk soil ^{14}C data by mass balance calculation. The results obtained for the Halle site in a highly industrialised region were compared with estimates for the field trials in rural areas, at Rothamsted and at Rotthalmünster.

5.2.1 Characterisation of the black particles by light microscopy, ^{14}C and ^{13}C analysis

An inspection of the black particles selected from topsoil samples by light microscopy (figure 5.6) proved again that this fraction is composed of different components such as shiny, angular particles, porous particles, and structured particles (figure 5.8) which looked like charred plant fragments. This observation and the variable, but very low ^{14}C values (figure 5.7) suggest that the black material is a mixture of mainly lignite fragments and minor proportions of combustion derived products and burned vegetation residues.

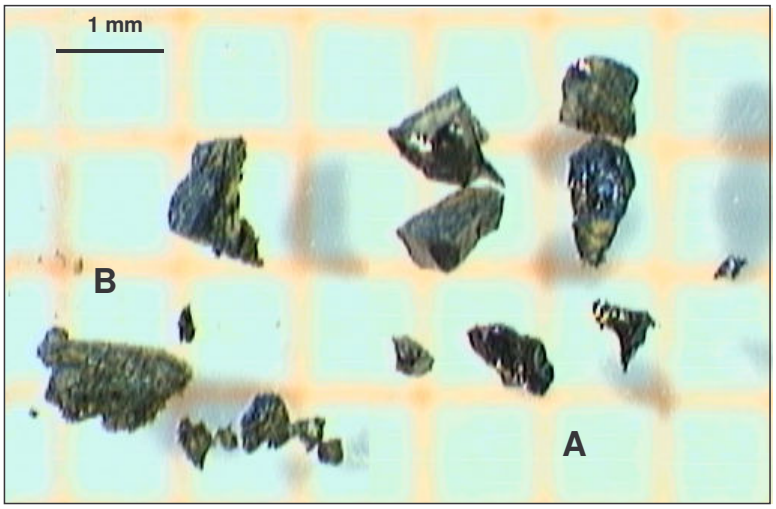

Figure 5.6: Photography of (A) shiny, angular and (B) porous black particles selected from the plough horizon of the rye monoculture at Halle.

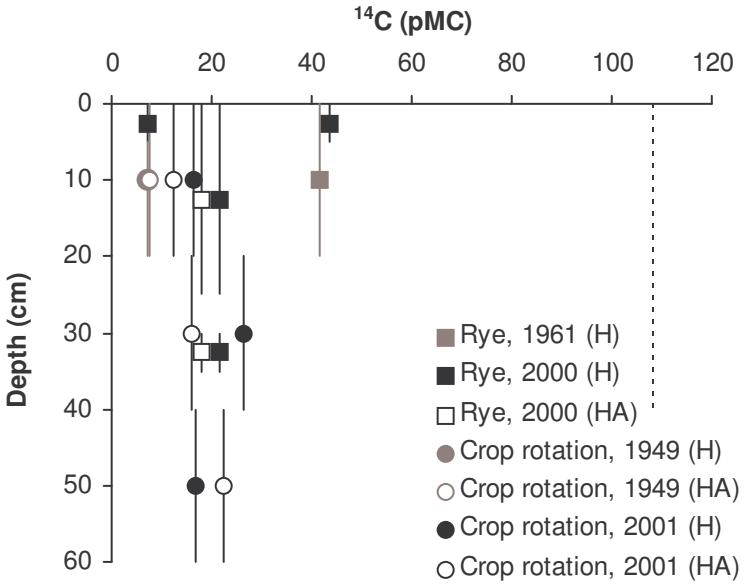

Figure 5.7: Radiocarbon concentrations of black particles, separated into humin (H) and humic acid fractions (HA), selected from recent and archived soil samples of field trials at Halle. The bar length represents the sampling interval and the dashed line the ^{14}C content of the atmosphere in 2001 (Levin et al., 2003).

$\delta^{13}C$ results for selected black particles indicate their origin from C_3 vegetation (table 5.3). Moreover, investigations of Wiesenberg et al. (2004a), who used 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 the old carbon at Halle. Black fragments may further originate from fly-ash, emitted by surrounding industries and power plants fuelled by lignite (Merbach et al., 1999; Enders et al., 1994), as well as from incomplete combustion of fossil fuel by traffic (see further results in chapter 5.2.2).

Table 5.3: Total organic carbon (TOC) contents and $\delta^{13}C$ values of black particles selected from a topsoil sample (0-25 cm depth) of the rye culture at Halle.

Sample	TOC (%)	$\delta^{13}C$ (‰ PDB)
1	63.2	-24.1
2	43.8	-25.4
3	42.6	-25.9

Carbon and $\delta^{13}C$ measurements by Bettina John, University of Göttingen

The separated particles yielded total organic carbon contents (TOC) of about 43 % and about 63 % (table 5.3). This suggests their origin from lignite, which has TOC contents

between 60 and 70 % (Killops and Killops, 1993), while the lower values propose less carbonised material or lignite particles contaminated by humic substances such as fulvic acids (TOC: 41-51 %; Stevenson, 1994), as possible source.

5.2.2 Morphological and chemical characterisation of black particles by SEM/EDX analysis

Scanning electron microscopy (SEM) coupled to energy-dispersive X-ray spectroscopy (EDX) analyses were applied to optically different black particles of > 200 µm grain size selected by hand-picking from surface soil samples of the rye culture at Halle and the trial with continuous wheat at Rothamsted. The particles were cleaned by sonication in ultra pure water. SEM was successfully applied in previous investigations for the recognition of combustion residues derived from vegetation burning, fossil fuel combustion or combustion condensates occurring as soot, such as charcoal, soot and charred plant residues (Fernandes et al., 2003; Brodowski et al., 2004). Here, this technique is used to identify the morphology of the different particles, and, by this, possible sources of the black particles.

The particle shown in figure 5.8-A was recognised as plant fragment according to analysis of Fernandes et al. (2003). The pores with their thick, circular surrounding are supposed to be bordered pits which are characteristics of xylem structures. Figure 5.8-B shows a fragment, which is thought to be a lignite fragment, with sections looking like a shell-like fracture, and porous material on the left side and at the bottom of this picture. The size of the selected analysed particle, which was about 1-2 mm, excludes soot, which occurs in much smaller particle-sizes, as a possible source.

EDX analysis was applied to selected sections on each particle to derive an additional parameter, the atomic O/C ratio, as criterion for the differentiation between possible sources of the separated material. The O/C ratios were calculated from the analysed elemental composition with the help of equation 15 described in chapter 4.6. This parameter can be used to determine the degree of graphitisation via the oxygen loss of the continuum of carbon combustion products where pure graphite is the strongest carbonised material, which has an O/C ratio of about 0.1 (Killops and Killops, 1993). Stoffyn-Egli et al. (1997) and Brodowski et al. (2004), who examined charred vegetation residues and fossil fuel combustion products, showed that O/C ratios of <0.15 and ≤ 0.33 respectively are indicative for material produced by incomplete combustion. However, since only small sections of each particle were analysed by

EDX, these data can only be seen as additional information to the results of the SEM analysis.

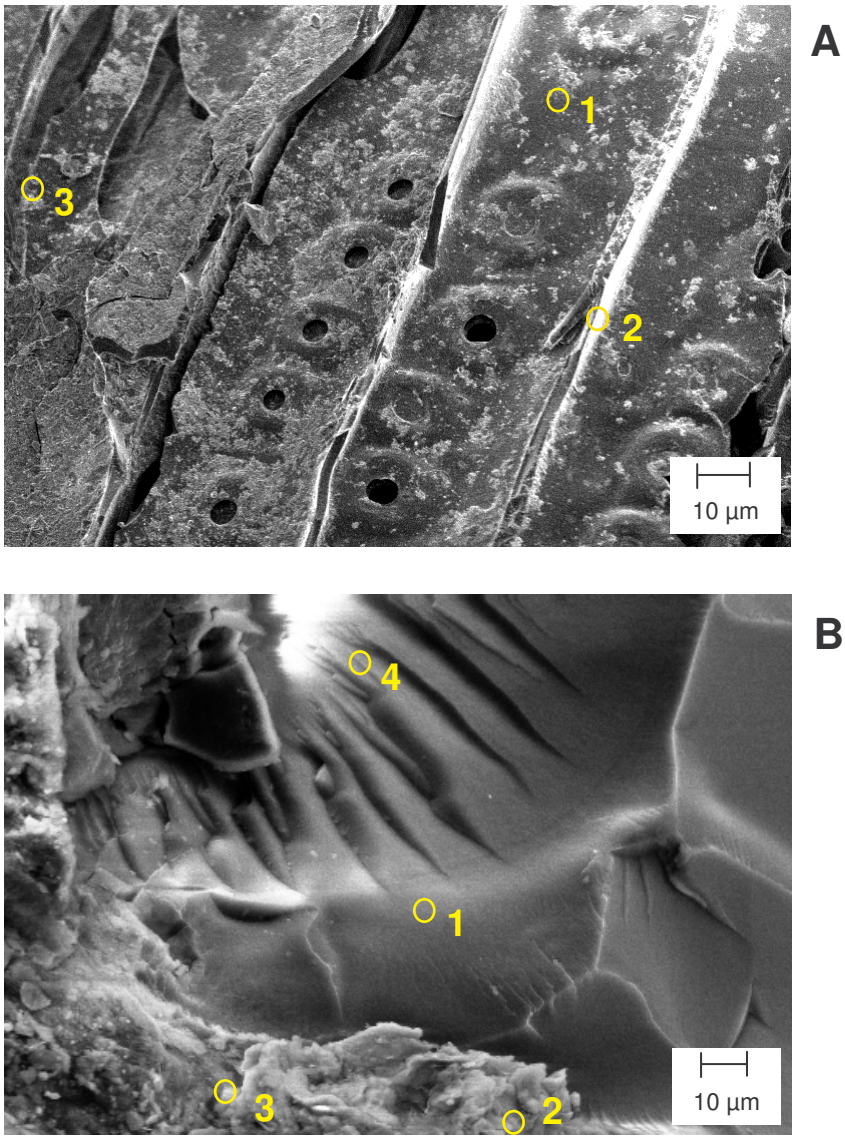

Figure 5.8: Scanning electron microscope images of two different black particles selected from the topsoil of the Halle site. The EDX spectra, marked by the circles, were recorded on a supposed charred plant fragment (A) and a lignite particle (B).

The spectra 2 and 3 of the supposed charred plant (rye?) residue shown in figure 5.8-A, yielded an O/C ratio of 0.32 and 0.26 respectively, agreeing with findings of Brodowski et al. (2004). They produced charred rye straw by heating the plant material in closed stainless steel containers in a muffle furnace (350°C) and determined an average O/C ratio of 0.3 ± 0.08. Spectrum 1 had a higher ratio of 0.47 indicating that this part is less charred or increasingly oxidised. A further explanation for this finding is

that other material may adhere to this fragment. The material shown in figure 5.8-B exhibited lower O/C ratios between 0.08 and 0.23 (spectra 1, 3, 4). Particularly the glassy (amorphous) parts (spectra 1 and 4) yielded extremely low ratios of 0.08 indicating that these parts of this fragment are strongly carbonised. This suggests combustion of the assumed lignite particle. However, spectrum 3, showed a higher O/C ratio of 0.2 and spectrum 2 an extremely high ratio of about 9.5. The latter again is thought to reflect sorption of less transformed organic material characterised by high oxygen and low carbon contents. The variability in O/C ratio within a few µm suggests the structural diversity of this material. Investigations of Chabbi et al. (2004) revealed a high structural variability of lignite, which was examined by solid state ^{13}C nuclear magnetic resonance spectroscopy (NMR). This complicates a precise identification of the origin of the analysed particle.

Additionally, black particles selected from the topsoil of the continuous wheat trial at Rothamsted (UK) were analysed by this method. Different spectra recorded on these particles yielded similar low O/C ratios of 0.08 to 0.17 characteristic for highly carbonised material such as coal fragments. However, a considerably higher ^{14}C value of 88.6 pMC and 105.7 pMC of the soil humin and humic acid fraction respectively (chapter 5.1.3) indicates only a minor contribution of fossil carbon to SOM at the Rothamsted site.

In summary, these data and the microscopic observations confirm that the black fragments with high but variable ^{14}C ages in the soil at Halle derive from lignite and its combustion products. A minor portion is assumed to originate from biomass burning in historical times. A differentiation between combusted and non-combusted lignite fragments is difficult. Higher O/C ratios and rougher structures may indicate the presence of non-combusted lignite fragments. Fossil fuel-derived carbon was not only found in the soil at Halle, but also in the topsoil of a continuous wheat trial at Rothamsted, most probably due to its proximity to London.

5.2.3 ^{14}C-based quantification of fossil carbon in soils

A quantification of fossil carbon in soils based on ^{14}C data has the advantage that the total amount of this fraction, including microbially transformed compounds and material interacting with soil minerals, is detected. As shown by investigations of Schmidt and Noack (2000) and Schmidt et al. (2001) in some soils high amounts of combustion-derived material are found in the fraction of less than 5 µm size, which is not detectable

by conventional optical methods (Skjemstad et al., 1996; Schmidt and Noack, 2000; Schmidt et al., 2001). As a result, the quantity of this old material in soils remains under-estimated by these methods.

The admixture of fossil carbon (X), containing no ^{14}C, at the Halle site was estimated from the measured ^{14}C content of bulk SOM and the ^{14}C concentration of recent plant material entering the soil since the establishment of the site by a simple mass balance calculation:

$$X = [1 - (^{14}C_{measured} / {}^{14}C_{recent})] \cdot 100 \quad (16)$$

with:

X: the amount of fossil carbon (%)

$^{14}C_{measured}$: the ^{14}C concentration of bulk SOM (pMC)

$^{14}C_{recent}$: the ^{14}C concentration of recent plant material entering the soil in a certain period of time (pMC; estimated via equation 17)

The ^{14}C value of recent plant material, $^{14}C_{recent}$, has to be estimated, since (i) the atmospheric $^{14}CO_2$ level changed drastically in the time span between 1954 until today due to the release of bomb-^{14}C, (ii) the plant material entering the soil of the agricultural trials each year is derived from atmospheric CO_2 of that years growing season, and (iii) the plant residues underlie decomposition. The estimation was made by combining the atmospheric $^{14}CO_2$ record (Levin and Kromer, 1997; Levin et al., 2003; Levin and Kromer, 2004) with a simple decomposition model similar to Hsieh (1993) but consisting of two pools with different inputs of new organic carbon and different turnover rates (equation 17). The value of $^{14}C_{recent}$ was calculated for the period since the starting of the trials in 1878 until the year 2000, assuming recent plant material to reflect the atmospheric ^{14}C level and to be composed of 75 % easily decomposable organic components with a mean residence time (MRT) of 2 years and of 25 % more resistant components with 10 years MRT. Equation 17 calculates the amount of organic carbon of a particular year (i) remaining in each pool in the year of soil sampling (y) multiplied with its decay corrected ^{14}C concentration. $^{14}C_{recent}$ is the weighted average of the contributions of both pools since the establishment of the field trial until the year of soil collection.

$$^{14}C_{recent} = \frac{\sum_{i=b}^{y} I_{i,1} \exp[-k_1(y-i)] \times {}^{14}C_i \exp[-(y-i)/8267] + \sum_{i=b}^{y} I_{i,2} \exp[-k_2(y-i)] \times {}^{14}C_i \exp[-(y-i)/8267]}{\sum_{i=b}^{y} I_{i,1} \exp[-k_1(y-i)] + \sum_{i=b}^{y} I_{i,2} \exp[-k_2(y-i)]} \quad (17)$$

with:

$I_{i,1}$, $I_{i,2}$: the amount of new SOC input in pool 1 (= 0.75) and pool 2 (= 0.25) in the year i

$^{14}C_i$: the ^{14}C content of the atmosphere (pMC) in the year i

y: the year of soil sampling

b: the year of site establishment

k_1, k_2: first-order decomposition rates (1/MRT) of SOM pools 1 (= 0.5) and 2 (= 0.1)

8267: the ^{14}C mean-life in years

A contribution of fossil carbon of about 52 % was calculated for the untreated surface soil (0-25 cm depth) at Halle (table 5.4). The humin fraction contained an even higher proportion of 72 % fossil carbon in the upper 25 cm which decreases to about 52.7 % in 60 to 68 cm soil depth. Lower estimates of fossil carbon contributions were determined for the humin and humic acid fraction of the ‘selected’ surface soil, which was cleaned from identifiable coal particles and plant residues. These data indicate that a mechanical separation of the fossil material is not effective. In 30 cm to 68 cm soil depth bulk and selected soil yielded closely similar amounts of fossil carbon, suggesting that the size of the fossil material decreases with increasing soil depth and its association with the mineral matrix is stronger, which both hinder a mechanical separation. Based on the benzenepolycarboxylic acid method (modified according to Glaser et al., 1998) Brodowski (2004) estimated the proportion of charred black carbon in Halle topsoil only at about 15 %. This lower estimate indicates that only a small proportion of the lignite carbon is detected by this method, which is based on the assumption that charred organic material is converted to benzenepolycarboxylic acids whereas uncharred organic substances are not (Glaser et al., 1998, 2001). Moreover, this lower value may be caused by losses of carbon during the conversion of SOM (hydrolysis, oxidation, cleaning steps) to benzenepolycarboxylic acid (Brodowski, 2004). The high apparent ^{14}C age of about 6,000 years of the humic acid fraction confirms the results of Rumpel and Kögel-Knabner (2002) who found high portions of lignite in this fraction in 14 to 37 year old cultivated mine soils. They concluded that lignite is oxidised during biodegradation becoming part of the humic acid fraction.

At Rotthamsted, which is located at about 40 km distance from the city centre of London, SEM/EDX analysis indicated that a possible source of fossil carbon

responsible for a ^{14}C age of the soil humin fraction of 970 years BP may as well be coal fragments. Compared to Halle a mass balance calculation yields a much smaller contribution of fossil carbon to the surface soil at Rothamsted of about 23 % in the soil humin and only 8.1 % in the humic acid fraction. A closely modern ^{14}C value of 107.6 pMC of the bulk soil (0-10 cm) at Rotthalmünster, located in a rural area, suggests a negligible contribution of fossil fuel-derived carbon to SOM at this site. Only 4.9 % of the organic carbon in the humin fraction may be derived from old, fossil sources.

The calculation above is approximate because in reality SOM is a continuum of fresh plant residues to highly altered organic substances and thus will consist of more than two pools with different turnover rates. Furthermore, the ratio of slow to fast cycling organic matter will be different in the plough horizon and at greater soil depth and stabilisation of SOM via chemical and physical mechanisms is ignored.

Table 5.4: ^{14}C values and ^{14}C-based estimates of fossil carbon contribution to soil organic matter at Halle, Rothamsted, and Rotthalmünster.

Site [1]	Depth (cm)	Soil fraction	^{14}C (pMC)		Fossil carbon [2] (%)
Halle: rye	0-25	Bulk	U: 54.5 ± 0.5		U: 52.0
(KIA 12773)		Bulk	H: 31.6 ± 0.3	HA: 49.9 ± 0.2	H: 72.3, HA: 56.3
		Selected	H: 38.9 ± 0.3	HA: 52.2 ± 0.3	H: 65.8, HA: 54.1
Halle: rye	30-35	Bulk	H: 41.1 ± 0.2*	HA: 60.7 ± 0.2	H: 63.8, HA: 46.5
(KIA 13723)		Selected	H: 43.0 ± 0.2*	HA: 62.9 ± 0.2	H: 62.1, HA: 44.6
Halle: rye	60-68	Bulk	H: 53.6 ± 0.3*	HA: 65.3 ± 0.4	H: 52.8, HA: 42.5
(KIA 13724)		Selected	H: 53.6 ± 0.7*	HA: 67.5 + 0.3	H: 52.8, HA: 40.6
Rothamsted: wheat (KIA 15533)	0-20	Bulk	H: 88.6 ± 0.5	HA: 105.7 ± 0.3	H: 23.0, HA: 8.1
Rotthalmünster:	0-20	Bulk	U: 107.6 ± 0.3		U: 3.6
maize (KIA 15532)		Bulk	H: 106.1 ± 0.3	HA: 111.8 ± 0.5	H: 4.9, HA: bomb-^{14}C

'Bulk soil' is the <2 mm sieved soil and the 'selected soil', the bulk soil cleaned from microscopically identifiable plant residues and black particles. (U) designates the untreated soil, (H) and (HA) the humin and humic acid fractions extracted from the bulk soil.

[1] Dates of soil sampling are: 2000 at Halle, 1997 at Rothamsted and 2002 at Rotthalmünster.

[2] Calculated according to equation 17 with $^{14}C_{recent}$ = 113.5 pMC (Halle), 115.0 pMC (Rothamsted), 111.6 pMC (Rotthalmünster).

* Weighted average for two graphite targets prepared from a composite soil sample.

These results demonstrate the importance of an old carbon fraction in soils which can be more than 50 % of the total soil organic carbon. Comparable results were reported by Schmidt et al. (1999a), who estimated the proportion of black carbon in a German Chernozem to be up to 45 % of the soil organic carbon. Glaser et al. (2000) analysed soils of the Brazilian Amazonian region and found concentrations of carbon derived

from charred organic material in topsoil organic matter of up to 35 % in Terra Preta and of about 15 % in Oxisols.

However, until now it is still not clear if the old carbon is preserved due to the recalcitrant chemical properties of the material or if it is protected by physical and chemical stabilisation processes. Studies of Rumpel et al. (2001) and Petsch et al. (2001) examining microbial degradation of lignite and geogenic carbon indicate that this material is not totally inert as it is used by soil microorganisms as carbon source. This could be confirmed by our own investigations of individual phospholipid fatty acids which are described in chapter 5.3.2.

5.3 Radiocarbon analysis of soil lipids

The results obtained on the field trials on the Halle site illustrate the heterogeneous mixture of organic compounds in physically and chemically defined soil organic matter fractions and show their susceptibility to contamination, such as a variable contribution of fossil fuel-derived carbon, even in more specific lipid fractions (chapters 5.1.1 to 5.1.4). Similar observations of vegetation fire and fossil fuel-derived carbon contributing to soil fractions were reported by Schmidt et al. (1996, 2001), Skjemstad et al., (1999), and Glaser et al. (2000). The addition of old, refractory carbon to SOM results in an underestimation of carbon turnover rates based on ^{14}C analyses. A more precise determination of 'natural' soil organic carbon dynamics is only achievable by the examination of biomarker substances, which can be attributed to specific inputs (chapter 3.1), thereby excluding contaminating carbon sources. Their ^{14}C content, makes it possible to evaluate the origin and transformation of the individual compounds in soils as shown by compound-specific radiocarbon data of marine sediments (Eglinton et al., 1997; Ohkouchi et al., 2003).

Lipids are a relatively stable and diverse group of organic compounds in soils, ranging from relatively simple molecules, such as organic acids, to more complex substances like sterols, hydrocarbons, fats, waxes, and resins (Dinel et al., 1990; Stevenson, 1994) which contain several diagnostic markers. Although they only represent about 4 to 8 % of the soil organic carbon (Dinel et al., 1990), they are assumed to be of high importance for SOM stabilisation, e.g. aggregate formation (Amblès et al., 1993). Soil lipids mainly originate from vegetation but also from animals and soil microorganisms (Dinel et al., 1990). Specific lipid compound classes can be used as proxies to investigate the input of vegetation-derived components to the soil and to study their transformation (Amblès et al., 1994; Bol et al., 1996; van Bergen et al., 1997; Bull et al., 2000). Individual lipid compounds, such as certain short-chain fatty acids derived from phospholipids, which originate from bacterial cell membranes (Tunlid and White, 1992; Zelles, 1999), have shown to be the most useful biomarkers for the investigation of carbon assimilation and degradation by soil microorganisms, being key agents in organic carbon transformation and mineralisation in soils (chapter 2.3). Their structural diversity and biologic specificity make it possible to characterise microbial communities and their reaction to changing soil conditions (Zelles et al., 1992; Frostegård et al., 1993; Frostegård and Bååth, 1996; Pankhurst et al., 2001). Compound-specific ^{13}C and ^{14}C data of phospholipid fatty acids (PLFAs) were proven to reflect the substrate

metabolised by soil microbes (Abraham et al., 1998; Burke et al., 2003; Petsch et al., 2001, 2003).

The analysis of easily degradable substances, like PLFAs, excludes the use of archived pre-bomb samples and thus quantification of turnover rates by tracing bomb-^{14}C in a time interval covering the bomb-spike. However, ^{14}C results for such compounds in recent samples still give information on the contribution of different organic carbon sources: (i) fresh plant residues, with ^{14}C contents corresponding to 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 figure 3.1, and (iii) a smaller pool of 'old', biologically refractory organic components with depleted ^{14}C values (<100 pMC), which may possibly be of fossil origin (Lichtfouse et al., 1997). The objectives of the ^{14}C analyses of lipid compounds described in this chapter were:

- Radiocarbon analysis was applied to nine different lipid compound classes, isolated from the surface soil of the study sites at Halle and at Rotthalmünster, to identify different carbon sources contributing to these fractions and, at the Halle site, the proportion of fossil carbon in certain compound classes. The extraction of these compounds was done by an automated procedure used in organic geochemical studies which is capable of separating complex substrates into clean, well-defined classes.

- Compound-specific radiocarbon analysis (CSRA) of individual microbial-derived PLFAs from fossil carbon contaminated and uncontaminated field trials was used, to identify substrate utilisation by soil microorganisms, playing a key role in the transformation of soil organic carbon. ^{14}C results were compared with relative abundances of the PLFAs at both sites which reflect the presence of certain groups of microbes and the substrate used by these groups.

5.3.1 Identification of organic carbon sources in lipid compound classes

Soil lipids can be extracted and split into different compound classes with organic solvents of different polarity. In this study an automated accelerated solvent extraction was used to extract total lipids from surface soil samples. The subsequent separation into different compound classes, defined by their different polarity was done by a hetero-compound medium pressure liquid chromatography which is described in chapter 4.3.1. The lipid isolation was done by Guido Wiesenberg, Geological Institute,

University of Cologne, as part of his Ph.D. thesis. The ^{14}C concentrations of the different lipid compound classes from soil samples of the maize culture at Rotthalmünster and the continuous rye trial Halle are compared in figure 5.9 and in table 8.7.

^{14}C values of the lipid compound classes from Rotthalmünster soil were close to the modern atmospheric ^{14}C level (figure 5.9-A). The total lipid extract (Ex) was 3.3 ± 0.7 % depleted in ^{14}C compared to the bulk soil (S) (106.5 ± 0.3 pMC). Radiocarbon concentrations of the isolated compound classes ranged from 98.7 ± 0.3 pMC of the low polarity fraction (N), with closely similar values for the acid (H) and basic fraction (Q), to 105.5 ± 0.2 pMC of the high polarity, high molecular weight (HMW) fraction (W). Aliphatic (A) and aromatic hydrocarbons (B), isolated from the low polarity fraction (N), had a low ^{14}C concentration of 43.6 ± 0.3 pMC (A) and 25 ± 1.7 pMC (B). Since the bulk soil of this site had a nearly recent ^{14}C concentration, the low values of the fractions A and B are most probably not caused by fossil carbon but possibly reflect the contribution of microbially synthesised resistant compounds which were also detected by Lichtfouse et al. (1998a). Lichtfouse et al. (1998b) found that *n*-alkanes, synthesised by microbes, are stabilised via physical and/or chemical mechanisms. This may explain the relatively high ^{14}C age of 6670 years BP of the aliphatic hydrocarbon fraction (A) which mainly consists of straight-chain alkanes. However, these ^{14}C depleted fractions represent only a small proportion (ca. 0.5 % (B) to 6.0 % (A)) of the total extract. Main constituents (ca. 85 %) of the low polarity fraction are low polar heterocompounds (C) with a relatively high ^{14}C content of 103.4 ± 0.3 pMC.

In contrast to the rural, uncontaminated Rotthalmünster site, lipid compound classes from surface soil samples of the rye plots at Halle showed highly depleted ^{14}C values (figure 5.9-B). The lowest ^{14}C concentrations were measured for the aliphatic hydrocarbons (A) with 19.4 ± 0.2 pMC (~13,170 years BP) and for the aromatic hydrocarbons (B) having 5.7 ± 0.3 pMC (~21,860 years BP) which were both isolated from the low polarity fraction (N). These values can be attributed to a high contribution of fossil, ^{14}C-free carbon. Wiesenberg et al. (2004a), who compared distribution patterns and $\delta^{13}C$ signatures of *n*-alkane and *n*-carboxylic acids from Halle soil with that of coal from a nearby lignite deposit, identified lignite as source of non-vegetation derived carbon in the aliphatic hydrocarbon and the acid fraction at Halle. Lignite-derived compounds thus may also be responsible for the considerably higher proportion of aliphatic (A: 9.4 %) and aromatic hydrocarbons (B: 1.2 %) of the total

extract (Ex) from Halle soil in comparison to that from Rotthalmünster (A: 6.0 % and B: 0.4 %). ^{14}C concentrations of ca. 55.4 ± 0.4 pMC, measured in the acid (H) and the high molecular fraction (W), reflect a lower contamination of these more functionalised fractions which, however, still include about 50 % of fossil carbon.

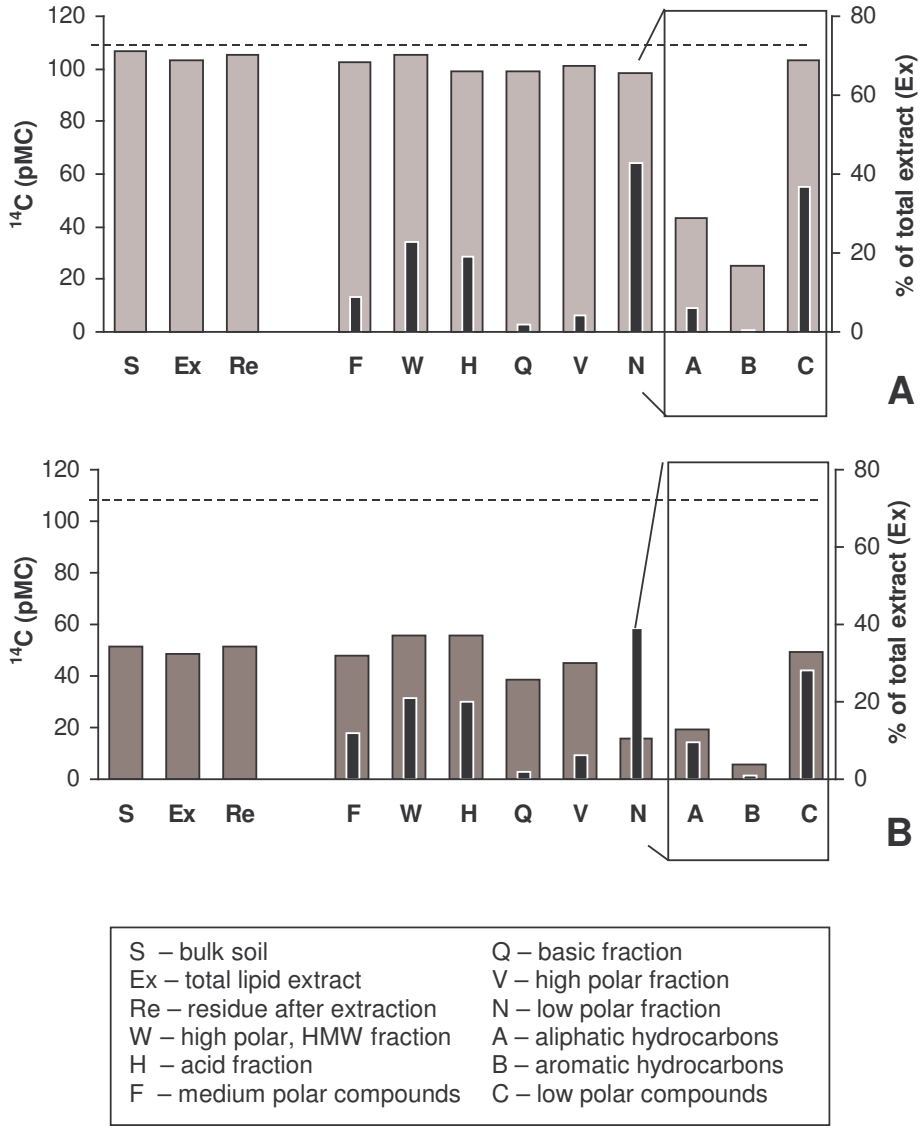

Figure 5.9: ^{14}C values of lipid compound classes from 0-30cm soil depth of (A) the rural site at Rotthalmünster (maize), and (B) the urban site at Halle (rye). The percentage of each compound class of the total extract is shown by the black bars refering to the right axis. Fraction N was separated into fraction A, B and C. The dashed line indicates the atmospheric ^{14}C level in 2002 (A) and 2000 (B; Levin et al., 2003).

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 as well as from anthropogenic sources with quite different ^{14}C

contents. These fractions have shown to be highly susceptible to contaminations such as fossil fuel-derived carbon. To exclude contaminations as observed at the Halle site, it is essential to isolate and analyse compounds at the molecular level.

5.3.2 Microbial substrate usage indicated by ^{14}C values of individual phospholipid fatty acids

Phospholipids are integral components of cell membranes which are composed of a hydrophilic glycerophosphate headgroup and two hydrophobic fatty acid-derived tails. By regulating membrane fluidity they enable the transport of nutrients into the cell and the elimination of metabolic substances (Kaneda, 1991). Results of laboratory experiments showed that intact phospholipids are hydrolysed within weeks after cell death by removal of the phosphate group and thus they are assumed to turn over rapidly (White et al., 1979b; Harvey et al., 1986). Phospholipids are not found in storage lipids or in anthropogenic contaminants (Zelles et al., 1992). Specific fatty acids derived from phospholipids with 14 to 20 carbon atom chain-lengths are characteristic of microorganism groups that produce them (Frostegård et al., 1993; Zelles, 1999). Their occurrence and abundance has been used for the identification of different groups of micoorganisms in soils and marine sediments and their reaction to changed conditions (Baird et al., 1985; Frostegård and Bååth, 1996; Pankhurst et al., 2001; Ponder and Mahasin, 2002). Whereas the straight-chain C16:0 and C18:0 phospholipid fatty acids (PLFAs) are relatively unspecific, since they are found in most bacteria and eukaryotes, the branched-chain, monounsaturated, and cyclopropyl saturated PLFAs are attributed purely to soil bacteria (Kaneda, 1991; Zelles, 1999). The unspecific *n*-C16:0 and *n*-C18:0 PLFAs may also originate from fine roots which were not completely separated from the soil samples. Compound-specific ^{13}C and ^{14}C analysis of individual PLFAs can give information on carbon sources and pathways in soil microorganisms (Abraham et al., 1998; Boschker et al., 1998; Petsch et al., 2001, 2003).

Individual PLFAs were isolated from fresh topsoil samples collected on the wheat and rye cultures at Rotthalmünster (0-35 cm), and on the rye and maize trials at Halle (0-25 cm) by preparative capillary gas-chromatography as described in chapter 4.3.3. The isolation was done by Christiane Kramer at the Max Planck Institute for Biogeochemistry in Jena as part of her Ph.D. thesis. To keep the uncertainty of the ^{14}C results relatively small, the most abundant PLFAs shown in figure 5.10, yielding >70 µg carbon, were isolated for micro-scale AMS ^{14}C measurements.

Since the sample quantities of individual PLFAs were extremely small (40 to 250 µg of carbon), the uncertainties of the AMS ^{14}C results were relatively large, and no statistically significant differences (2 σ-criterion) were visible between PLFAs from the maize and wheat trials at Rotthalmünster and from maize and rye cultures at Halle (table 8.10 and 8.11). Thus, weighted averages of individual PLFAs from both plots of each site were calculated according to:

$$\bar{A} \pm \sigma_{\bar{A}} = (A_1 \cdot 1/\sigma_1^2 + A_2 \cdot 1/\sigma_2^2) / (1/\sigma_1^2 + 1/\sigma_2^2) \pm 1/(1/\sigma_1^2 + 1/\sigma_2^2)^{0.5} \quad (18)$$

where $\bar{A}$ is the weighted average and $\sigma_{\bar{A}}$ its standard error calculated from ^{14}C data of individual PLFAs from (A_1) maize, and (A_2) wheat (Rotthalmünster) and rye (Halle) respectively. σ_1 and σ_2 are the respective standard errors.

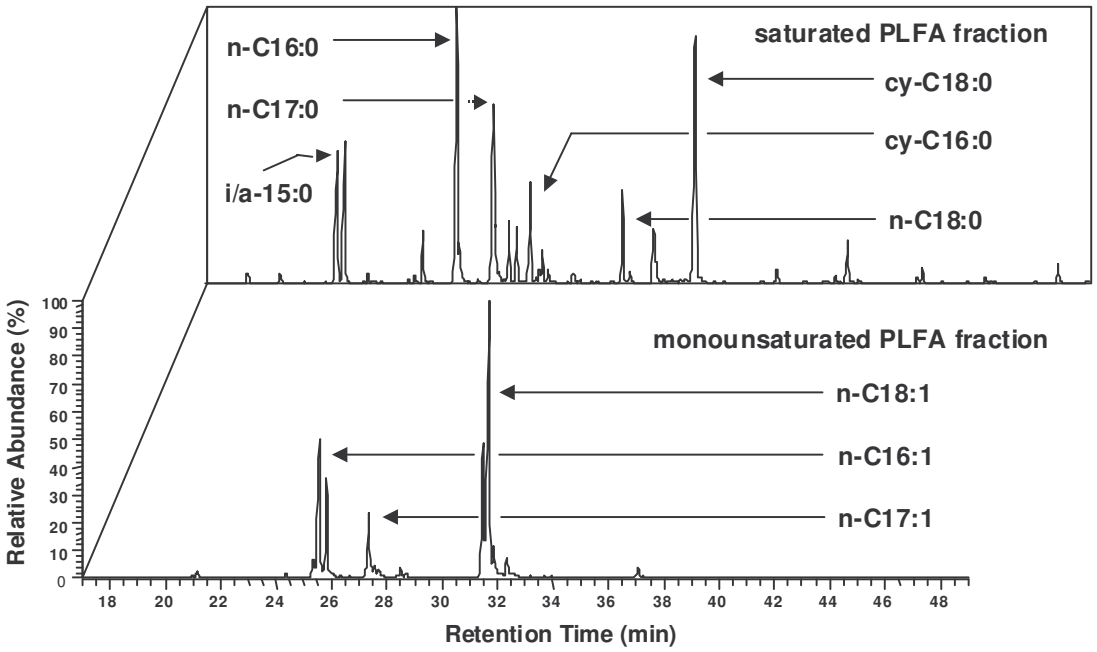

Figure 5.10: Individual PLFAs isolated by preparative capillary gas-chromatography for AMS ^{14}C analysis. PLFAs from the Ap-horizon of the maize trial at Rotthalmünster. Gas-chromatograms from Christiane Kramer. The time axes of the two chromatograms are independent.

The weighted averages of the ^{14}C content of individual PLFAs from Rotthalmünster and Halle topsoil are given with their (reduced) 1-σ measurement uncertainty for each site in figure 5.11, displayed in relation to the atmospheric ^{14}C level of about 108 pMC in 2001. In contrast to the extremely low radiocarbon concentration of the bulk soil (55 pMC) and the phospholipid fraction (53 pMC; figure 5.4) at Halle, the ^{14}C values of its

PLFAs were surprisingly high, ranging from about 93 pMC to 108 pMC. The relatively low radiocarbon contents of saturated PLFAs, particularly of *n*-C17:0 (93.0 ± 0.6 pMC) and *cy*-C18:0 (93.8 ± 0.4 pMC), suggest an assimilation of fossil, most probably lignite-derived carbon by soil microorganisms as suggested by the ^{14}C results of the phospholipid fraction (chapter 5.1.4). This assumption is supported by the findings of Petsch et al. (2001) who identified the microbial uptake of organic carbon from fossil shale by ^{13}C and ^{14}C analysis of individual PLFAs. 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 (equation 16, chapter 5.2.3) to amount to about 18 % in *n*-C17:0 and *cy*-C18:0, and about 13 % in *n*-C18:0, *cy*-C16:0, and *i/a*-C15:0 PLFAs from Halle soil.

The comparison of PLFA ^{14}C values from the topsoil of both study sites revealed considerable differences:

- The saturated PLFAs *i/a*-C15:0, *n*-C16:0, and *cy*-C18:0 at Rotthalmünster yielded ^{14}C levels (111.0 to 113.5 ± 1.0 pMC) significantly (2-σ measurement uncertainty) above that of the atmosphere at the time of soil sampling. This reflects their metabolism of organic material from the last about 40 years which contain relatively high levels of bomb-^{14}C. At Halle an even older carbon source was assimilated as reflected by the relatively low radiocarbon values of *n*-C17:0 and *cy*-C18:0 PLFAs (93.0 ± 1.2 and 93.7 ± 1.5 pMC). These data suggest the incorporation of fossil, supposedly refractory carbon. The *i/a*-C15:0 PLFA yielded a less depleted ^{14}C concentration of 99.8 ± 1.0 pMC.
- The radiocarbon values of the *n*-C18:0 and *cy*-C16:0 PLFAs at Halle and at Rotthalmünster, showing comparable ^{14}C concentrations (98.7 to 104.0 pMC), both were significantly (2-σ criterion; except *n*-C18:0 from Rotthalmünster) below atmospheric radiocarbon level. This suggests the contribution of pre-bomb SOM to these compounds as a fossil carbon contamination is low or absent at Rotthalmünster.
- At both sites, the monounsaturated PLFAs showed no significant difference from each other (2-σ criterion), nor from the atmospheric ^{14}C level at their time of growth. These PLFAs are apparently synthesised exclusively from fresh organic carbon derived from recent photosynthesis.

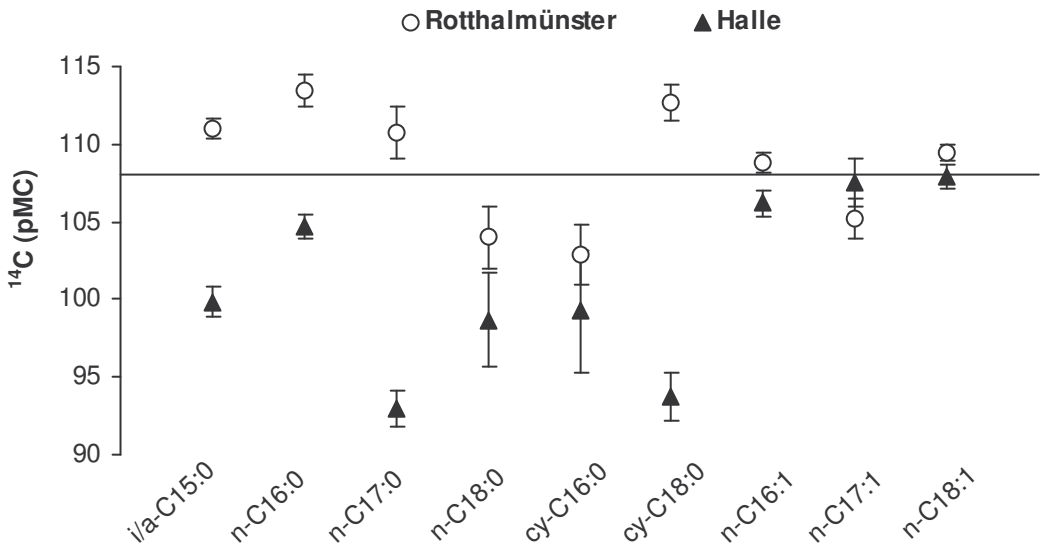

Figure 5.11: Comparison of ^{14}C in individual PLFAs from surface soil samples of trials at Rotthalmünster (0-35 cm depth, average of maize and wheat values) and at Halle (0-25 cm depth, average of maize and rye). The error bars show the 1-σ measurement uncertainties calculated for these averages and 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 2001 (Levin et al., 2003).

All isolated PLFAs yield much higher relative abundances at Halle compared to Rotthalmünster (figure 5.12):

- High relative abundances were determined for the *n*-C16:0 PLFA from the surface soil at Halle as well as at Rotthalmünster which will not be discussed due to its ubiquity and possible origin from plant cell membranes.
- In topsoil samples from Rotthalmünster, the *cy*-C18:0 PLFA yielded a high relative abundance followed by the monounsaturated PLFAs *n*-C16:1 and *n*-C17:1 which all can be attributed to Gram-negative bacteria assuming the predominance of aerobic microbes (Zelles, 1999). Comparing these data with the ^{14}C values of these PLFAs suggests that different microbes are abundant in the topsoil of this site: microbes assimilate recent carbon (monounsaturated) and those using several year old organic substrate (*i*/*a*-C15:0, *cy*-C18:0), characterised by a relatively high contribution of bomb-^{14}C.
- At Halle, high relative abundances were determined for the two monounsaturated PLFAs (Gram-negative biomarkers), which are synthesised from recent carbon sources as shown by their nearly modern ^{14}C values. Contrasting to Rotthalmünster

the saturated PLFAs *i/a*-C15:0 (Gram-positive biomarker; Zelles, 1999) and *cy*-C18:0 showed similar high relative abundances at Halle, which both yielded low ^{14}C levels reflecting the incorporation of relatively old and fossil carbon respectively. These findings agree with the supposed ability of some Gram-positive bacteria to oxidise hydrocarbons (Petsch et al., 2001). The results obtained in the surface soil at Halle indicate the presence of both, microbes using recent organic substrate and, due to the high contribution of fossil carbon to SOM at Halle, microorganisms which are able to assimilate lignite-derived, recalcitrant carbon.

- The comparison of both study sites revealed the largest difference in relative abundance for the *n*-C17:1 PLFA, which however, was not significantly different in ^{14}C on both sites. The PLFAs *i/a*-C15:0 and *cy*-C18:0 yielded considerably higher relative abundances and low ^{14}C concentrations in the Halle soil compared to Rotthalmünster. The data of these saturated PLFAs suggest (i) an increase in microorganisms which are able to assimilate old carbon sources in the plough horizon at Halle and (ii) a lower substrate specificity of the microbes producing these PLFAs.

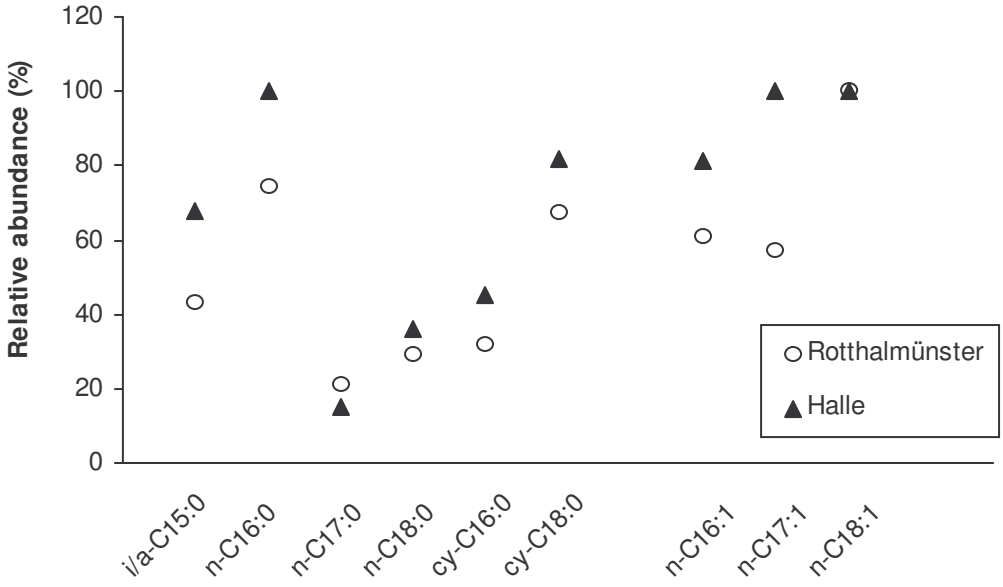

Figure 5.12: Relative abundances of the individual PLFAs (normalised to *n*-C18:1 = 100 %) isolated from surface soil with continuous maize at Rotthalmünster (0-35 cm depth) and at Halle (0-25 cm depth).

5.4 Vertical transformation of organic carbon in soils

Radiocarbon analyses of organic matter fractions as well as of microbial PLFAs were used to investigate the transformation of organic carbon in soil profiles of agricultural long-term experimental sites. The investigations described in this chapter focus on the field trials at Rotthalmünster, since they are not contaminated by fossil carbon and thus make it possible to investigate the dynamics of plant-derived organic carbon in the soil.

As demonstrated by previous investigations, the ^{14}C data of organic matter from different soil horizons or, more detailed, from thin-layers in soil profiles, are helpful to study the transformation of organic carbon. Decreasing ^{14}C concentrations with increasing soil depth are assumed to reflect (i) a lower contribution of recent, photosynthetically-derived organic carbon, (ii) an enrichment of stable organic compounds (Harrison et al., 1993a), but also (iii) a decline of decomposition activity and increased protection of organic matter with depth in the soil (Becker-Heidmann and Scharpenseel, 1986; Scharpenseel and Becker-Heidmann, 1989; Trumbore et al., 1995). Moreover, ^{14}C data of SOM fractions from different soil depths may reflect organic matter transport processes such as bioturbation, leaching, or root growth (Martel and Paul, 1974; Becker-Heidmann and Scharpenseel, 1986; Scharpenseel and Becker-Heidmann, 1989; Trumbore et al., 1989).

Whereas many studies on soil microorganisms focus on the surface soil (0-30 cm), very little is known about a possible large number of subsoil microbes (Ringelberg et al., 1997; Blume et al., 2002), controlling soil carbon transformation. In contrast to surface soils, which are rich in fresh, easily degradable organic material, such as plant debris and root exudates, the carbon input into the subsoil is generally lower and the organic material is more stable as proven by the results shown in figure 5.14. ^{14}C data of individual PLFAs from deeper parts of the soil are expected to reflect these quantitative and qualitative changes of the subsoil organic matter and to yield information on (i) the carbon pools preferentially metabolised at greater depth and (ii) the changes in the microbial groups identifiable by the analysed PLFAs (chapter 5.3.2).

- The AAA-extraction method was applied to bulk soil collected from depth intervals down to about 60 cm soil depth to examine sources contributing to the separated humin and humic acid fraction as well as to identify vertical transport of the organic matter. The comparison of the wheat and maize cultures with the grassland trial, which has not been ploughed since 1961, was intended to provide information on

the effect of soil cultivation on organic matter stability and translocation in the soil profile.

- To identify the availability of organic matter to microbial decomposition in the subsoil as well as the presence of microbial groups, characterised by certain PLFAs, the ^{14}C distribution and relative abundance of individual PLFAs were analysed. The comparison of these PLFA data with those of the surface soil (chapter 5.3.2) is expected to reveal similarities of microbial metabolism, e.g. between the older subsoil organic matter at Rotthalmünster and the fossil carbon contaminated surface soil at Halle.

5.4.1 Depth related ^{14}C distribution of chemical soil fractions

Figure 5.14 and 5.15 and table 8.4 and 8.5 show changes in ^{14}C concentration with increasing depth in the humin and the humic acid fraction extracted from samples which were taken on the different cultures at Rotthalmünster. ^{14}C concentrations of SOM fractions from the surface soil were close to the atmospheric $^{14}CO_2$ level of about 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 both, a low contribution of young compounds and a relative enrichment of resistant organic components in the humin fraction (Rice, 2001) with soil depth and, in consequence, an increasing apparent mean residence time as demonstrated by Trumbore et al. (1989) tracing the infiltration of bomb-^{14}C in SOM fractions. The strong correlation of ^{14}C values of this fraction with the total organic carbon (TOC; John, 2003) content of the bulk soil in the sampled profiles of the trials with wheat, maize, and grassland (figure 5.13), reflects that high TOC contents derive from high portions of recent organic carbon (O'Brien and Stout, 1978). It also indicates a low contribution of recent, plant-derived organic carbon to the humin fraction at greater depth.

The vertical ^{14}C distribution of the humic acid fraction reflects a minor impact of the soil TOC content (all trials: $r^2 = 0.60$, $n = 11$) as indicated by a less intensive ^{14}C decline in deeper parts of the soil. This result suggests the predominantly modern origin of this fraction and moreover, may reflect a downward transport of young, soluble carbon from the surface and/or from root exudates, promoted by soil tillage.

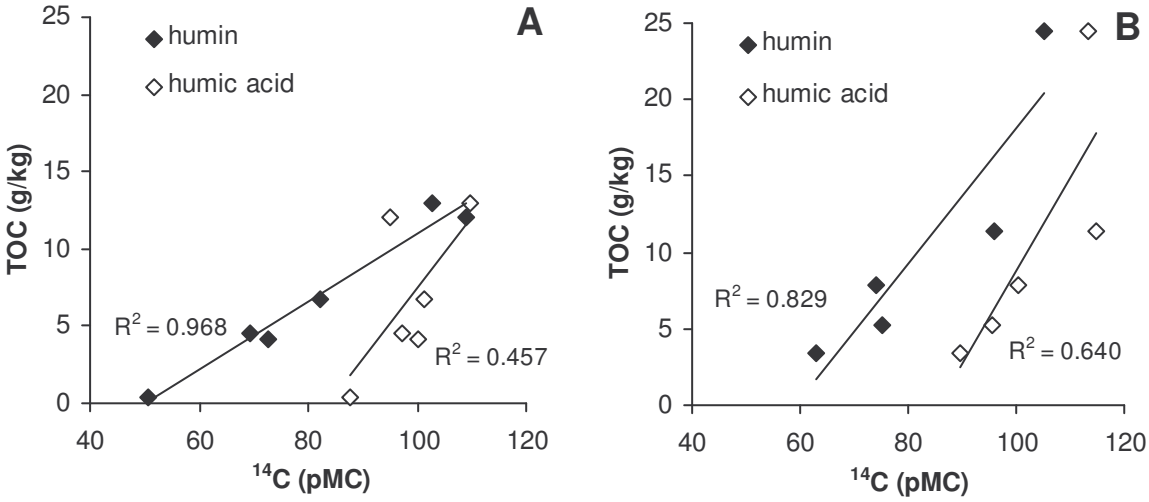

Figure 5.13:Correlation of total soil organic carbon (TOC) with the ^{14}C concentration of humin and humic acid fractions in (A) ploughed (wheat and maize) and (B) no-tillage soils (grassland, both from 0-65 cm depth).

^{14}C distribution of SOM in ploughed soil profiles

On the maize field the ^{14}C concentration of the humin fraction ranged from 102.8 ± 0.3 pMC in the plough horizon (0-35 cm depth) to 72.6 ± 0.4 pMC in 45-60 cm soil depth (figure 5.14). In the ploughed topsoil under continuous wheat cropping a higher value of 109.0 ± 0.3 pMC was determined 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 depth). The relatively high concentrations of ^{14}C and TOC, measured in the topsoil, reflect the large contribution of modern, vegetation-derived organic carbon. Decreased values of both parameters below the plough horizon indicate an enrichment of SOM in more resistant organic compounds. 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 (plough 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, soluble organic carbon (Martel and Paul, 1974), which is most probably translocated downward in the profile by processes such as leaching or bioturbation (Scharpenseel and Becker-Heidmann, 1989; Wang et al., 1999). In contrast, considerably lower values of 95.2 ± 0.4 pMC (plough horizon) to 87.5 ± 0.4 pMC (45-65 cm) were measured on the wheat trial. The results obtained for the subsoil of the wheat culture probably 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., 2004).

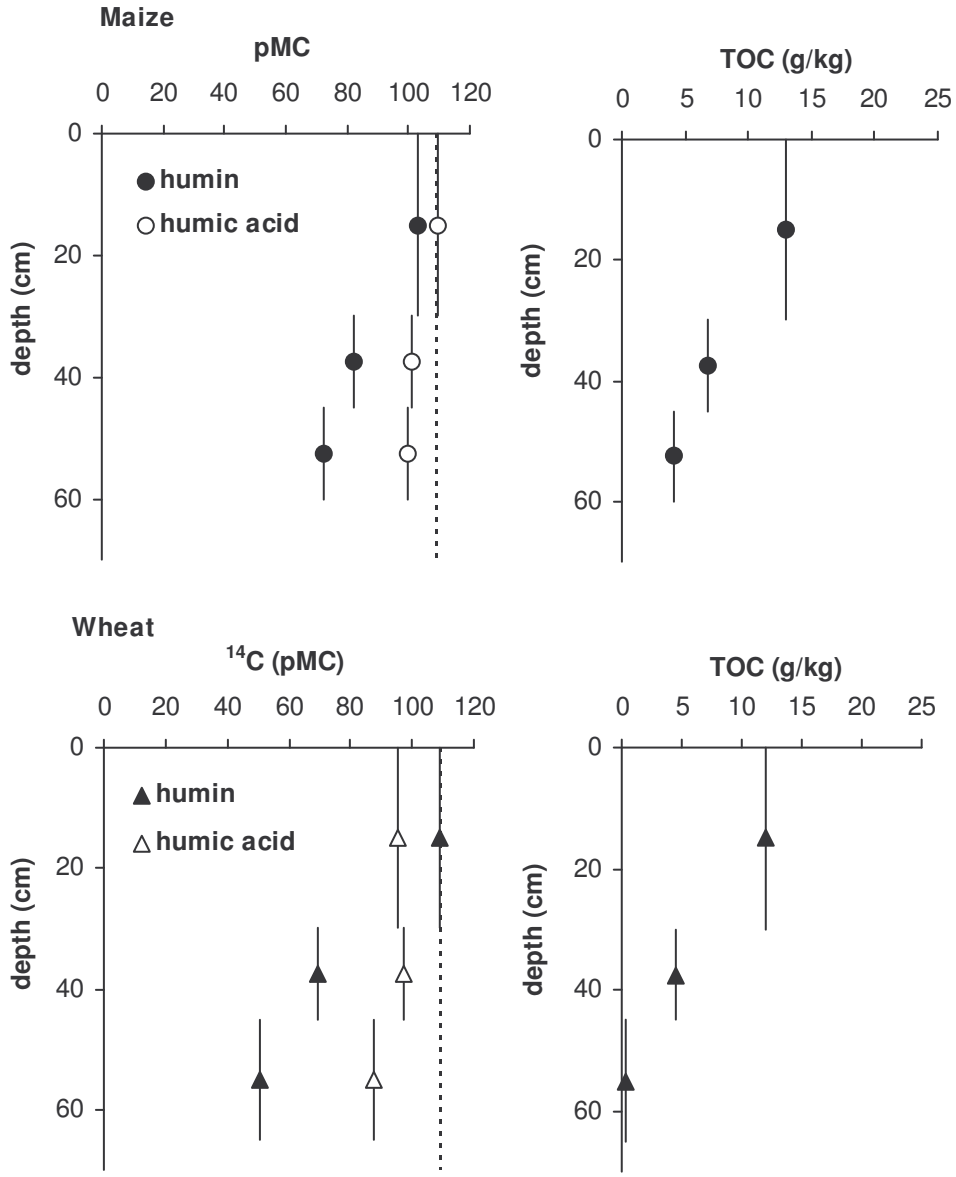

Figure 5.14: ^{14}C concentration of humin and humic acid fractions and total organic carbon content (TOC) of the bulk soil in depth profiles under maize and wheat cultures at Rotthalmünster. The vertical bars represent the sampling interval and the dashed line the atmospheric ^{14}C content in 2002 (Levin et al., 2003). The ^{14}C uncertainty is smaller than the symbol size.

^{14}C distribution of SOM in soil profiles without tillage

The radiocarbon concentrations of the humin fraction extracted from the grassland soil decreased in the upper 30 cm from 105.2 ± 0.3 pMC (0-10 cm depth) to 74.2 ± 0.4 pMC (20-30 cm depth) associated with a decrease in TOC (figure 5.15). The high values of ^{14}C and TOC in the upper 10 cm are assumed to result from the accumulation of plant-derived carbon in the soil during the past 40 years since the site was established. Since the grass is cut about four times per year and the hay is removed from the field, the high TOC content of 24.5 g/kg in 0-10 cm supposedly derives mainly from root biomass.

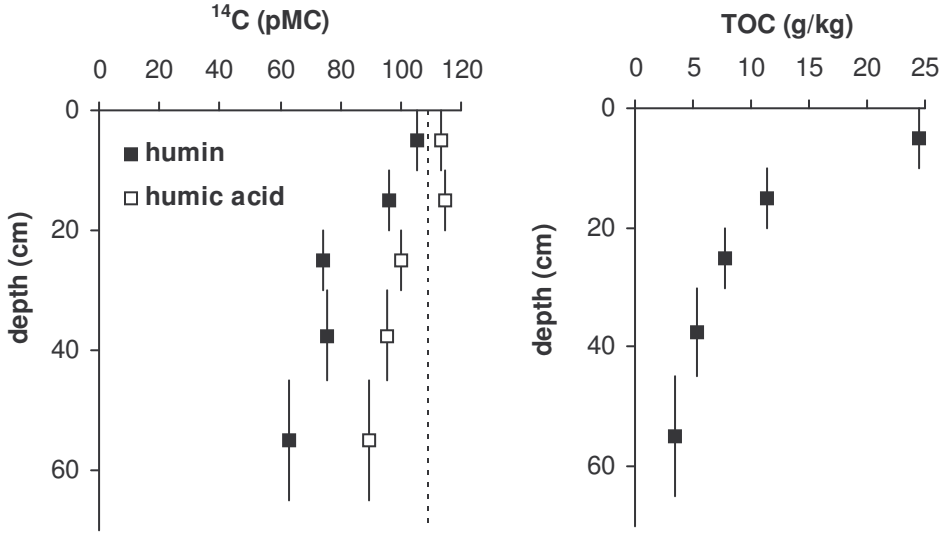

Figure 5.15: ^{14}C values of humin and humic acid fractions and total organic carbon content (TOC) of the bulk soil under grassland at Rotthalmünster. The vertical bars represent the sampling interval and the dashed line the atmospheric ^{14}C level in 2002 (Levin et al., 2003).

The humic acid fraction in 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 about 40 years, with increasing bomb-^{14}C levels back to 1963, to this fraction (figure 3.1). Thus, the humic acid fraction in the grassland topsoil apparently comprised only a small proportion of carbon derived from recent root excudates. The high ^{14}C value may reflect a preferential degradation of young, labile organic compounds by soil microbes, such as dissolved carbohydrates and amino acids, leaving relatively stable organic matter. This was suggested by Kalbitz et al. (2003) who investigated changes in DOM compositions in incubation

experiments. The ^{14}C decline of this fraction below 20 cm depth is comparable to that in the soil cultivated with wheat receiving a lower straw input than the maize culture. This indicates a higher contribution of carbon from microbially transformed humic substances than from recent plant litter.

5.4.2 Distribution of microbial phospholipid fatty acids in surface and subsoils

The ^{14}C distribution of individual PLFAs was studied in soil samples from the plough horizon (0-35 cm depth) and from 35 to 45 cm soil depth of the maize and the wheat monoculture at Rotthalmünster. These data were compared to the results obtained in the fossil carbon contaminated topsoil at Halle (figure 5.17). The PLFAs isolation was limited to a depth of 45 cm due to the low carbon content below the ploughed surface soil (figure 5.14). This required the extraction of 500 g of soil and a time consuming PLFA isolation procedure (up to 50 injections of the phospholipid methyl ester fraction in the PCGC system, each GC run taking about one hour) to obtain about 100 µg carbon of an individual PLFA. Thus it was not possible to isolate PLFAs from below 45 cm soil depth.

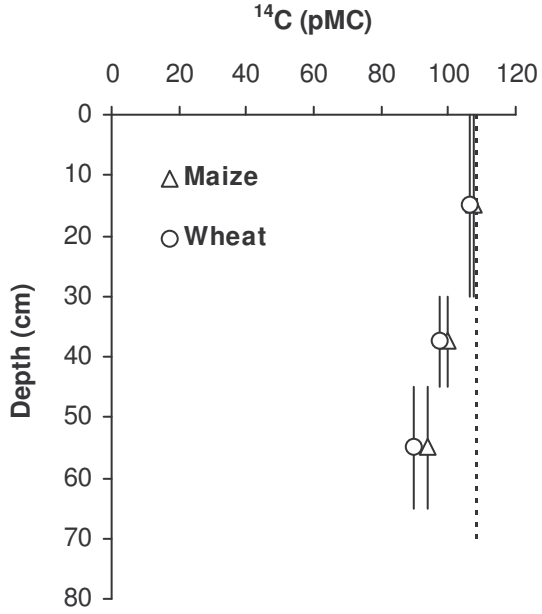

Figure 5.16: ^{14}C distribution of the phospholipid fraction in soil profiles cultivated with wheat and maize at Rotthalmünster (KIA 20168, 20169). The sampling intervals are represented by the bar length and the atmospheric $^{14}CO_2$ level by the dashed line.

The ^{14}C data, given in table 8.9, as well as the vertical ^{14}C distribution of the phospholipid fraction (figure 5.16), indicate no significant difference between both cultures investigated. Thus, weighted average ^{14}C concentrations of the individual PLFAs were calculated from data of the maize and wheat cultures at Rotthalmünster as described in chapter 5.3.2. The vertical ^{14}C distribution of the phospholipid fraction, obtained by solvent extraction and solid phase chromatography, in 0-65 cm soil depth

is comparable to that of the soil humic acid fraction shown in figure 5.15 (maize and wheat) in the previous chapter 5.4.1. This indicates that predominantly young organic compounds contribute to this fraction even at greater soil depth. Assuming the majority of the phospholipid fraction to originate from microbial cell membranes, it also reflects that the vertical transport of DOM provides the carbon taken up by soil microbes (Kalbitz et al., 2000).

Surface soil ^{14}C values of individual PLFAs from Rotthalmünster

In the surface soil (0-35 cm depth) at Rotthalmünster, relatively high ^{14}C concentrations of 104.0 pMC to 113.5 pMC were found for individual PLFAs as described in chapter 5.3.2. The saturated PLFAs *i/a*-C15:0, *n*-C16:0, *cy*-C18:0 showed ^{14}C concentrations significantly (2-σ citerion) above that of the atmospheric $^{14}CO_2$ concentration at the time of soil collection in 2002. The ^{14}C value of the *n*-C17:0 also showed a higher level of bomb-^{14}C than the atmosphere in 2002, but the difference was not statistically significant. This indicates microbial incorporation of organic carbon from the last about 40 years, containing elevated levels of bomb-^{14}C. The *n*-C18:0 (104.0 ± 2.1 pMC), and *cy*-C16:0 (105.4 ± 2.5 pMC) saturated PLFAs yielded levels which were below the atmospheric $^{14}CO_2$ content, most probably reflecting the assimilation of older, pre-bomb organic carbon. The ^{14}C concentrations of the monounsaturated PLFAs were close to the atmospheric ^{14}C level at their time of growth, suggesting a high, maybe exclusive, incorporation of recent organic carbon.

Subsoil ^{14}C values of individual PLFAs from Rotthalmünster

In 35-45 cm soil depth, a decrease in ^{14}C concentration was observed, which was higher for the saturated PLFAs, *i/a*-C15:0, *n*-C16:0, and *n*-C17:0, than for the unbranched monounsaturated PLFAs *n*-C16:1, *n*-C17:1, and *n*-C18:1 (figure 5.17). The *n*-C16:0 (103.2 ± 0.7 pMC) and *n*-C17:0 (101.1 ± 0.8 pMC) PLFAs showed the highest depletion of about 9 % in 35-45 cm soil depth. In contrast the unbranched monounsaturated PLFAs, that yielded radiocarbon concentration close to that of the atmosphere in the surface soil, showed only a small decrease in ^{14}C of about 1.8 % to 3.6 % in the subsoil.

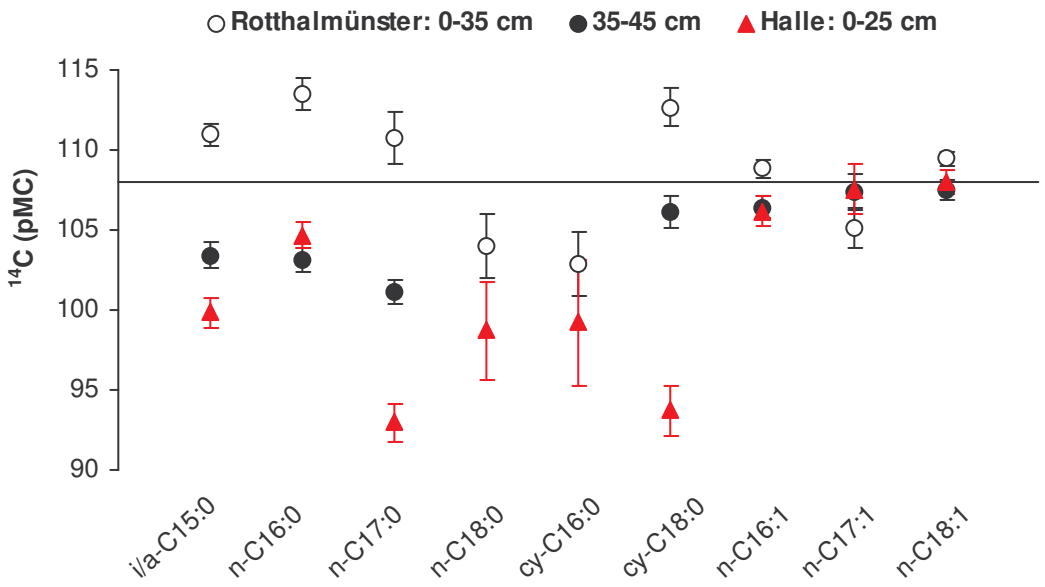

Figure 5.17: ^{14}C concentration of PLFAs from the surface and the subsoil at Rotthalmünster compared with data of the topsoil at Halle. Weighted averages for data of trials with maize and wheat (Rotthalmünster) and maize and rye (Halle) were calculated for each depth. Single values are given for Rotthalmünster, 0-35 cm depth: *n*-C17:0 (wheat), *n*-C17:1 (maize), all unsaturated PLFAs from 35-45 cm depth (maize), and for Halle: *cy*-C16:0 and *n*-C18.0 (maize). The error bars show the 1-σ measurement uncertainties for single values and calculated averages. The x-axis represents the atmospheric $^{14}CO_2$ level in 2002 (Levin et al., 2003).

The pattern of the differences in ^{14}C concentration between surface and subsoil is consistent with the differences in ^{14}C content observed between individual PLFAs in the surface soil:

- The monounsaturated PLFAs are predominantly derived from fresh organic matter, and this continues to be the case below the plough horizon at Rotthalmünster. The high ^{14}C concentrations of the monounsaturated PLFAs in the subsoil suggest the selective use of fresh organic substrate by the producers of these PLFAs (Gram-negative bacteria). This is confirmed by likewise high ^{14}C values of the unsaturated PLFAs in the fossil carbon contaminated surface soil (0-25 cm depth) at Halle, indicating no incorporation of fossil carbon by microbes (chapter 5.3.2). A potential young carbon source is mobile, dissolved organic matter, which may also be responsible for the relatively young humic acid fraction below the plough horizon at Rotthalmünster (chapter 5.4.1). This carbon may derive from root exudates, which are preferentially used by Gram-negative bacteria as shown by ^{13}C-labelling

experiments of Butler et al. (2003) examining the incorporation of rhizodepositions into PLFAs.

- The *i/a*-C15:0, *n*-C16:0, and *n*-C17:0 PLFAs, on the other hand, are synthesised from organic matter that is somewhat older, showing higher bomb-^{14}C values in the surface soil and lower ^{14}C values of older, pre-1954 material in the subsoil at Rotthalmünster. A comparable result was achieved for the fossil carbon contaminated surface soil at Halle reflecting the synthesis of the *n*-C17:0 and *cy*-C18:0 PLFAs from old and fossil carbon respectively (chapter 5.3.2).

In the subsoil at Rotthalmünster an increase in relative abundance was found for the monounsaturated PLFAs *n*-C17:1 and *n*-C16:1 and a minor increase for the saturated PLFAs *i/a*-C15:0 and *n*-C17:0 (figure 5.18).

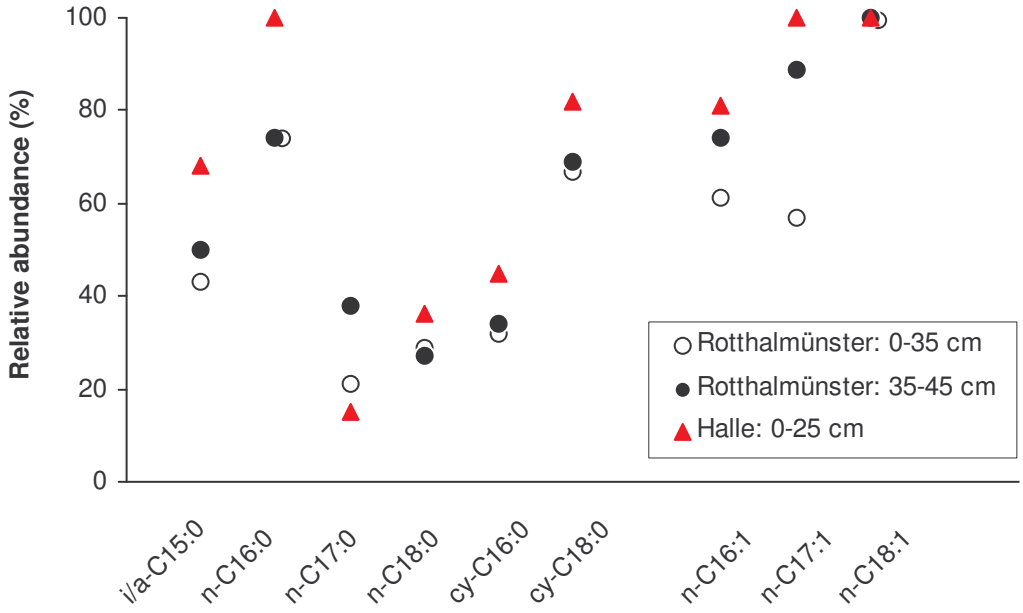

Figure 5.18: Relative abundances of individual PLFAs (normalised to *n*-C18:1 = 100 %) from 0-35 cm and 35-45 cm soil depth at Rotthalmünster compared to data of PLFAs from 0-25 cm depth at Halle. All PLFAs were isolated from soil samples taken on the continuous maize cultures of both sites.

This change in relative abundance and the ^{14}C information of these PLFAs suggest:

- A high relative abundance of microorganisms preferentially assimilating recent, most probably translocated plant and/or root-derived organic carbon, in the subsoil at Rotthalmünster, represented by the monounsaturated PLFAs (Gram-negative bacteria).

- A relative increase of microbes, which use older carbon sources, represented by the two saturated PLFAs (*i*/*a*-C15:0, *n*-C17:0) which are indicators for Gram-positive bacteria. This result is similar to the findings of Fierer et al. (2003), who used principal component analyses of PLFAs from soil profiles, and determined a higher abundance of Gram-positive biomarker PLFAs at greater soil depth but, in contrast, decreased abundances of PLFAs representing Gram-negative bacteria.

Although the subsoil organic matter at Rotthalmünster is considerably younger than the fossil carbon contaminated Halle topsoil (figures 5.2 and 5.14), the data of PLFAs from 35-45 soil depth at Rotthalmünster correspond to the results for PLFAs from the surface soil at Halle and revealed similarities in organic substrate usage of soil microbes (chapter 5.3.2).

5.5 Assessment of physical carbon stabilisation in surface soils

The interaction of organic matter with the mineral soil matrix is considered to be one of the most important controls over the degradation of soil organic carbon (Oades, 1988; Six et al., 2002). Physical protection is thought to reduce access of microbes, which mainly control SOM decomposition, but also of oxygen to SOM and, as a consequence, to lower degradation rates of protected organic carbon (Six et al., 2002). Different physical fractionation methods, such as sieving, sedimentation, or density separation, have been applied to SOM to study these effects (Tisdall and Oades, 1982; Golchin et al., 1994b; Puget et al., 2000; Six et al., 2001). The advantage of these different methods over chemical separation techniques is that they are less destructive, avoiding chemical changes in SOM (Christensen, 2001). Analyses of stable carbon isotopes and a limited number of radiocarbon analyses of physically-defined SOM fractions revealed differences between organic carbon dynamics in these separates and their relation to soil structure (Balesdent et al., 1998; Römkens and Hassink, 1998; Trumbore and Zheng, 1996; Baisdent et al., 2002).

Radiocarbon analyses were applied to organic matter fractions from the surface soil, separated according to particle-size, density, and aggregate-size. The fractionation was done by Bettina John (Institute for Forest Nutrition and Soil Science, University of Göttingen) as part of her Ph.D. thesis. The different methods used are expected to differentiate between organic matter that is located in different positions of the soil structure and thus stabilised by mechanisms of different intensity based on the aggregate hierarchy concept of Tisdall and Oades (1982): Primary organo-mineral complexes, the first stage in aggregate formation, are formed by humic material adhering to clay and silt size soil minerals via persistent chemical stabilisation mechanisms (Baldock and Skjemstadt, 2000). These depend on the mineralogy of the soil particles, in particular of the clay fraction, which is characterised by a large specific surface area and a high effective cation exchange capacity (Baldock and Skjemstad, 2000; Torn et al., 1997). These primary complexes are bound together with silt particles into small microaggregates (<20 µm) to form somewhat larger microaggregates (20-250 µm). Larger macroaggregate (>250 µm) are bound together with microaggregates via less stable, microbially decomposable binding agents such as fungal hyphae, fine roots or microbial-derived compounds (van Veen and Paul, 1981; Oades, 1993; Golchin et al., 1997). Because of the degradability of the binding agents larger aggregates are less stable than microaggregates and are more influenced by

soil cultivation (Tisdall and Oades, 1982). The aims of the analyses described in this chapter are:

- The analysis of ^{14}C concentrations and of maize-derived carbon, based on natural ^{13}C labelling (chapter 3.1.1), in particle-size fractions from the plough horizon at Halle has the objective to identify the effect of primary stabilisation mechanisms on soil carbon dynamics. The radiocarbon data were used to quantify the fossil carbon contamination in each size fraction as well as to correct the results of natural ^{13}C labelling. The comparison of these data with total organic carbon contents is thought to reveal the significance of certain size-fractions for organic carbon stabilisation in cultivated surface soils.

- The difference in ^{14}C content of free particulate organic matter (POM) of low density (<1.6 g/cm^3) and POM occluded in soil aggregates, which were separated after soil dispersion by sonication, is considered to reflect different levels of organic matter association with soil minerals. Density fractions were separated from soil samples of the plough horizon of trials at Halle and at Rotthalmünster.

- The effects of tillage on the stabilisation of organic carbon in soil aggregates is investigated by ^{14}C analysis of water-stable aggregates. Aggregates of different size were separated by wet sieving from cultivated trials and from continuous grassland, which had not been ploughed, at Rotthalmünster.

5.5.1 Carbon dynamics in particle-size fractions

Organic matter associated with soil minerals is less susceptible to microbial degradation than free organic material. The stability of these organo-mineral complexes varies, depending on the grain-size, having different mineralogical properties, and generally increases with decreasing particle-size (Hassink, 1997). Clay and silt size fractions were found to contain highly stabilised organic carbon (Balesdent et al., 1987; Hassink, 1997). To determine the composition and dynamics of organic carbon in six size fraction, separated from the rye and maize monocultures at Halle, total organic carbon contents, $\delta^{13}C$ values (both as well as nitrogen contents measured by Bettina John, University of Göttingen) and ^{14}C concentrations (figure 5.19) were determined. The size fractionation was done by wet sieving and centrifugation after disaggregation of the air-dried soil samples by sonication (chapter 4.2.1). The vegetation change on the maize trial at Halle, which has continuously been cultivated

with rye until 1961, was used to calculate the percentage of maize-derived carbon from the $\delta^{13}C$ data of the particle-size fractions according to equation 12 in chapter 4.5. Furthermore, the proportion of fossil carbon in the size fractions was estimated by their ^{14}C concentrations based on a mass balance calculation (equation 16, chapter 5.2.3).

Carbon distribution in particle-size fractions

The surface soil at Halle consisted of about 70 % sand, 21 % silt and 8 % clay on the rye culture and 65 % sand, 25 % silt and 9 % clay indicating a small loss of soil material during fractionation and small differences in soil texture between both cultures. Whereas the coarse sand fraction contained only about 4 to 5 % (maize, rye) of the soil material, the largest amount of about 60 % was included in medium (200-630 µm) and fine sand (6-200 µm) fractions. All sand-sized fractions accounted for 18 to 21 % (rye, maize) of the total organic carbon, which is twice as much as determined for other arable soils (Christensen, 1992). This may be due to the addition of a large amount of lignite carbon as suggested by the ^{14}C-based estimates which are given in table 5.5. On both cultures the majority of the carbon was present in the medium plus fine silt (2-20 µm) and the clay (<2 µm) fractions (2.6 to 4.4 % C) making up more than 58 to 65 % (maize, rye) of the total organic carbon (figure 5.19). Carbon enrichment in silt and clay fractions is attributed to their large specific surface areas and variable charge (Baldock and Skjemstad, 2000). The high carbon contents of these fine-grained fractions were inversely related to the proportion of soil material in both fractions (Christensen, 2001), each containing only about 10 % of the total soil material. The coarse silt fraction from the maize trial contained about 8 % more carbon compared with the wheat cultivated soil indicating minor differences in soil texture between both cultures.

Radiocarbon distribution in particle-size fractions

The radiocarbon concentrations of particle-size fractions from the maize and rye cultures at Halle ranged from 84 pMC to 19 pMC suggesting a variable contribution of fossil, lignite-derived carbon (chapter 5.2) to these fractions. The sum of the ^{14}C values of all six fractions weighted with their carbon content yields a value of 52.6 pMC, which corresponds well to the bulk soil radiocarbon concentration of 54.5 pMC. This suggests that no considerable changes or losses of SOC occurred during fractionation and sample preparation for AMS measurement. ^{14}C values showed a strong decrease from relatively high concentrations of 84.1 pMC (rye) and 47.6 pMC (maize) in the coarse sand fraction to extremely low values of 23.7 pMC and 31.9 pMC in the fine sand and

coarse silt (20-63 µm) fractions of the rye culture and the medium sand fraction (200-630 µm: 17.6 pMC) on the maize trial at Halle. The silt and clay fractions had almost similar values on both cultures increasing from about 32 pMC in coarse silt to 69.5 pMC in the clay fraction which corresponded to an increase in total organic carbon content in these fractions.

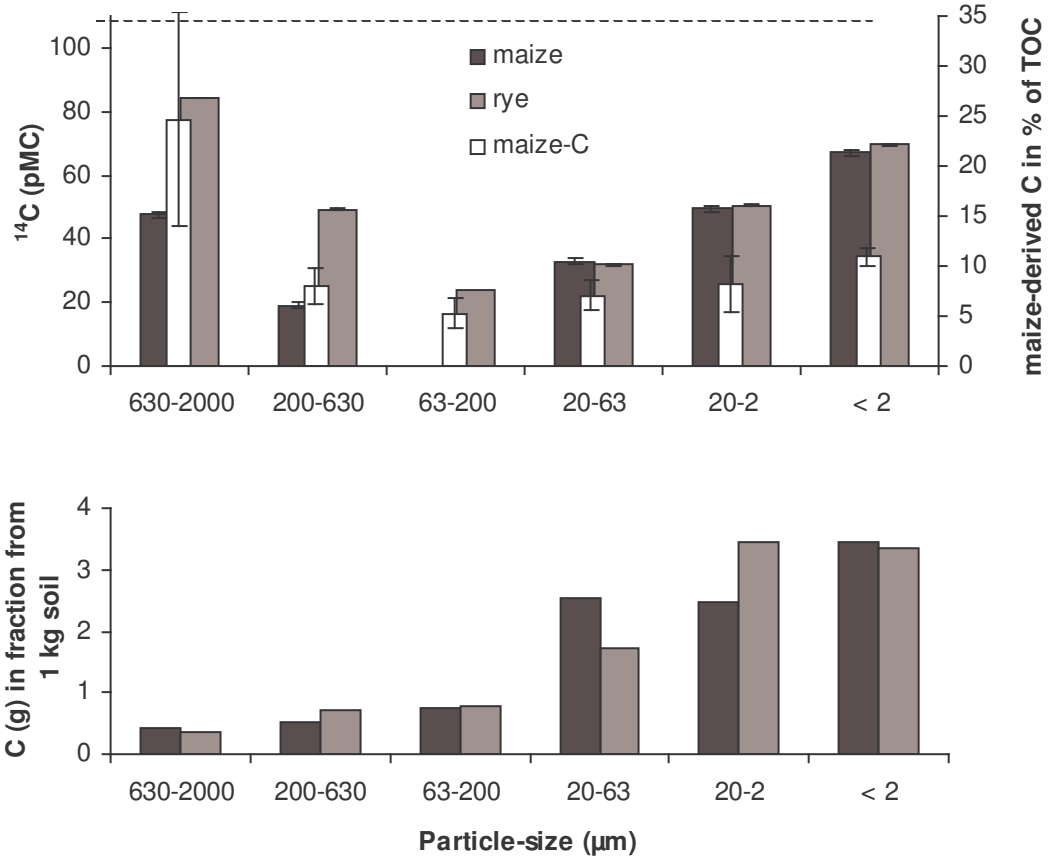

Figure 5.19: Comparison of ^{14}C concentrations with maize-derived C and total organic C (TOC) contents of particle-size fractions from maize and wheat trials (0-25 cm depth) at Halle. The error bars represent 1-σ uncertainty of the AMS measurement and the standard deviation of the maize-derived C, calculated according to equation 13 (chapter 4.5). The atmospheric ^{14}C content in 2000 (Levin et al., 2003) is shown by the dashed line. Maize-derived carbon data and carbon contents from John (2003).

In comparison to the rye culture, a considerably lower ^{14}C concentration was determined for the sand fractions from the surface soil cultivated with maize. This most probably results from the contribution of black particles, which were analysed separately, yielding an extremely low ^{14}C value of 0.9 ± 0.1 pMC in coarse- and 4.4 ± 0.1 pMC in medium-grained sand. The coarse sand fraction showed a considerably

higher C/N ratio of 42 under maize compared to 28 under rye cropping, which suggests a higher proportion of material rich in C and low in N content in this fraction from the soil with maize cultivation. The relatively high C/N ratio may indicate either, a higher contribution of plant residues (C/N straw: 50-100; leaves and roots: 30-55; Scheffer and Schachtschabel, 1998) or the addition of organic material derived from fossil fuels (C/N: >50) or vegetation fires (C/N: ~40; Fernandes et al., 2003).

The extremely high apparent mean residence times of carbon in the fine sand and coarse silt fractions of roughly 9,000 and 11,500 years (maize, rye) respectively suggest a high input of up to 79 % fossil fuel-derived carbon (table 5.5). Moreover, these fractions showed relatively high C/N ratios of about 20 to 28 as well as low O/C ratios (chapter 5.2.1) of 0.16 which is characteristic of highly carbonised material. The latter was determined in a 63-250 µm particle-size fraction from the surface soil at Halle (Brodowski, 2004). These data and results of Goldberg (1985) and Schmidt et al. (1996) suggest a large quantity of 20-200 µm sized, airborne lignite particles in SOM and their transport over several km distance. The old material is presumably strongly associated with soil minerals and thus difficult to separate as indicated by an unsuccessful test to separate the material via sonication and density fractionation.

The highest ^{14}C content of about 70 pMC, i.e. a low apparent mean residence time of about 3,000 years - except of 84 pMC determined for 2.0-0.63 mm, wheat - was obtained for the clay fraction from the topsoil of both cultures. It implies on the one hand a lower contamination by fossil carbon, which however, still amounts to about 40 %. On the other hand this ^{14}C value suggests the contribution of relatively young organic carbon and its apparent fast turnover, which was previously observed by Anderson and Paul (1984) for fine clay associated organic matter via ^{14}C analysis. A C/N ratio of about 9.6, being the lowest of all size fractions (Christensen, 1992) and typical of microbial biomass, indicates a high transformation (i.e. loss of carbon upon oxidation by soil microbes) of the organic matter associated with <2 µm particles. This agrees with results of Guggenberger et al. (1995) who examined the composition of SOM in different particle-size fractions and found increased decomposition of the organic matter with decreasing particle-size. The small C/N ratio as well as results of previous investigations indicate a high microbial activity and the accumulation and stabilisation of microbial products, such as microbial polysaccharides and amino sugars, in the clay fraction (Six et al., 2001; Schmidt and Kögel-Knabner, 2002; Kiem and Kögel-Knabner, 2003). The stabilisation of microbial products is thought to occur via interactions with clay-sized mineral surfaces as indicated by the relationship

between polysaccharide carbon and high specific surface area (Kiem and Kögel-Knabner, 2003). Poll et al. (2004), examining microbial biomass and microbial activity parameters in the topsoil of the rye culture at Halle, determined the highest proportion of microbial biomass in the silt and clay fractions. Moreover, data of Laird et al. (2001) showed that microbial fatty acids are major components of the clay fraction in agricultural soils, making up about 12 % of the organic carbon in this fraction. These fatty acids comprised high proportions of fungal and bacterial biomarkers.

Table 5.5: Distribution of organic carbon, radiocarbon, and fossil carbon in particle-size fractions from the rye and maize trials (0-25 cm depth) at Halle.

Fraction (µm)	C (g) from 1 kg soil [1]	^{14}C (pMC)	Fossil C [2] (%)	C (g) from 1 kg soil [1]	^{14}C (pMC)	Fossil C [2] (%)
	rye*			maize*		
630-2000	0.34	84.1 ± 0.2	25.9	0.41	47.6 ± 0.2	58.0
200-630	0.72	49.2 ± 0.2	56.7	0.52	19.1 ± 0.2	83.2
63-200	0.78	23.7 ± 0.2	79.1	0.76	---	---
20-63	1.73	31.9 ± 0.2	71.9	2.54	32.8 ± 0.2	71.2
2-20	3.46	50.6 ± 0.2	55.4	2.48	49.6 ± 0.2	56.3
< 2	3.35	69.5 ± 0.3	38.8	3.45	67.2 ± 0.2	40.8

[1] Data from John (2003)
[2] $^{14}C_{recent}$ = 113.5 pMC, calculated according to equation 17, chapter 5.2.2
[*] Rye: KIA 15531, maize: KIA 18404

Distribution of maize-derived carbon

The contribution of carbon derived from maize, which has been cultivated on the former rye trial since 1961, to the particle-size fractions in the plough horizon of the maize culture showed a similar strong decline from coarse to fine sand as observed for ^{14}C (figure 5.19). The highest proportion of 24.7 % maize-derived carbon, calculated from $\delta^{13}C$ values, was found in the coarse sand fraction. This value reflects a high input of recent crop residues and a relatively fast turnover of organic material in this size class (table 5.6). Apparently only 5.3 % maize-derived carbon was accumulated in fine sand which suggests an extremely slow rate (>700 years) of carbon cycling. The proportion of maize-derived carbon showed a less pronounced increase from coarse silt to clay than the distribution of ^{14}C in these fine-grained fractions, indicating that only part of the old C_3-label (rye) was replaced by the new C_4-signature (maize).

However, the ^{13}C-based data are considered to be misleading due to the high proportion of lignite carbon in the soil at Halle (chapter 5.2). The presence and continuous addition of the fossil material, which has a C_3 isotopic signature (table 5.3), most probably led to a drastic underestimation of the contribution of the new C_4 vegetation to natural SOM. In consequence, unrealistically low turnover rates of carbon in particle-size fractions from the maize trial were calculated, based on the natural ^{13}C labelling method as defined in equation 14 in chapter 4.5. These ranged from 138 years (coarse sand) to 718 years (fine sand; table 5.6). Hence, the proportion of fossil carbon, estimated by the ^{14}C data of the particle-size fractions (table 5.5), was used to correct the underestimated accumulation of maize carbon in each size fraction according to equations 19 to 21.

$$f^*_{bulk} = \frac{\delta^{13}C_{C4-soil} - X_{C4-soil} \times \delta^{13}C_{coal} - (1 - X_{C4-soil}) \times \delta^{13}C_{C3-soil}{}^*}{\delta^{13}C_{C4-plant} - \delta^{13}C_{C3-soil}{}^*} \times 100 \qquad (19)$$

with:

f^*_{bulk}: the proportion of maize-derived C (%) corrected for the fossil C contribution in the C_4-cropped soil and related to the total soil organic carbon in each fraction

$X_{C4\text{-}soil}$: the proportion of fossil C (%) in each fraction of the C_4-cropped soil (estimated from ^{14}C values of the respective fraction: equation 16 and 17, chapter 5.2.2)

$\delta^{13}C_{C3\text{-}soil}{}^*$: the $\delta^{13}C$ value of the soil cultivated with C_3 crops, corrected for the fossil C contribution (‰ PDB; equation 20)

$\delta^{13}C_{C4\text{-}soil}$: the $\delta^{13}C$ value of the fossil C contaminated soil cultivated with C_4 crops (‰ PDB)

$\delta^{13}C_{coal}$: the $\delta^{13}C$ value of lignite particles (‰ PDB; table 5.3)

$\delta^{13}C_{C4\text{-}plant}$: the $\delta^{13}C$ value of the C_4 plants (‰ PDB; table 4.6)

The $\delta^{13}C$ value of coal ($\delta^{13}C_{coal}$) was derived from the $\delta^{13}C$ values of the selected black particles given in table 5.3 (chapter 5.2.1). The average $\delta^{13}C$ values of -25.1 ‰ PDB corresponds to data of Wiesenberg et al. (2004a) who determined a $\delta^{13}C$ of -25.0 ‰ PDB and of -25.8 ‰ PDB for a briquette and for lignite taken from the main seam of a nearby mine at Beuna.

The corrected $\delta^{13}C$ value of the soil cultivated with C_3 crops ($\delta^{13}C_{C3\text{-}soil}$*) was obtained by mass balance calculation as:

$$\delta^{13}C_{C3-soil}{}^{*} = \frac{\delta^{13}C_{C3-soil} - X_{C3-soil} \times \delta^{13}C_{coal}}{1 - X_{C3-soil}} \qquad (20)$$

with:

$\delta^{13}C_{C3\text{-}soil}$: the $\delta^{13}C$ value of the soil cultivated with C_3-crops (‰ PDB)

$\delta^{13}C_{coal}$: the $\delta^{13}C$ value of lignite particles (‰ PDB)

$X_{C3\text{-}soil}$: the proportion of fossil C (%) in each fraction of the C_3-cropped soil (estimated from ^{14}C values of the respective fraction: equation 16)

The proportion of maize-derived carbon (f^*_{bulk}) thus corrected is related to the total organic carbon in the soil, including carbon derived from lignite and from C_3 and C_4 crops. To relate the corrected f^*_{bulk} value to the amount of carbon only derived from plant material, f^*_{free}, the lignite-derived carbon is subtracted from the total amount of soil organic carbon as:

$$f^*_{free} = f^*_{bulk} / (1 - X_{C4\text{-}soil}) \qquad (21)$$

with:

f^*_{free}: the proportion of maize-derived C (%) related to SOC without lignite C

$X_{C4\text{-}soil}$: the proportion of fossil C (%) in each fraction from the C_4-cropped soil (estimated from ^{14}C values of the respective fraction: equation 16)

The corrected proportions of maize-derived carbon seem to be more realistic and indicate decreasing proportions of maize-derived carbon with decreasing particle size (table 5.6). A contribution of 59.2 % and 50.1 % maize carbon in the coarse and medium-sized sand fractions respectively, which is about 35 to 42 % higher than the uncorrected value, suggests a relatively fast turnover of carbon within about 40 to 60 years. This indicates a low stabilisation of organic carbon associated with these large soil particles, which is probably caused by soil tillage destroying macroaggregates and preventing organic matter to form stable associations with soil minerals (chapter 5.5.3). The silt and clay fractions yielded considerably lower, corrected amounts of about 21 % maize carbon and a slow turnover time of 170 and 160 years respectively. Since the medium plus fine silt and the clay fraction, which stored more than half of the

total soil organic carbon, seems highly protected by interactions with the soil minerals, these fractions are most important for carbon sequestration in cultivated surface soils.

Table 5.6: Distribution of $\delta^{13}C$ and maize-derived carbon in particle-size fractions from rye and maize cultivated surface soils at Halle.

Fraction (µm)	$\delta^{13}C$ [1] (‰ PDB)	$\delta^{13}C$ [1] (‰ PDB)	Maize C [2] (%)	Turnover [2] (years)	Maize $C_{corr.}$ [3] (%)	$Turnover_{corr.}$ [3] (years)
	rye			maize		
630-2000	-26.2 ± 1.6	-22.0 ± 0.8	24.7 ± 5.3	138	59.2	43
200-630	-25.3 ± 0.1	-24.0 ± 0.3	8.1 ± 0.9	465	50.1	56
63-200	-24.9 ± 0.2	-24.0 ± 0.2	5.3 ± 0.8	718	---	
20-63	-25.2 ± 0.2	-24.0 ± 0.0	7.1 ± 0.7	531	30.1	109
2-20	-25.5 ± 0.1	-24.2 ± 0.5	8.2 ± 1.4	454	20.5	170
< 2	-25.4 ± 0.1	-23.6 ± 1.0	10.9 ± 0.4	337	21.6	160

[1] $\delta^{13}C$ data from John (2003).

[2] Maize-derived C and turnover times calculated from $\delta^{13}C$ values with $\delta^{13}C_{C3\text{-}plant}$ = -28.4 ‰ PDB and $\delta^{13}C_{C4\text{-}plant}$ = -11.6 ‰ PDB (equations 12 and 14, chapter 4.5).

[3] Maize-derived C and turnover times corrected for fossil C contribution (table 5.5) as in equations 19 to 21.

5.5.2 Organic carbon turnover in density fractions

The location of organic matter in the secondary soil structure, i.e. in aggregated organo-mineral complexes (= aggregates), is a further mechanism that limits microbial or oxidative degradation of organic carbon. Intra-aggregate organic matter is physically stabilised by incorporation within macroaggregates, while free organic matter is located between aggregates (Golchin et al., 1994a; Six et al., 1998) and thus more easily accessible for soil microorganisms and oxygen diffusion.

To characterise the stability of organic matter located between and within soil aggregates, ^{14}C concentrations of four density fraction, obtained by separation with sodium polytungstate of 1.6 g/cm³ density, were determined (figure 5.20). Free particulate organic matter ($fPOM_{<1.6}$) was recovered from the supernatant of the density solution after centrifugation. The light occluded particulate organic matter ($oPOM_{<1.6}$) and heavy $oPOM_{1.6\text{-}2.0}$ were separated after the dispersion of soil aggregates by sonication followed by centrifugation as described in detail in chapter 4.2.2. The $mineral\ fraction_{>2.0}$ is the remainder of this procedure. The density fractionation was applied to soil sampled in the plough horizon of the study sites at Rotthalmünster (0-35 cm depth: maize and wheat) and at Halle (0-25 cm depth: maize and rye).

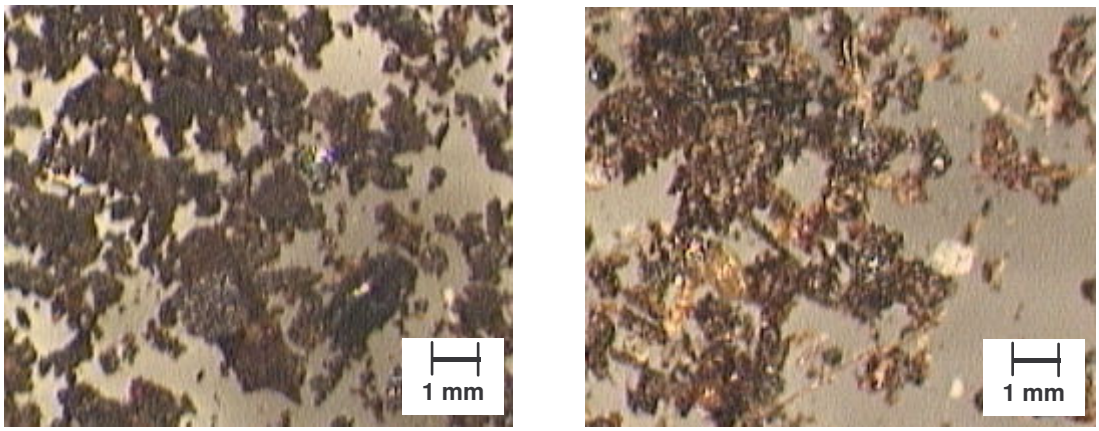

Figure 5.20: Photographs of the light occluded particulate organic matter (<1.6 g/cm³) from the plough horizon of the maize cultures at Halle (left) and at Rotthalmünster (right).

The inspection of the density fractions by light microscopy showed that the $fPOM_{<1.6}$ fraction contained relatively large plant residues and coarse organic material. In contrast, the light occluded particulate organic matter fraction ($oPOM_{<1.6}$) had a much darker colour and looked more homogeneous, consisting of fine organic material with few identifiable plant structures. The $oPOM_{<1.6}$ fraction from the soil at Halle compared to Rotthalmünster soil showed a considerable difference in colour (figure 5.20). Investigations of Golchin et al. (1994a, 1994c), who used ^{13}C and NMR analysis, and John et al. (2004), who applied natural ^{13}C labelling, indicate a higher degree of degradation of the occluded (light $oPOM_{<1.6}$ > heavy $oPOM_{1.6\text{-}2.0}$) compared to the free particulate organic matter.

^{14}C distribution in density fractions from Rotthalmünster

The ^{14}C values of the mineral, $fPOM_{<1.6}$, and $oPOM_{1.6\text{-}2.0}$ fractions from surface soil sampled on the maize and wheat trials at Rotthalmünster ranged from 102.7 to 107.7 pMC (figure 5.21, table 5.7). The ^{14}C results for presumably mineral-free $fPOM_{<1.6}$, which is thought to be mainly composed of fresh to slightly decomposed plant residues located between aggregates (Golchin et al., 1994a), 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 microbially transformed organic matter by occlusion in soil aggregates as also indicated by the results of ^{13}C studies by Golchin et al. (1994a). However, as pointed out by John et al. (2004), turnover rates derived from carbon isotopes do not give information on

the mechanisms responsible for physical SOC protection. Since only a small proportion of the total organic carbon (figure 5.21) was contained in the light oPOM fraction (John et al., 2004), total carbon cycling in the maize and wheat surface soils is dominated by the faster turning organic matter fractions.

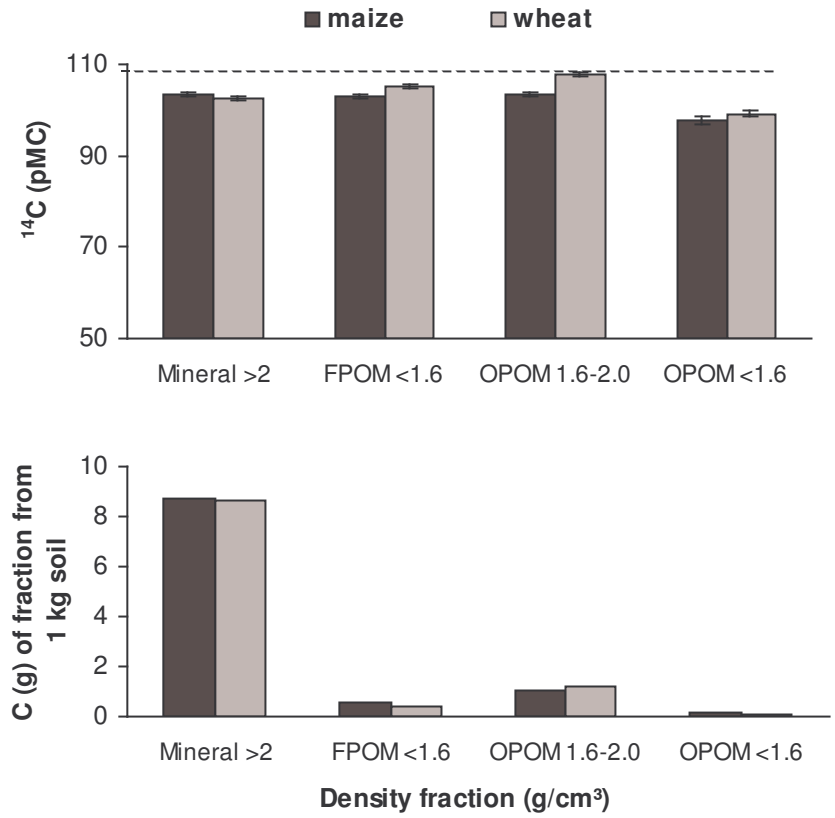

Figure 5.21: ^{14}C and organic carbon contents of free particulate organic matter (fPOM), occluded POM, and a mineral fraction separated by density fractionation from topsoil samples (0-30 cm depth) of maize and wheat trials at Rotthalmünster. The error bars represent the 1-σ measurement uncertainty and the dashed line the atmospheric ^{14}C level in 2002 (Levin et al., 2003).

The $oPOM_{1.6\text{-}2.0}$ fraction, which contains organic components working as binding-agents between aggregates (Golchin et al., 1994b), showed the highest ^{14}C values on both cultures. These results agree with the higher apparent turnover rate (50 years) determined for the heavy $oPOM_{1.6\text{-}2.0}$ fraction under maize from ^{13}C data by John et al. (2004) compared to a considerably slower turnover of the light oPOM (84 years; table 5.7). The much higher amount of organic carbon of about 10 to 12 % (wheat, maize) stored in this fraction emphasizes the importance of the heavy oPOM fraction for organic carbon storage in cultivated soils.

Table 5.7: ^{14}C and maize-derived carbon distribution in density fraction from the maize trial (0-30 cm depth) at Rotthalmünster.

Density fraction (g/cm³)	^{14}C (pMC)	^{14}C (pMC)	Maize C [1] (%)	Turnover [1] (years)
	wheat*	maize*		
$Mineral_{>2.0}$	102. ± 0.4	103.5 ± 0.3	31.12 ± 3.0	64
$fPOM_{<1.6}$	105.4 ± 0.5	102.9 ± 0.5	60.28 ± 3.8	26
$oPOM_{1.6-2.0}$	107.7 ± 0.3	103.5 ± 0.3	38.3 ± 2.7	50
$oPOM_{<1.6}$	99.3 ± 0.9	97.6 ± 0.8	24.8 ± 3.9	84

[1] Maize-derived C and turnover times calculated from $\delta^{13}C$ values (John, 2003) with $\delta^{13}C_{C3\text{-}plant}$ = -26.8 ‰ PDB, and $\delta^{13}C_{C4\text{-}plant}$ = -12.7 ‰ PDB (equations 12 and 14, chapter 4.5).
* Wheat: KIA 19936, maize: KIA 19937.

^{14}C distribution in density fractions from Halle

The data obtained at Rotthalmünster were compared with measurements of density fractions separated from rye and maize cultures at the Halle study site.

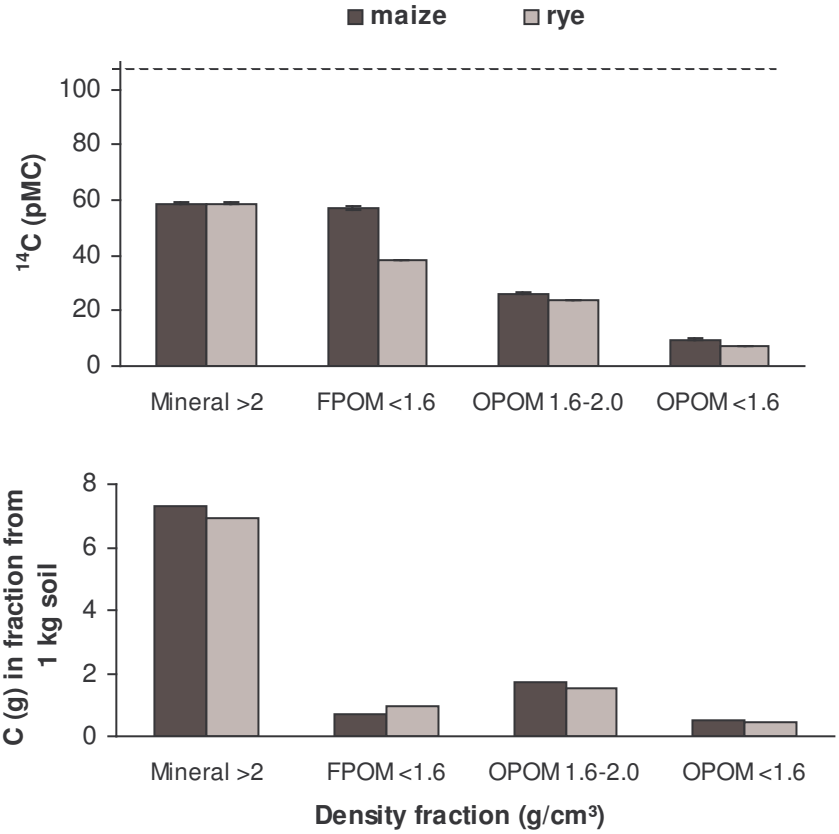

Figure 5.22: ^{14}C and carbon contents of density fractionated free particulate organic matter (fPOM), and occluded POM, and a mineral fraction from topsoil samples (0-30 cm depth) of field trials at Halle. The atmospheric ^{14}C level in 2000 (Levin et al., 2003) is represented by the dashed line.

The low radiocarbon values of all fractions (figure 5.22) are caused by the contribution of up to 94 % fossil carbon (table 5.8) to the separated organic matter. The mineral >2.0 g/cm³ fraction had a similar ^{14}C concentration of 58.9 ± 0.2 pMC under maize and rye cultivation, exceeding that of the bulk surface soil (54.5 pMC, ~ 4,880 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), suggesting a high but variable contribution of fossil carbon to this otherwise 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 $oPOM_{1.6-2.0}$ fraction yielded ^{14}C values of 23.9 ± 0.2 pMC (rye) and 26.3 ± 0.2 pMC (maize), 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 $oPOM_{<1.6}$, containing the highest proportion of fossil carbon. This apparent enrichment of fossil carbon in the oPOM fractions confirms the assumed protective potential of soil aggregates. This interpretation is substantiated by a much higher amount of carbon, particularly in $oPOM_{<1.6}$ comprising about 5 % of the total organic carbon compared to only 0. 6 to 1.4 % in this fraction at Rotthalmünster (wheat and maize). However, the low ^{14}C content of the light oPOM fraction may also indicate a preferential enrichment of the lignite-derived carbon, due to the low density of the particles (Trumbore and Zheng, 1996).

Table 5.8: ^{14}C and maize-derived carbon distribution in density fractions from rye and maize cultures (0-25 cm) at Halle.

Fraction (µm)	^{14}C (pMC)	Fossil C [1] (%)	^{14}C (pMC)	Fossil C [1] (%)
	rye*		maize*	
$Mineral_{>2.0}$	58.9 ± 0.2	48.1	58.9 ± 0.2	48.1
$fPOM_{<1.6}$	38.5 ± 0.2	66.1	57.2 ± 0.4	49.6
$oPOM_{1.6-2.0}$	23.9 ± 0.2	79.0	26.3 ± 0.2	76.9
$oPOM_{<1.6}$	7.1 ± 0.1	93.7	9.6 ± 0.2	91.5

[1] Calculated according to equation 16, chapter 5.2.2 with $^{14}C_{recent}$ = 113.5 pMC.
* Rye: KIA 19634, maize: KIA 19635.

As previously shown for the particle-size fractions (chapter 5.5.1), the fossil carbon contamination resulted in a drastic overestimation of carbon stability. Again ^{14}C-based estimates of fossil carbon in each density fraction were used to correct the amount of maize-derived carbon which accumulated in the respective fraction after the vegetation change from rye to maize cropping in 1961 (table 5.9). The data corrected according to equations 19 to 21 indicate a much higher input of maize-derived carbon to all density

fractions. The shortest turnover time of about 50 and 80 years were determined for organic carbon in the $fPOM_{<1.6}$ and the $oPOM_{1.6-2.0}$ fraction respectively. The $oPOM_{<1.6}$ fraction yielded the lowest proportion of maize-derived carbon and highest turnover time, which, however, contains only a minor amount of total soil organic carbon. Most of the organic carbon was stored in the $mineral_{>2}$ fraction which was found to turn over within 126 years and thus is most important with respect to soil carbon sequestration. Although the corrected ^{13}C-based turnover times at Halle were still about twice as high as at Rotthalmünster, which most probably is caused by differences in soil properties as well as in climate (chapters 4.1.1 and 4.1.2), the distribution of maize-derived carbon in the separated density fractions at Halle was comparable to that of Rotthalmünster.

Table 5.9: Distribution of $\delta^{13}C$, maize-derived carbon in density fractions of rye and maize trials at Halle.

Fraction (µm)	$\delta^{13}C$ [1] (‰ PDB)	$\delta^{13}C$ [1] (‰ PDB)	Maize C [1] (%)	Turnover[2] (years)	Maize $C_{corr.}$[3] (%)	$Turnover_{corr.}$[3] (years)
	rye			maize		
$Mineral_{>2.0}$	-25.6 ± 0.1	-23.6 ± 0.0	11.4 ± 0.5	322	26.6	126
$fPOM_{<1.6}$	-26.2 ± 0.4	-22.3 ± 0.7	22.9 ± 5.0	150	52.6	52
$oPOM_{1.6-2.0}$	-25.2 ± 0.1	-24.0 ± 0.3	7.4 ± 2.0	507	37.4	83
$oPOM_{<1.6}$	-25.7 ± 0.1	-25.5 ± 0.1	1.3 ± 0.6	2980	21.0	166

[1] $\delta^{13}C$ and maize-derived C data from John (2003).
[2] Turnover times calculated from percentage of maize-C (equation 14, chapter 4.5).
[3] Values corrected for fossil C contribution (estimated from ^{14}C data: equation 16) with $\delta^{13}C_{coal}$ = -25.1 ‰ PDB, and $\delta^{13}C_{C4\text{-}plant}$ = -11.6 ‰ PDB (equation 19 to 21, chapter 5.5.1).

5.5.3 Carbon dynamics in water-stable aggregates

As shown by Tisdall and Oades (1982), aggregate structures can be related to their water stability. The wet sieving procedure, which is used to separate water-stable aggregates of different size, accounts for the disruptive effect of soil wetting. Moreover, this method is less abrasive compared to dry sieving. Stable macroaggregates have been found to contain much higher proportions of carbon compared with smaller aggregates (Puget et al., 1995; John et al., 2004). However, the organic matter in macroaggregates is more easily decomposable than that in microaggregates (Puget et al., 1995). The stability of macroaggregates is related to the incorporation of plant residues such as fine roots and fungal hyphae, which can form associations between microaggregates (Tisdall and Oades, 1982). Macroaggregate turnover has an indirect effect on the formation of microaggregates (Six et al., 1998), which most effectively

stabilise organic components for relatively long periods of time and thus are important for the sequestration of SOC (John et al., 2004).

The disturbance of soils by tillage has significant effects on organic carbon dynamics. Tillage results in the disruption of aggregates and leads to the decomposition of carbon which was protected within them. Six et al. (1999) reported a carbon depletion in the intra-aggregate fraction (53-250 µm) in a conventionally cultivated soil of about 50 % compared to a no-tillage soil. This was attributed to a reduced formation of aggregates and their lower stability resulting in an increased decomposition. Moreover, tillage modifies the location of SOM in the profile, by the mixing of recent crop-derived carbon with older subsoil organic matter, and causes changes in soil climate affecting microbial activity (Balesdent et al., 2000).

The effect of soil tillage on organic carbon dynamics in aggregates was investigated by ^{14}C analyses of five aggregate size classes, recovered by wet sieving according to the method described in chapter 4.2.3, from cultivated and not ploughed soils at Rotthalmünster (figure 5.23, table 5.10).

Carbon contents and ^{14}C values of aggregate fractions in ploughed soils

Considerable differences were found in the distribution of soil material in macroaggregate fractions from the plough horizon under wheat and under maize cropping at Rotthalmünster. Whereas on the trial with maize about 11 % of the soil material was contained in the largest fraction (>2 mm), on the wheat culture this fraction comprised only 5.6 % of the soil material (John, 2003). The higher carbon content (3.5 %) and much higher C/N ratio of 15.5 (compared to 9.0 under maize) of these megaaggregates from the wheat culture suggest that recent crop residues are the main constituents, which is, however, not reflected by a ^{14}C concentration of only 104.4 pMC, being below the atmospheric level in 2002. In contrast to the megaaggregates, the 1-2 mm sized macroaggregates from the wheat trial contained about twice the amount of total soil material (7.7 %) of the maize culture (3.4 %). These differences between both cultures may reflect both, the effects of different soil cultivation and of small differences in soil texture.

The small macro- and microaggregate fractions of 53 to 1000 µm size on both cultures included the highest amount of soil material, both fractions together about 65 %, and about the same high proportion of total soil organic carbon (John, 2003). John et al.

(2004) estimated the turnover of carbon in 53-1000 µm sized aggregates in the maize topsoil at 50 to 60 years compared to a much lower carbon turnover rate of 100 years in the fraction <53 µm. However, the silt-clay fraction contained only about 12 % and 15 % of the total organic carbon in soils with wheat and maize cropping respectively. Hence, the intermediate 53 to 1000 µm aggregate fraction is most important for carbon storage in cultivated soils.

^{14}C concentrations of all aggregate fractions from both trials ranged between 103.8 to 106.6 pMC and yielded no significant (2-σ criterion) differences from each other (figure 5.23, table 5.10).

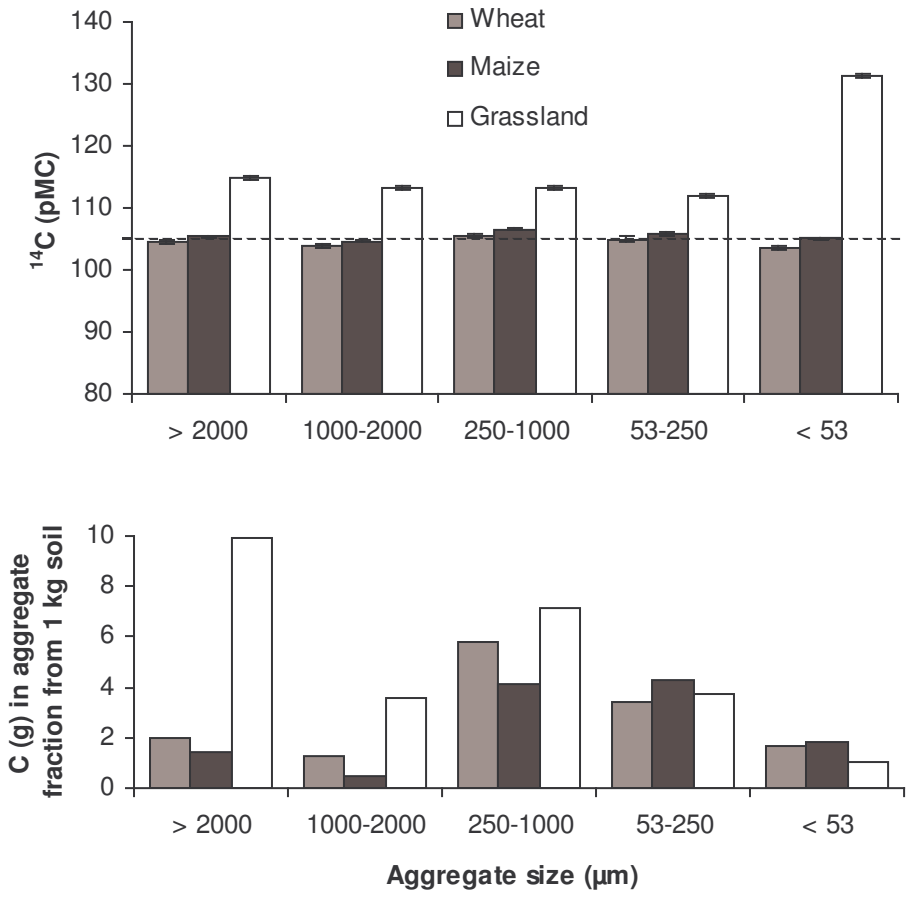

Figure 5.23: Radiocarbon and organic carbon concentration in water-stable aggregate fractions from ploughed (maize, wheat: 0-30 cm depth) and not ploughed (grassland: 0-10 cm depth) topsoils at Rotthalmünster. The error bars show the 1-σ measurement uncertainties and the dashed line the atmospheric ^{14}C content in 2002 (Levin et al., 2003) Carbon contents from John (2003).

In comparison to a ^{14}C value of 107.6 ± 0.3 pMC of the bulk soil under maize cultivation (table 8.4) these concentrations may indicate the loss of a younger carbon fraction by acid hydrolysis, which was applied to the aggregate fraction. However, the macroaggregates of 1-2 mm size showed a small depletion in ^{14}C on both trials which indicates a contribution of older, pre-1954 carbon to this fraction. Microscopic inspection revealed a high amount of mineral particles encrusted with presumably iron (Fe) oxides in the 1-2 mm fraction. These particles, which were selected and analysed separately, yielded a ^{14}C concentration of 95.2 ± 0.7 pMC suggesting stabilisation of pre-1954 carbon by adsorption to the Fe loaded mineral surface (Oades, 1988). The silt-clay fraction, which can also contain significant amounts of Fe oxides (Kaiser and Guggenberger, 2000; Kahle et al., 2003), also showed depleted ^{14}C values of 103.5 and 105.0 pMC in the soil of the wheat and maize trial respectively.

Carbon and ^{14}C distribution in aggregate fractions in a no-tillage soil

On the grassland site at Rotthalmünster, which was established in 1961, the distribution of carbon in aggregate fractions from 0-10 cm soil depth was considerably different from that in the plough horizon of the maize and wheat cultures. Due to the much higher organic carbon content of the grassland surface soil (figure 5.15, chapter 5.4.1), all aggregate fractions, except the silt-clay fraction (<53 µm), yielded substantially higher carbon contents. Moreover, all fractions showed ^{14}C values above that of the present atmosphere in the range of 111.9 to 131.4 pMC. These high values indicate a low turnover of organic carbon, probably due to a minor mixing of recent vegetation residues into the surface soil as well as a lower aeration of the soil both as a result of no-tillage. In consequence, organic carbon has accumulated in soil aggregates over the past decades (Trumbore and Druffel, 1995).

The megaaggregates of >2 mm size showed a much higher stability, i.e. resistance during wet sieving, in the not ploughed grassland soil than in the uncultivated soils, thus comprising the largest amount of soil material (38 %) as well as of organic carbon (39 %). This was previously shown by investigations of Tisdall and Oades (1982) and Six et al. (2000). The organic carbon contained in this fraction was about three times higher than in the cultivated surface soils. Tisdall and Oades (1982) attributed similar observations to the development of an extensive fibrous root system in soils with permanent grass promoting the formation of stable macroaggregates via associations between roots and microaggregates. Carbon analyses of aggregate fractions from below the main root zone (10-30 cm depth) of the grassland trial at Rotthalmünster

showed a very different carbon distribution with drastically decreased carbon contents of macroaggregates (1 to >2 mm size) but higher amount in microaggregates (250-1000 µm; John et al., 2004). A relatively high (bomb-) radiocarbon content of 114.9 pMC of these megaaggregates (>2 mm) and low C/N ratio of 8.8 suggest the contribution of microbially transformed, several decades old material to mega- as well as macroaggregates (113.4 pMC).

About 28 % of total organic carbon was contained in the small macroaggregates (250-1000 µm) under grassland which indicates their importance for carbon storage in both, cultivated and not ploughed soils (Six et al., 2000). Whereas in the ploughed soils ^{14}C data close to the atmospheric level suggest a fast exchange of organic carbon in this fraction, no-tillage seems to result in an enrichment of several years to decades old organic material caused by a slower turnover rate of SOC as also indicated by likewise high ^{14}C concentrations of 113.1 pMC of the large macroaggregates. This result agrees with the ^{13}C-based turnover times of about 50 years (250-1000 µm; table 5.10).

Table 5.10: ^{14}C contents in aggregate fractions from grassland (0-10 cm depth), wheat, and maize trials (0-30 cm depth) at Rotthalmünster and percentage of maize-derived carbon derived from ^{13}C data.

Fraction (µm)	^{14}C (pMC)	^{14}C (pMC)	^{14}C (pMC)	Maize C[1] (%)	Turnover[1] (years)
	grassland	wheat		maize	
> 2000	114.9 ± 0.3	104.4 ± 0.3	105.5 ± 0.2	47.2 ± n.d	38
1000-2000	113.4 ± 0.3	103.8 ± 0.3	104.6 ± 0.5	46.9 ± 2.8	38
250-1000	113.1 ± 0.3	105.6 ± 0.3	106.6 ± 0.3	38.2 ± 1.2	50
53-250	111.9 ± 0.3	105.0 ± 0.4	105.9 ± 0.3	32.7 ± 1.5	61
< 53	131.4 ± 0.4	103.5 ± 0.4	105.0 ± 0.3	21.1 ± 0.7	101

[1] Data from John et al. (2004), calculated from ^{13}C values.

The proportion of carbon stored in microaggregates and in the silt-clay fraction under no-tillage was considerably smaller than in the cultivated soils. The silt-clay fraction <53 µm stored only 4 % of the total organic carbon in the grassland surface soil compared to 12 % and 15 % in the cultivated soils (wheat and maize). This reflects that microaggregates are less influenced by soil management than macroaggregates (Six et al., 1999, 2000). The silt-clay fraction showed an extremely high contribution of bomb-^{14}C yielding an ^{14}C value of 131.4 ± 0.4 pMC and a low C/N ratio of 7.3. This high concentration can only be explained by the stabilisation of organic carbon derived from the period with high atmospheric ^{14}C concentrations. Probably organic carbon from the

starting period of the trial in 1961, characterised by extremely high atmospheric ^{14}C levels around 1964 (figure 3.1, chapter 3.2.1), was microbially transformed and stabilised in clay-sized organo-mineral complexes because of the termination of soil ploughing. If this is the case it reflects that soil cultivation prevents the formation of stable organo-mineral complexes.

These results of the aggregate fractions from the ploughed and no-tillage soils confirm the aggregate formation concept discussed by Golchin et al. (1997) and Six et al. (1999). The authors suppose that the destruction of megaaggregates, as observed in the ploughed soils, leads to a reduced rate of microaggregate formation and a lower stabilisation of SOC. This is shown by almost similar ^{14}C values of all fractions under wheat and maize cropping. It is assumed that within stable macroaggregates uncomplexed organic matter is progressively transformed by soil microbes resulting in the formation of new microaggregates (<250 µm) (Golchin et al., 1997; Six et al., 1998). The high amount of bomb-^{14}C of the <53 µm fraction from the grassland soil and its low C/N ratio indicate the microbial transformation and stabilisation of SOC associated with silt and clay particles. However, in the no-tillage grassland soil microaggregates of 53-250 µm size showed no significant difference in ^{14}C nor in C/N ratio from aggregates >250 µm suggesting similar binding agents to be responsible for their stabilisation.

6 Conclusions

The radiocarbon analysis of operationally-defined physical and chemical organic matter fractions as well as of individual compounds from agricultural soils provided information on the origin, transformation, and stabilisation of organic carbon. The data also revealed difficulties associated with the investigation of mixed carbon pools, due to their susceptibility to contamination by non-soil derived carbon, and indicate new perspectives of compound-specific radiocarbon analysis in soil science.

^{14}C distribution in recent and archived soil fractions from long-term field trials at Halle

The contribution of bomb-^{14}C to soil fractions from recent and archived soil samples compared with pre-1954 samples should have provided information on carbon turnover rates in agricultural soils. At Halle, this approach was hindered by a high amount of about 55 % fossil carbon in the surface soil, which decreased in the subsoil as shown by a depth related decrease of ^{14}C ages. Fossil carbon contributions to soil humin and humic acid fractions at about 60 cm depth suggest downward transport of a high proportion of particulate organic matter and a lower amount of soluble fossil carbon by bioturbation and leaching respectively. Differences in ^{14}C concentration between soil sampled in 1949, 1961, and 2001 reflect the increase in industrial activity, i.e. lignite use, since 1949.

The fossil contamination was found to extend to all physically and chemically-defined SOM fractions, even to more specific lipid compound classes isolated by solvent extraction and different chromatographic techniques. This result reveals the difficulties associated with the investigation of carbon dynamics in mixed SOM pools, as fossil fuel related, ^{14}C-free carbon can strongly influence the composition and dynamics of the soil carbon reservoir, and points out the need for more specific separation methods.

Origin and quantity of fossil carbon in soil organic matter at Halle

The ^{14}C data of SOM fractions and selected black particles were used to detect and quantify the fossil carbon admixture at Halle. The fossil material was assumed to originate mainly from lignite fragments and residues after their incomplete combustion, since the study site is located in a highly industrialised region of Germany with open cast lignite mining, power plants and a railway track adjacent to the field trials. Extremely low ^{14}C concentrations of black particles (7 pMC), selected from archived

and recent surface soil samples, and of fine sand to coarse silt fractions (20-200 µm; 24 to 33 pMC) confirm this hypothesis and suggest a large portion of fossil, airborne particles. Results of SEM/EDX analyses indicate differences in the morphology as well as in sources of the selected black fragments, suggesting the presence of non- and incompletely combusted lignite particles as well as burned vegetation residues.

The proportion of fossil carbon in surface SOM from different long-term study sites, quantified from SOM ^{14}C data by mass balance calculation, was dependent on the proximity of the study site to fossil fuel using industries and traffic. Highest contributions were determined for the soil at Halle, considerably lower values for Rothamsted, and a negligible fossil carbon input was found in the soil from Rotthalmünster, which thus is most suitable for the investigation of 'natural', vegetation-derived soil organic carbon cycling. Since Rotthalmünster could not provide archived pre-bomb soil samples, further investigations of SOM dynamics by ^{14}C analysis should concentrate on the trials at Rothamsted.

Transformation of organic carbon in uncontaminated soil profiles

^{14}C analyses were applied to humin and humic acid fractions, separated by standard acid-alkali extraction from depth intervals of different cultures with and without ploughing at Rotthalmünster. A strong ^{14}C depletion of the humin fraction in 0 to 60 cm depth of about 30 to 50 % in both, ploughed and the non-cultivated soils, reflects an accumulation of resistant organic components, depending on total organic carbon content but independent of the kind of soil cultivation. In contrast, the humic acid fraction was influenced by soil tillage promoting downward transport of young, soluble SOC contributing to this fraction at greater depth. Permanent grassland cultivation and no-tillage produced a humic acid fraction comprising several decades old carbon supposedly originating from transformed humic substances. These compounds may derive from SOM stabilised in macroaggregates as reflected by similar ^{14}C concentrations (about 113 pMC).

The ^{14}C values of individual microbial PLFAs from the plough horizon and the subsoil at Rotthalmünster and their comparison with PLFA ^{14}C data from the fossil carbon contaminated surface soil at Halle suggest (i) synthesis of straight-chain monounsaturated PLFAs (*n*-C16:1, *n*-C17:1, *n*-C18:1) from recent, most probably dissolved organic carbon by Gram-negative bacteria, and (ii) a lower substrate specificity of microbes producing saturated PLFAs. The latter interpretation is based on

higher bomb-^{14}C contents of *i/a*-C15:0, *n*-C16:0, and *n*-C17:0 PLFAs in the surface soil and lower ^{14}C values in the subsoil at Rotthalmünster, both indicating the assimilation of older SOM from the past years to decades and pre-1954 material respectively. At Halle ^{14}C values of *n*-C17:0 and *cy*-C18:0 PLFAs even indicate metabolism of fossil carbon by Gram-positive bacteria.

Physical organic carbon protection

Particle-size fractions, separated from surface soils with rye and maize cultivation at Halle, were influenced by high portions of fossil, lignite-derived carbon. The contamination was highest in fine sand and coarse silt fractions (63-200 µm) making up 72 to 79 % of the total organic carbon. Because of the C_3 isotopic label ($\delta^{13}C$: -24 ‰ to -26 ‰ PDB) of the fossil material, ^{14}C as well as data of natural ^{13}C labelling underestimate 'natural' organic carbon turnover times. Hence, the proportion of maize-derived carbon in size separates was corrected for the contribution of lignite carbon via their ^{14}C concentrations by mass balance calculation. Corrected percentages of maize carbon yielded more realistic turnover times in the range of 43 to 170 years increasing from coarse sand to clay compared to 138 to 718 years suggested by the uncorrected data. On this basis the medium plus fine silt and the clay fraction are supposed to be most important for carbon sequestration in cultivated soils, since they stored >50 % of the total organic carbon turning over within >150 years.

Carbon and radiocarbon distributions in density fraction, separated from the plough horizon of different cultures at Halle and at Rotthalmünster, again revealed their susceptibility to the fossil carbon contamination at Halle. Extremely depleted ^{14}C contents suggest an accumulation of lignite particles in the light occluded particulate organic matter (POM). The data of natural ^{13}C labelling indicate increasing turnover times of carbon in free-$POM_{<1.6}$ > occluded-$POM_{1.6\text{-}2.0}$ > $mineral_{>2.0}$-fraction > occluded-$POM_{<1.6}$ and confirm previous findings, which indicated a high stability of organic carbon within soil aggregates. Since the mineral-fraction, the remainder of the fractionation procedure, comprised most of the organic carbon and yielded slow turnover rates, it is thought to be most important for soil carbon sequestration in cultivated soils. The distribution of maize-derived carbon in density fractions from Rotthalmünster corresponded to the corrected proportions in the fossil carbon contaminated Halle soil. This demonstrates the reliability of this calculation.

The distribution of carbon and radiocarbon in water-stable aggregates of different size from ploughed and no-tillage soils confirms that tillage mainly destroys macroaggregates resulting in the exposure of formerly protected organic carbon and its loss by biodegradation and oxidation. In the not cultivated grassland soil megaaggregates and macroaggregates (1 to >2 mm) together stored 53 % of the total organic carbon which turns over within several decades as indicated by a relatively high contribution of bomb-^{14}C to these fractions (~114 pMC). A considerably lower amount of carbon in these large aggregates from the ploughed surface soils with wheat and maize cultivation indicates the loss of about 30 and 37 % organic carbon respectively from these large aggregates due to their repeated destruction by tillage. In the ploughed soils no significant difference in ^{14}C content was found between all aggregate fractions, suggesting similar organic components, such as slightly decomposed plant material, to work as binding agents between mineral particles and aggregates. Microaggregates were found to be less sensitive to soil cultivation since they comprised a higher portion of total SOC in the ploughed soils compared to the no-tillage grassland soil. Small macro- and microaggregates (53-1000 µm) on both, cultivated and no-tillage soils stored a major portion of the total organic carbon (40 to 70 %) which turns over within several decades as indicated by ^{14}C as well as ^{13}C data.

Further research

Since fossil carbon contamination at Halle hindered the determination of carbon turnover times in soil fractions via the bomb-^{14}C method, further research should concentrate on study sites providing archived soil samples, which are not or less influenced by fossil fuel-derived carbon, such as Rothamsted. Although the analysis of carbon dynamics in functionally-defined fractions is ambiguous, since they do not represent a homogeneous SOC pool, a quantification of organic carbon in the separated fractions and compounds would be helpful to determine their impact on total soil organic carbon dynamics. Further compound-specific ^{14}C analysis should focus on the identification of biomarkers that can be used as proxies of specific organisms or key processes of carbon transformation.

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7.1 Own publications including results of this thesis

This thesis includes results which have already been published or which are submitted for publication in the journals listed below:

Chapter: 5.1.2

Rethemeyer, J., Bruhn, F., Grootes, P.M. und Nadeau, M.-J., 2001. Bomben-^{14}C als Informationsquelle für die Mechanismen der Kohlenstoff-Stabilisierung in Böden: Inhomogenität des organischen Bodenmaterials. *Mitteilungen der Deutschen Bodenkundlichen Gesellschaft* 96 (1), 267-268.

Chapters: 5.1.3, 5.1.4, and 5.2.2

Rethemeyer, J., Bruhn, F., Kramer, C., Gleixner, G., Andersen, N., Nadeau, M.-J., and Grootes, P.M., 2004. Age heterogeneity of soil organic matter. *Nuclear Instruments and Methods in Physics Research B* 223-224, 521-527.

Chapters: 5.3.1, and 5.3.2

Rethemeyer, J., Wiesenberg, G., Schwark, L., Kramer, C., Gleixner, G. Andersen, N., Nadeau, M.-J., and Grootes, P.M., 2004. Complexity of soil organic matter: AMS ^{14}C analysis of soil lipid fractions and individual compounds. *Radiocarbon* 64 (1), 465-473.

Chapters: 5.4.1, 5.4.2, and 5.5.2

Rethemeyer, J., Kramer, C., Gleixner, G., John, B., Yamashita, T., Flessa, H., Andersen, N., Nadeau, M.J., and Grootes, P.M., 2004. Transformation of soil organic matter in agricultural long-term field trials: Radiocarbon concentration versus soil depth. *Geoderma*, in press.

8 Appendix

8.1 Abbreviations

AAA	acid-alkali-acid extraction
AMS	accelerator mass spectrometry
ASE	accelerated solvent extraction
BC	black carbon
BP	years before present (1950)
CSRA	compound-specific radiocarbon analysis
$\delta^{13}C$	^{13}C:^{12}C ratio in ‰ expressed relatively to the PDB standard
$\Delta^{14}C$	normalised fMC value given as ‰ of the absolute standard activity
DCM	dichloromethane
DOM	dissolved organic matter
EDX	energy-dispersive X-ray spectroscopy
FAME	fatty acid methyl ester
fMC	fraction modern carbon
fPOM	free particulate organic matter
GC	gas-chromatography
Gt	gigatonnes (1 Gt = 10^{15} g)
ha	hectare
IAEA	International Atomic Energy Agency
IOM	inert organic matter
IR-MS	isotope ratio-mass spectrometry
keV	kilo electron volts
MeOH	methanol
MeV	million electron volts
MV	million volts
MPLC	medium pressure liquid chromatography
MRT	mean residence time
NIST	National Institute of Standards and Technology
NMR	nuclear magnetic resonance spectroscopy
oPOM	occluded particulate organic matter

PDB	Pee Dee Belemnite, carbonate standard for ^{13}C analysis
PCGC	preparative capillary gas-chromatography
PLFA	phospholipid fatty acid
pMC	percent modern carbon
POM	particulate organic matter
ppm	parts per million
rpm	rotations per minute
SEM	scanning electron microscopy
σ	standard error
SOC	soil organic carbon
SOM	soil organic matter
TOC	total organic carbon

Table 8.1: ^{14}C values of archived and recent surface soil samples collected on the continuous rye trial at Halle. SOM was mechanically separated into different components and chemically into a humin and a humic acid fraction. $\delta^{13}C$ values (‰ PDB) were determined by AMS and are not comparable with IR-MS data (see chapter 4.4.3).

KIA-Nr.		Component	Fraction	C yield (mg)	^{14}C (pMC)	±	$\Delta^{14}C$ (‰)	±	^{14}C age (years BP)	±	$\delta^{13}C$ (‰)	±
				Rye, unfertilised: 0-20 cm (archived sample from 1961)								
18420		Selcted soil	Humin	9.44	26.94	0.14	-730.96	1.40	10540	45	-24.00	0.36
18420	II	Selected soil	Humic acid	3.48	47.41	0.18	-526.53	1.80	5985	30	-25.57	0.49
18420	B	???	Humin	0.95	41.68	0.24	-583.75	2.40	7030	50	-25.49	0.22
18420	C	Plant residues	Humin	0.31	117.68	0.62	175.24	6.20	modern		-33.62	0.16
				Rye, unfertilised: 0-25 cm, soil pit (September 2000)								
12773		Bulk soil	Untreated	4.31	54.49	0.45	-458.39	4.50	4880	70	-28.30	0.56
12773	A	Bulk soil	Humin	0.97	31.55	0.26	-686.40	2.60	9300	70	-25.13	0.15
12773	A II	Bulk soil	Humic acid	2.37	49.86	0.21	-504.41	2.10	5630	50	-26.10	0.07
12773	B	Selected soil	Humin	1.28	38.85	0.21	-613.84	2.10	7590	60	-23.15	0.12
12773	B II	Selected soil	Humic acid	1.93	52.15	0.30	-481.64	3.00	5230	50	-23.84	0.17
12773	C	Plant residues	Humin	4.24	108.74	0.39	80.84	3.90	modern		-28.77	0.15
12773	C II	Plant residues	Humic acid	2.35	108.29	0.34	76.37	3.40	modern		-30.51	0.29
12773	D	Roots	Treated with 1% HCl	4.29	107.91	0.29	72.59	2.90	modern		-29.08	0.07
12773	E	Black particles	Humin	1.31	21.44	0.19	-786.89	1.90	12350	90	-25.92	0.07
12773	E II	Black particles	Humic acid	0.15	17.87	1.06	-822.38	10.60	13660	480	-31.58	0.24
12774		Rye straw	Treated with 1% HCl	3.98	107.57	0.29	69.21	2.90	modern		-25.55	0.18
12775		Young rye plant	Treated with 1% HCl	3.84	106.44	0.36	57.98	3.60	modern		-28.90	0.06
15369	A	Bulk soil	Hot water extract	1.10	94.22	0.43	-63.48	4.30	480	40	-25.42	0.14
15369	B	Bulk soil	Hot water extract	0.51	96.34	0.46	-42.41	4.60	300	40	-30.17	0.16
15370	A	Bulk soil	Pyrophosphate extract	0.92	43.78	0.31	-564.84	3.10	6640	60	-26.82	0.20
15370	B	Bulk soil	Water extract	0.30	83.43	0.63	-170.73	6.30	1455	60	-33.05	0.11
15371	A	Bulk soil	Pyrophosphate extract	1.36	38.77	0.22	-614.64	2.20	7610	50	-26.94	0.15
15371	B	Bulk soil	Water extract	0.33	82.55	0.55	-179.48	5.50	1540	55	-33.37	0.08

Table 8.2: ^{14}C concentration of recent soil sampled in depth profiles of the rye trial at Halle. SOM was mechanically separated into different components and chemically into a humin and a humic acid fraction. $\delta^{13}C$ values (‰ PDB) were determined by AMS and are not comparable with IR-MS data (see chapter 4.4.3).

KIA-Nr.		Component	Fraction	C yield (mg)	^{14}C (pMC)	±	$\Delta^{14}C$ (‰)	±	^{14}C age (years BP)	±	$\delta^{13}C$ (‰)	±
				Rye, unfertilised: 0-5 cm (December 2000)								
13262	A	Bulk soil	Humin	1.31	29.51	0.20	-706.68	2.00	9805	55	-24.93	0.14
13262	A II	Bulk soil	Humic acid	3.88	45.67	0.20	-546.05	2.00	6295	35	-25.68	0.08
13262	B	Selected soil	Humin	1.69	28.34	0.21	-718.31	2.10	10130	60	-24.95	0.11
13262	B II	Selected soil	Humic acid	3.64	44.11	0.30	-561.56	3.00	6575	55	-24.76	0.10
13262	C	Plant residues	Humin	3.82	107.90	0.30	72.49	3.00	modern		-25.95	0.05
13262	C	Plant residues	Humic acid	0.43	109.33	0.51	86.71	5.10	modern		-34.57	0.11
13262	D	Roots	Humin	4.08	106.39	0.30	57.48	3.00	modern		-28.08	0.08
13262	D II	Roots	Humic acid	0.53	105.10	0.47	44.66	4.70	modern		-36.22	0.07
13262	E	Black particles	Humin	3.09	7.00	0.10	-930.42	1.00	21360	110	-24.12	0.05
				Rye, unfertilised: 30-35 cm (December 2000)								
13723	Aa	Bulk soil	Humin	1.39	42.00	0.23	-582.53	2.30	6970	45	-27.02	0.06
13723	Ab	Bulk soil	Humin	0.30	35.91	0.54	-643.07	5.40	8230	120	-31.70	0.10
13723	A II	Bulk soil	Humic acid	1.67	60.66	0.23	-397.06	2.30	4015	30	-25.46	0.13
13723	Ba	Selected soil	Humin	1.16	43.94	0.24	-563.25	2.40	6605	45	-23.82	0.05
13723	Bb	Selected soil	Humin	0.48	40.54	0.39	-597.04	3.90	7250	80	-28.72	0.13
13723	B II	Selected soil	Humic acid	1.95	62.89	0.23	-374.89	2.30	3725	30	-25.92	0.18
13723	C	Roots	Humin	1.26	106.97	0.31	63.25	3.10	modern		-27.64	0.10
13723	D	Black particles	Humin	0.51	21.51	0.33	-786.20	3.30	12340	130	-29.60	0.16
13723	D II	Black particles	Humic acid	0.41	18.10	0.40	-820.09	4.00	13730	180	-30.29	0.07
				Rye, unfertilised: 60-68 cm (December 2000)								
13724	Aa	Bulk soil	Humin	0.68	53.70	0.33	-466.24	3.30	4995	50	-25.75	0.20
13724	Ab	Bulk soil	Humin	0.09	51.89	1.65	-484.23	16.50	5270	260	-34.85	0.10
13724	A II	Bulk soil	Humic acid	1.73	65.31	0.36	-350.84	3.60	3420	45	-25.20	0.15
13724	Ba	Selected soil	Humin	0.34	54.26	1.05	-460.67	10.50	5120	160	-31.70	0.17
13724	Bb	Selected soil	Humin	0.16	52.84	1.05	-474.79	10.50	5120	160	-35.06	0.10
13724	B II	Selected soil	Humic acid	0.99	67.47	0.28	-329.37	2.80	3160	35	-26.98	0.11

Table 8.3: ^{14}C concentrations of archived and recent soil samples from soil profiles with crop rotation at Halle. Different SOM components were separated mechanically and a humin and a humic acid fraction were extracted chemically. $\delta^{13}C$ values (‰ PDB) were determined by AMS and are not comparable with IR-MS data (see chapter 4.4.3).

KIA-Nr.		Component	Fraction	C yield (mg)	^{14}C (pMC)	±	$\Delta^{14}C$ (‰)	±	^{14}C age (years BP)	±	$\delta^{13}C$ (‰)	±
Crop rotation, unfertilised: 0-20 cm (archived sample from 1949)												
14368	B	Selcted soil	Humin	4.48	51.08	0.21	-489.14	2.10	5395	35	-24.97	0.12
14368	B II	Selected soil	Humic acid	4.91	66.73	0.25	-332.62	2.50	3250	30	-26.25	0.08
14368	C	Black particles	Humin	2.32	7.00	0.11	-929.99	1.10	21360	120	-23.14	0.17
14368	C II	Black particles	Humic acid	0.34	7.70	0.47	-922.99	4.70	20590	500	-28.31	0.08
Crop rotation, unfertilised: 0-20 cm (March 2001)												
14365	A	Bulk soil	Humin	3.45	43.96	0.22	-563.10	2.20	6600	40	-25.25	0.09
14365	A II	Bulk soil	Humic acid	4.64	55.59	0.33	-447.52	3.30	4720	50	-26.53	0.19
14365	B	Selected soil	Humin	2.42	45.49	0.23	-547.90	2.30	6330	40	-24.33	0.14
14365	B II	Selected soil	Humic acid	4.26	55.34	0.25	-450.00	2.50	4750	40	-27.57	0.10
14365	C	Black particles	Humin	2.02	16.59	0.15	-835.12	1.50	14430	70	-25.15	0.09
14365	C II	Black particles	Humic acid	0.27	12.55	0.58	-875.27	5.80	16670	380	-31.00	0.12
Crop rotation, unfertilised: 20-40 cm (March 2001)												
14366	A	Bulk soil	Humin	3.59	41.79	0.21	-584.67	2.10	7010	40	-25.28	0.08
14366	A II	Bulk soil	Humic acid	3.98	57.62	0.36	-427.34	3.60	4430	50	-26.51	0.31
14366	B	Selected soil	Humin	4.29	44.89	0.20	-553.86	2.00	6435	36	-23.77	0.10
14366	B II	Selected soil	Humic acid	4.17	56.38	0.29	-439.67	2.90	4605	40	-24.75	0.10
14366	C	Black particles	Humin	1.79	26.57	0.19	-735.93	1.90	10650	60	-23.96	0.10
14366	C II	Black particles	Humic acid	0.43	16.15	0.40	-839.49	4.00	14640	200	-30.21	0.18
Crop rotation, unfertilised: 40-60 cm (March 2001)												
14367	A	Bulk soil	Humin	2.60	58.64	0.26	-417.21	2.60	4290	35	-24.87	0.11
14367	A II	Bulk soil	Humic acid	3.44	63.66	0.26	-367.32	2.60	3630	35	-26.05	0.20
14367	B	Selected soil	Humin	2.47	58.00	0.24	-423.57	2.40	4375	35	-24.94	0.14
14367	B II	Selected soil	Humic acid	3.48	64.42	0.32	-359.76	3.20	3530	40	-26.14	0.06
14367	C	Black particles	Humin	1.68	16.78	0.16	-833.23	1.60	14340	70	-22.26	0.08
14367	C II	Black particles	Humic acid	0.19	22.40	0.84	-777.38	8.40	12020	310	-31.34	0.09

Table 8.4: ^{14}C distribution of soil humin and humic acid fractions in a soil profile with continuous maize cultivation at Rotthalmünster. $\delta^{13}C$ values (‰ PDB) were determined by AMS and are not comparable with IR-MS data (see chapter 4.4.3).

KIA-Nr.		Soil depth (cm)	Component	Fraction	C yield (mg)	^{14}C (pMC)	±	$\Delta^{14}C$ (‰)	±	^{14}C age (years BP)	±	$\delta^{13}C$ (‰)	±
					Maize, NPK (September 2001)								
15532	A	0-10	Bulk soil	Untreated	4.09	107.63	0.30	69.68	3.00	modern		-21.09	0.11
15532	D	0-10	Bulk soil	Water extract	0.24	115.16	0.73	144.52	7.30	modern		-26.52	0.31
15532	D I	0-10	Bulk soil	Humin	3.20	106.09	0.29	54.38	2.90	modern		-21.85	0.20
15532	D II	0-10	Bulk soil	Humic acid	0.73	111.75	0.48	110.63	4.80	modern		-24.07	0.13
15532	C	0-10	Plant residues	Humin	3.53	108.45	0.35	77.83	3.50	modern		-11.08	0.22
15532	C II	0-10	Plant residues	Humic acid	1.07	106.91	0.41	62.52	4.10	modern		-13.34	0.29
15532	E	0-10	Black particles	Humin	0.17	22.14	0.90	-779.96	9.00	12110	335	-34.06	0.14
					Maize, NPK (September 2002)								
21145		0-35	Bulk soil	Humin	0.98	102.78	0.31	21.36	3.10	modern		-19.23	0.12
21145	II	0-35	Bulk soil	Humic acid	1.33	109.72	0.39	90.32	3.90	modern		-21.55	0.08
21146		35-45	Bulk soil	Humin	0.48	82.36	0.41	-181.56	4.10	1560	40	-26.66	0.16
21146	II	35-45	Bulk soil	Humic acid	0.64	101.17	0.37	5.36	3.70	modern		-27.91	
21147		45-60	Bulk soil	Humin	0.57	72.59	0.35	-278.65	3.50	2575	40	-25.18	0.22
21147	II	45-60	Bulk soil	Humic acid	0.94	100.06	0.35	-5.67	3.50	modern		-23.46	0.12

Table 8.5: ^{14}C distribution of soil humin and humic acid fractions in soil profiles on trials with continuous wheat and grassland at Rotthalmünster. $\delta^{13}C$ values (‰ PDB) were determined by AMS and are not comparable with IR-MS data (see chapter 4.4.3).

KIA-Nr.		Soil depth (cm)	Component	Fraction	C yield (mg)	^{14}C (pMC)	±	$\Delta^{14}C$ (‰)	±	^{14}C age (years BP)	±	$\delta^{13}C$ (‰)	±
					Wheat, NPK (September 2002)								
21142		0-30	Bulk soil	Humin	0.95	108.98	0.32	82.97	3.20	modern		-27.16	0.07
21142	II	0-30	Bulk soil	Humic acid	0.97	95.17	0.38	-54.27	3.80	400	30	-27.48	0.09
21143		30-45	Bulk soil	Humin	0.41	69.44	0.46	-309.95	4.60	2930	55	-30.60	0.20
21143	II	30-45	Bulk soil	Humic acid	0.64	97.40	0.36	-32.11	3.60	210	30	-28.27	0.14
21144		45-65	Bulk soil	Humin	0.47	50.56	0.40	-497.57	4.00	5480	60	-29.15	0.19
21144	II	45-65	Bulk soil	Humic acid	0.53	87.53	0.41	-130.19	4.10	1070	40	-29.16	0.26
					Grassland; NPK (September 2002)								
21148		0-10	Bulk soil	Humin	0.95	105.24	0.31	45.80	3.10	modern		-28.41	0.12
21148	II	0-10	Bulk soil	Humic acid	4.06	113.26	0.27	125.50	2.70	modern		-29.33	0.07
21149		10-20	Bulk soil	Humin	0.81	95.91	0.41	-46.91	4.10	335	35	-27.03	0.33
21149	II	10-20	Bulk soil	Humic acid	0.20	114.72	0.89	140.01	8.90	modern		-37.85	0.10
21150		20-30	Bulk soil	Humin	0.47	74.23	0.42	-262.35	4.20	2395	50	-29.66	0.31
21150	II	20-30	Bulk soil	Humic acid	0.71	100.19	0.40	-4.38	4.00	modern		-30.08	0.12
21151		30-45	Bulk soil	Humin	0.69	75.30	0.35	-251.72	3.50	2280	30	-27.39	0.24
21151	II	30-45	Bulk soil	Humic acid	0.76	95.50	0.33	-50.99	3.30	370	30	-27.42	0.15
21152		45-65	Bulk soil	Humin	0.65	63.01	0.59	-373.85	5.90	3710	80	-26.88	0.14
21152	II	45-65	Bulk soil	Humic acid	0.44	89.65	0.43	-109.12	4.30	880	40	-29.05	0.14

Table 8.6: ^{14}C concentration of bulk soil and selected components from a topsoil sample (0-20 cm, sampled in 1997) of the 'Broadbalk' continuous wheat trial at Rothamsted. $\delta^{13}C$ values (‰ PDB) were determined by AMS and are not comparable with IR-MS data (see chapter 4.4.3).

KIA-Nr.		Component	Fraction	C yield (mg)	^{14}C (pMC)	±	$\Delta^{14}C$ (‰)	±	^{14}C age (years BP)	±	$\delta^{13}C$ (‰)	±
					Wheat, NPK: 0-20 cm (1997)							
15533		Bulk soil	Water extract	0.29	104.08	0.62	34.90	6.20	modern		-29.06	0.22
15533	I	Bulk soil	Humin	3.15	88.6	0.45	-119.02	4.50	970	30	-25.39	0.07
15533	II	Bulk soil	Humic acid	0.49	105.71	0.33	51.11	3.30	modern		-29.47	0.15
15533	A	Plant residues	Humin	0.52	112.66	0.41	120.21	4.10	modern		-29.62	0.14
15533	B	Black particles	Humin	0.78	-0.14	0.20	-1001.39	2.00	44350		-22.76	0.13
15533	C	Oxides	Humin	0.76	0.55	0.22	-994.53	2.20	41795	4020	-21.69	0.74

Table 8.7: ^{14}C values of lipid compound classes separated from topsoil samples of the continuous rye trial at Halle and the continuous maize culture at Rotthalmünster. $\delta^{13}C$ values (‰ PDB) were determined by AMS and are not comparable with IR-MS data (see chapter 4.4.3).

KIA-Nr.		Fraction	% of Ex	C yield (mg)	^{14}C (pMC)	±	$\Delta^{14}C$ (‰)	±	^{14}C age (years BP)	±	$\delta^{13}C$ (‰)	±
HALLE - Rye, NPK: 0-25 cm (September 2000)												
16620	S	Bulk soil		13.18	51.64	0.21	-486.71	2.10	5310	30	-23.61	0.07
16620	Ex (a)	Total extract		0.70	48.52	0.33	-517.73	3.30	5810	55	-26.43	0.14
16620	Ex (b)	Total extract		0.71	48.11	0.33	-521.80	3.30	5875	55	-28.03	0.15
16620	Re	Residue of extraction		8.73	51.71	0.18	-486.02	1.80	5300	30	-23.11	0.23
16620	W (a)	High polar, HMW fraction	21.0	2.84	55.85	0.42	-444.87	4.20	4680	60	-25.98	0.19
16620	W (b)	High polar, HMW fraction	21.0	3.27	55.08	0.28	-452.52	2.80	4790	40	-24.54	0.14
16620	H (a)	Acid fraction	20.0	1.20	56.87	0.36	-434.73	3.60	4535	50	-25.00	0.13
16620	H (b)	Acid fraction	20.0	1.86	53.88	0.33	-464.45	3.30	4970	50	-23.98	0.16
16620	Q	Basic fraction	2.0	0.46	38.22	0.41	-620.10	4.10	7730	90	-27.89	0.18
16620	V	High polar fraction	6.0	0.55	45.16	0.38	-551.12	3.80	6390	70	-28.12	0.10
16620	F	Medium polar compounds	12.0	2.97	47.76	0.35	-525.28	3.50	5935	60	-24.81	0.18
16620	N	Low polar fraction	39.0	0.43	15.62	0.39	-844.74	3.90	14910	200	-29.97	0.08
16620	A	Aliphatic hydrocarbons	9.0	0.99	19.01	0.22	-811.05	2.20	13340	90	-28.61	0.15
16620	B	Aromatic hydrocarbons	1.0	0.49	5.68	0.33	-943.54	3.30	23040	480	-28.89	0.21
16620	C (a)	Low polar compounds	28.0	2.04	49.25	0.25	-510.47	2.50	5690	40	-24.71	0.15
16620	C (b)	Low polar compounds	28.0	2.17	49.57	0.28	-507.29	2.80	5640	45	-25.22	0.17
ROTTHALMÜNSTER - Maize, NPK: 0-35 cm (September 2002)												
20178	S	Bulk soil		2.18	106.52	0.28	58.78	2.80	modern		-22.18	0.42
20178	Ex	Total extract		1.91	103.24	0.62	26.17	6.20	modern		-25.44	0.12
20178	Re	Residue of extraction		2.16	105.37	0.24	47.35	2.40	modern		-22.48	0.16
20178	W	High polar, HMW fraction	23.0	1.91	105.47	0.23	48.34	2.30	modern		-21.46	0.23
20178	H	Acid fraction	19.0	1.40	98.84	0.26	-17.56	2.60	95	20	-23.82	0.21
20178	Q	Basic fraction	2.0	0.51	99.36	0.39	-12.39	3.90	50	30	-23.43	0.08
20178	V	High polar fraction	4.0	0.67	101.05	0.32	4.41	3.20	modern		-22.55	0.09
20178	F	Medium polar compounds	9.0	2.29	102.78	0.24	21.60	2.40	modern		-22.40	0.13
20178	N	Low polar fraction	43.0	1.51	98.69	0.27	-19.05	2.70	105	20	-24.89	0.07
20178	A	Aliphatic hydrocarbons	6.0	0.66	43.59	0.27	-566.73	2.70	6670	50	-31.70	0.07
20178	B	Aromatic hydrocarbons	0.5	0.09	25.00	1.71	-751.51	17.10	11130	570	-35.32	0.31
20178	C	Low polar compounds	37.0	2.19	103.44	0.31	28.16	3.10	modern		-20.42	0.30

Table 8.8: Radiocarbon concentration of total- and phospholipids extracted from surface and subsoil samples of trials with rye and maize at Halle. $\delta^{13}C$ values (‰ PDB) were determined by AMS and are not comparable with IR-MS data (see chapter 4.4.3).

KIA-Nr.		Soil depth (cm)	Trial	Fraction	C weight (mg)	^{14}C (pMC)	±	$\Delta^{14}C$ (‰)	±	^{14}C age (years BP)	±	$\delta^{13}C$ (‰)	±
					Rye (September 2000)								
15918	A	0-30	Unfertilised	Total lipids	1.20	42.13	0.23	-576.14	2.30	6940	45	-29.36	0.08
20173	A	0-30	Unfertilised	Total lipids	4.10	40.99	0.18	-587.61	1.80	7165	35	-31.64	0.74
15918	C	0-30	Unfertilised	Phospholipids	0.55	53.06	0.35	-466.18	3.50	5090	50	-31.13	0.15
20173	B	30-45	Unfertilised	Total lipids	3.41	46.76	0.21	-529.56	2.10	6110	40	-30.56	0.29
20173	C	45-60	Unfertilised	Total lipids	1.24	57.76	0.23	-418.90	2.30	4410	30	-29.47	0.21
15919	A	0-30	NPK	Total lipids	1.66	47.80	0.21	-519.10	2.10	5930	40	-30.69	0.09
15919	B	0-30	NPK	Phospholipids	0.61	60.66	0.34	-389.72	3.40	4015	30	-31.55	0.16
15920	A	0-30	Manure	Total lipids	1.89	61.31	0.24	-383.18	2.40	3930	30	-28.41	0.17
15920	B	0-30	Manure	Phospholipids	0.60	74.41	0.36	-251.39	3.60	2375	40	-31.08	0.08
					Maize (September 2000)								
15921	A	0-30	Unfertilised	Total lipids	1.16	38.28	0.21	-614.88	2.10	7715	45	-28.42	0.10
20172	A	0-30	Unfertilised	Total lipids	1.46	40.03	0.19	-597.27	1.90	7355	40	-27.31	0.15
15921	C	0-30	Unfertilised	Phospholipids	0.55	50.68	0.35	-490.13	3.50	5460	55	-29.11	0.08
20172	B	30-45	Unfertilised	Total lipids	2.08	51.39	0.2	-482.98	2.00	5350	30	-28.99	0.18
20172	C	45-60	Unfertilised	Total lipids	0.74	74.38	0.29	-251.69	2.90	2380	30	-27.68	0.29
15922	A	0-30	NPK	Total lipids	1.26	43.79	0.21	-559.44	2.10	6635	40	-27.01	0.11
15922	B	0-30	NPK	Phospholipids	0.74	54.74	0.29	-449.28	2.90	4840	40	-28.50	0.11
15923	A	0-30	Manure	Total lipids	1.27	57.89	0.30	-417.59	3.00	4390	40	-27.29	0.10
15923	B	0-30	Manure	Phospholipids	0.70	64.40	0.31	-352.09	3.10	3535	40	-28.12	0.14

Table 8.9: ^{14}C concentration of total- and phospholipids extracted from surface and subsoil samples of the wheat and maize cultures at Rotthalmünster. $\delta^{13}C$ values (‰ PDB) were determined by AMS and are not comparable with IR-MS data (see chapter 4.4.3).

KIA-Nr.		Soil depth (cm)	Trial	Fraction	C weight (mg)	^{14}C (pMC)	±	$\Delta^{14}C$ (‰)	±	^{14}C age (years BP)	±	$\delta^{13}C$ (‰)	±
					Maize (September 2002)								
20170	A a	0-30	NPK	Total lipids	4.60	103.04	0.29	36.90	2.90	modern		-21.73	0.20
20170	A b	0-30	NPK	Total lipids	3.66	100.54	0.24	11.74	2.40	modern		-23.76	0.16
20168	A	0-30	NPK	Phospholipids	1.09	107.34	0.36	80.17	3.60	modern		-23.97	0.14
20170	B	30-45	NPK	Total lipids	1.89	90.47	0.24	-89.59	2.40	805	20	-25.51	0.23
20168	B	30-45	NPK	Phospholipids	1.40	100.08	0.26	7.11	2.60	modern		-23.52	0.30
20170	C	45-65	NPK	Total lipids	3.01	93.82	0.23	-55.88	2.30	510	20	-24.69	0.23
20168	C	45-65	NPK	Phospholipids	0.96	93.88	0.28	-55.28	2.80	510	25	-24.01	0.26
					Wheat (September 2002)								
20171	A	0-30	NPK	Total lipids	3.92	102.28	0.24	29.25	2.40	modern		-30.02	0.28
20169	A	0-30	NPK	Phospholipids	0.65	106.52	0.35	71.92	3.50	modern		-26.26	0.19
20171	B	30-45	NPK	Total lipids	2.74	93.17	0.26	-62.42	2.60	570	20	-27.45	0.13
20169	B	30-45	NPK	Phospholipids	1.01	97.34	0.28	-20.46	2.80	220	25	-27.36	0.09
20171	C	45-65	NPK	Total lipids	1.46	80.59	0.24	-189.01	2.40	1730	25	-28.88	0.20
20169	C	45-65	NPK	Phospholipids	0.25	89.88	0.72	-95.53	7.20	860	65	-33.47	0.15

Table 8.10: ^{14}C concentration of individual PLFAs from surface (0-35 cm) and subsoil (30-45 cm) samples of wheat and maize cultures at Rotthalmünster. $\delta^{13}C$ values are determined by AMS and are not comparable with IR-MS data (chapter 4.4.3).

KIA-Nr.		PLFA	C yield (mg)	^{14}C (pMC)	±	$\Delta^{14}C$ (‰)	±	$\delta^{13}C$ (‰)	±
Maize, unfertilised: 0-35 cm (September 2002)									
20821	E5	i/a-C15:0	0.105	112.93	1.58	122.24	15.80	-25.47	0.19
20821	E6	n-C16:0	0.105	113.61	1.45	128.95	14.50	-24.92	0.14
20821	E7	n-C17:0	0.055	no current	---	---	---	---	---
20821	E8	*cy*-C16:0	0.040	97.62	3.45	-29.96	34.50	-22.43	0.23
20821	E9	n-C18:0	0.040	103.01	3.45	23.60	34.50	-26.62	0.24
20821	E10	*cy*-C18:0	0.065	113.74	2.24	130.28	22.40	-27.62	0.26
21088	D8	n-C16:1	0.180	109.45	1.12	87.66	11.20	-21.86	0.08
21088	D9	n-C17:1	0.115	111.41	1.88	107.16	18.80	-24.22	0.25
21088	D10	n-C18:1	0.240	110.18	0.76	94.93	7.60	-20.33	0.05
Maize, unfertilised: 35-45 cm (September 2002)									
21584	D1	i/a-C15:0	0.180	107.73	0.89	70.51	8.90	-20.51	0.23
21584	D2	n-C16:0	0.175	104.74	0.97	40.78	9.70	-21.79	0.23
21584	D3	n-C17:0	0.165	101.38	1.02	7.41	10.20	-22.30	0.22
21584	D4	*cy*-C18:0	0.125	106.09	1.29	54.23	12.90	-19.99	0.49
21583	C8	n-C16:1	0.290	106.42	0.66	57.57	6.60	-20.49	0.13
21583	C9	n-C17:1	0.145	107.36	1.11	66.86	11.10	-21.62	0.76
21583	C10	n-C18:1	0.275	107.46	0.63	67.87	6.30	-20.87	0.29
Wheat, unfertilised: 0-35 cm (September 2002)									
21082	A1	i/a-C15:0	0.215	110.53	0.77	98.39	7.70	-31.08	0.11
21082	A2	n-C16:0	0.125	113.32	1.39	126.09	13.90	-33.01	0.53
21082	A3	n-C17:0	0.090	110.75	1.67	100.53	16.70	-33.05	0.11
21082	A4	*cy*-C16:0	0.060	105.42	2.4	47.54	24.00	-31.99	0.13
21082	A5	n-C18:0	0.060	104.52	2.41	38.60	24.10	-32.70	0.19
21082	A6	*cy*-C18:0	0.115	112.23	1.43	115.28	14.30	-33.63	0.20
21083	A8	n-C16:1	0.270	108.56	0.72	78.79	7.20	-30.13	0.08
21083	A9	n-C17:1	0.115	100.06	1.7	-5.64	17.00	-36.26	0.27
21083	A10	n-C18:1	0.340	108.95	0.62	82.66	6.20	-31.77	0.17
Wheat, unfertilised: 35-45 cm (September 2002)									
21582	B1	i/a-C15:0	0.175	86.20	1.78	-143.39	17.80	-19.81	1.20
21582	B2	n-C16:0	0.150	101.27	1.05	6.36	10.50	-27.70	0.30
21582	B3	n-C17:0	0.125	100.79	1.22	1.62	12.20	-25.64	0.17
21582	B4	*cy*-C18:0	0.090	106.15	1.70	54.86	17.00	-25.45	0.76

[1] ^{14}C values were corrected according to equation 11.

Table 8.11: ^{14}C concentration of individual PLFAs from surface soil (0-25 cm) of trials cultivated with rye and maize at Halle. $\delta^{13}C$ values are determined by AMS and are not comparable with IR-MS data (chapter 4.4.3).

KIA-Nr.		PLFA	C yield (mg)	^{14}C (pMC)	±	$\Delta^{14}C$ (‰)	±	$\delta^{13}C$ (‰)	±
			Maize, unfertilised: 0-25 cm (September 2000)						
21086	C1	i/a-C15:0	0.105	100.00	1.48	-5.99	14.80	-23.11	0.10
21086	C2	n-C16:0	0.145	105.35	1.17	47.16	11.70	-25.84	0.12
21086	C3	n-C17:0	0.075	94.56	2.02	-60.13	20.20	-23.98	0.17
21086	C4	*cy*-C16:0	0.035	99.22	3.94	-13.78	39.40	-23.52	0.52
21086	C5	n-C18:0	0.050	98.69	3.08	-19.06	30.80	-25.55	0.35
21086	C6	*cy*-C18:0	0.075	92.13	1.99	-84.22	19.90	-24.86	0.11
21087	C8	n-C16:1	0.105	105.90	1.56	52.65	15.60	-23.62	0.37
21087	C9	n-C17:1	0.050	106.09	2.88	54.49	28.80	-23.93	0.25
21087	C10	n-C18:1	0.135	106.57	1.31	59.31	13.10	-19.03	0.05
			Rye, unfertilised: 0-25 cm (September 2000)						
21084	B1	i/a-C15:0	0.145	99.69	1.28	-9.06	12.80	-30.38	0.14
21084	B2	n-C16:0	0.145	104.03	1.12	34.06	11.20	-33.40	0.10
21084	B3	n-C17:0	0.110	92.07	1.5	-84.86	15.00	-32.56	0.23
21084	B4	*cy*-C16:0	---	---	---	---	---	---	---
21084	B5	n-C18:0	0.090	no current	---	---	---	---	---
21084	B6	*cy*-C18:0	0.065	95.98	2.27	-46.03	22.70	-29.36	0.40
21085	B8	n-C16:1	0.160	106.30	1.05	56.56	10.50	-32.70	0.14
21085	B9	n-C17:1	0.090	108.09	1.84	74.38	18.40	-33.34	0.20
21085	B10	n-C18:1	0.200	108.54	0.9	78.83	9.00	-31.45	0.13

[1] ^{14}C values were corrected according to equation 11.

Erklärung

Hiermit versichere ich an Eides statt, die vorliegende Arbeit ausschließlich unter Anleitung meiner wissenschaftlichen Lehrer und unter Verwendung der angegebenen Hilfsmittel angefertigt zu haben. Die Arbeit hat noch keinem anderen Prüfungsausschuß vorgelegen. Teile der Arbeit wurden nach § 7, Absatz 5 der Promotionsordnung der Mathematisch-Naturwissenschaftlichen Fakultät der Christian-Albrechts-Universität zu Kiel während des Promotionsverfahrens in Fachzeitschriften veröffentlicht.

Kiel, 05.12.2004

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