Input and turnover of plant-derived lipids in arable soils

I n a u g u r a l - D i s s e r t a t i o n

zur

Erlangung des Doktorgrades

der Mathematisch-Naturwissenschaftlichen Fakultät

der Universität zu Köln

vorgelegt von

Guido Lars Bruno Wiesenberg

aus Köln

2004

Dissertation an der
Mathematisch-Naturwissenschaftlichen Fakultät
der Universität zu Köln

Berichterstatter: HD Dr. Lorenz Schwark
Prof. Dr. Ulrich Radtke
Prof. Dr. Michael W.I. Schmidt

Tag der mündlichen Prüfung: 10.11.2004

Forschen heißt:

Herausforderung,
sich Ziele suchen,
Horizont erweitern,
Gelder erschließen,
Bekanntes screenen,
Diskussionen führen,
nach Neuem streben,
die Erfolge genießen,
ein Thema abgrenzen,
interdisziplinär denken,
neue Fragen aufwerfen,
Rückschläge verkraften,
mit Entbehrungen leben,
sich beschränken lernen,
Ergebnisse publik machen,
Etabliertes in Frage stellen,
Einschränkungen vornehmen,
den Tatsachen ins Auge blicken,
vielerlei Unbekanntes kennen lernen,
und natürlich dann rechtzeitig den Absprung schaffen!?!

Guido L.B. Wiesenberg, im August 2004

Abstract

Soil organic matter plays a central role for the global and especially for the terrestrial carbon cycle. As a result of climate problems, due to the significant increase of greenhouse gases (e.g. CO_2), suitable possibilities are needed to regulate the global carbon cycle. Especially the terrestrial part of the global carbon cycle seems to be suitable for regulative interventions. Until now, there is rare knowledge, which mechanisms are responsible for the fixation and mobilisation of carbon in soils. In this study, the unanswered questions shall be discussed to make recommendations, which measures can be forced in order to increase soil carbon pools sustainably.

Analyses in this study were performed on soil and plant samples of several long-term agricultural trials of either urban (Halle/Saale, Germany) or rural areas (e.g. Rotthalmünster, Germany). Within the enforcement of this study a lot of new findings were made concerning the dynamics of carbon in agro ecosystems of temperate climates. Previously, predominantly forest ecosystems have been subjected to intensive analyses. In contrast to forests, agro ecosystems are more dynamic due to tillage practises with annual mixing of the ploughed horizon and new annual plantings. A lot of parallels could be obtained for forest and arable ecosystems, but there are still numerous significant differences. Hence, most of the initial hypotheses could be confirmed, but some must be disproved. For example plant-derived lipids are not stable in arable soils of temperate climates for several thousand years. The most important results are as follows:

i. A reproducible, inexpensive, automated and fast extraction and separation procedure for soil lipids was successfully adopted and optimised.

ii. Long-chain n-carboxylic acids were found to be best suitable for the molecular differentiation between cereal crops following distinct photosynthesis pathways (C3- and C4-plants) and the corresponding monoculture cropped soils. Alkanes and short-chain carboxylic acids are only of limited use for the differentiation between these plant types. This is due to similar distribution patterns in both plant types. Additionally, other sources of short-chain carboxylic acids in soils, like bacteria and fungi as well as fossil sources of alkanes like brown coal with similar distribution patterns, make it difficult to use these compounds as differentiation parameters.

iii. As determined with the use of archived arable soil samples, significant soil carbon modifications as a result of the atmospheric Suess effect, like isotopic changes and amount of biomass incorporation, are expectable in the medium-term in arable soils. Suess effect induced modifications of soils mainly depend on amounts of biomass incorporation in soils and tillage practises applied. Within a few years of practising or low

biomass incorporation these effects can be determined poorly, because marginal modifications in soil management, e.g. ploughing depth variations, may overlay modifications caused by the Suess effect in short periods.

iv. For the first time turnover times of several individual lipids and lipid fractions were determined in this study. Within a few decades (20-60 years) lipids are turned over in agricultural soils, while bulk carbon is turned over significantly slower. Determined turnover times based on stable and radioactive carbon isotopes could be compared hardly, because low contributions of fossil ^{14}C-free carbon cause high turnover times, when applying the radioactive method. For soils of the low contaminated Rotthalmünster site several lipid fractions like carboxylic acids showed similar turnover times based on stable (27 years) and radioactive (42 years) carbon isotopes. Hence, especially carboxylic acids can be classified as an uncontaminated lipid fraction, yielding realistic turnover times for predominantly plant-derived and microbial organic matter. Especially the alkane fraction, which is widely used for the calculation of new carbon proportions and source apportionment of C3- and C4-plant-derived organic matter, show high ^{14}C ages resulting by pollution with fossil carbon (e.g. fossil fuel burning or direct input of oil and brown coal dust) for rural sites, too. Thus, alkanes can be used for turnover time calculations only with care. Additionally, actual contributions of different plant compartments to soil organic matter and their isotopic compositions directly before harvest are required for realistic turnover time determinations on a molecular level. Otherwise major errors could not be avoided.

v. In contrast to previous determinations for lipids in peaty and acidic soils, lipids were found to be less stable in agricultural soils. Generally, lipids are part of the intermediate stable carbon pool in soils. As demonstrated for bulk agricultural soils, incorporation and turnover of carbon mainly depends on soil management like tillage and biomass contribution. In tilled agricultural soils a fast turnover is expectable due to annual mixing of the ploughed horizon with surficial plant residues. Contrastingly, in soils with high biomass contributions and surficial tillage, a carbon and lipid enrichment can be expected, similar to grassland or forest soils.

Summarizing, a diagnostic plant biomarker for the differentiation between C3- and C4-crops was found. Generally, and in contrast to the initial hypothesis, lipids are not part of the stable carbon pool within soils, but are of intermediate stability. Sustainable management of agricultural soils in combination with large biomass contributions may lead to an increasing fixation of carbon and lipids in soils. Contrastingly, an intensive usage of agricultural soils with low biomass contributions causes a decrease of carbon in soils, which additionally produces disadvantages in soil management, e.g. a decline of the soil fauna and hence a reduced nutrient availability for crops.

Zusammenfassung

Die organische Bodensubstanz spielt eine zentrale Rolle für den globalen und vor allem für den terrestrischen Kohlenstoffkreislauf. Im Rahmen der Klimaproblematik, beruhend auf einer gravierenden Zunahme der Treibhausgase (u.a. CO_2), wird nach geeigneten Möglichkeiten zur Regulation des globalen Kohlenstoffkreislaufs gesucht. Vor allem der terrestrische Teil des globalen Kohlenstoffkreislaufs scheint geeignet zu sein, regulierend, das heißt z.B. in Form von nachhaltigen Bodenbewirtschaftungsmethoden, auf ihn einzuwirken und somit vermehrt Kohlenstoff zu fixieren. Allerdings gibt es bisher kaum Erkenntnisse, welche Mechanismen für den Einbau und die Freisetzung von Kohlenstoff im Boden verantwortlich sind. Ein Teil der bislang offenen Fragen soll in dieser Studie näher erörtert werden, um nach Möglichkeit Empfehlungen zu geben, welche Maßnahmen in Bezug auf eine nachhaltige Erhöhung der Kohlenstoffvorräte in Böden gemäßigter Breiten ergriffen werden können.

Im Rahmen der durchgeführten Arbeiten an Böden und Nutzpflanzen mehrerer statischen, ackerbaulicher Versuchsflächen sowohl stadtnaher (Halle/Saale, Deutschland), als auch ländlicher Standorte (z.B. Rotthalmünster, Deutschland) haben sich zahlreiche neue Erkenntnisse in Bezug auf die Dynamik des Kohlenstoffs in Agrikulturökosystemen gemäßigter Breiten ergeben. Nachdem bisher bevorzugt die Kohlenstoffdynamik in forstwirtschaftlichen Ökosystemen Gegenstand der Untersuchungen gewesen sind, haben die Erkenntnisse im Bereich der ackerbaulichen Ökosysteme, die aufgrund der angewendeten Pflugmethoden und der jährlich neuen Bepflanzungen ein deutlich dynamischeres System darstellen, zahlreiche Parallelen, aber auch Unterschiede erkennen lassen. Viele der anfangs aufgestellten Hypothesen ließen sich zwar bestätigen, einige konnten allerdings auch widerlegt werden. So wurde beispielsweise widerlegt, dass von Pflanzen eingetragene Lipide in Ackerböden gemäßigter Breiten nicht Jahrtausende stabil sind. Die wichtigsten Resultate ergeben sich wie folgt:

i. Ein gut reproduzierbares, kostengünstiges, automatisiertes, schnelles Extraktions- und Auftrennungsverfahren für Lipide in Böden ließ sich erfolgreich adaptieren und optimieren, wie unter Wiesenberg *et al*. (2004a) bereits publiziert wurde.

ii. Es zeigte sich, dass langkettige *n*-Carboxylsäuren hervorragend zur molekularen Differenzierung zwischen ackerbaulichen Gräsern mit unterschiedlichen Photosynthesemechanismen (C3- und C4-Pflanzen) geeignet sind. Diese Differenzierung ist sowohl zwischen den Pflanzen selbst als auch zwischen den mit den entsprechenden Pflanzen genutzten Böden möglich. Alkane und kurzkettige Carboxylsäuren eignen sich hingegen nur bedingt zur Differenzierung zwischen diesen Pflanzentypen, da die Verteilungsmuster der unterschiedlichen Pflanzen sehr ähnlich sind. Außerdem gibt es in Böden zahlreiche weitere Quellen für kurzkettige Carboxylsäuren wie z.B. Bakterien, Pilze und für Alkane auch fossile Quellen wie Braunkohle, die für diese Komponenten in Böden eine Differenzierung zwischen den Pflanzentypen erschweren.

iii. Untersuchungen und Abschätzungen an Ackerböden aus Langzeitarchiven zeigten, dass in diesen durchpflügten Böden mit hohen Biomasseeinträgen Veränderungen infolge des atmosphärischen Suess Effekts mittelfristig zu erwarten sind. Über Beobachtungszeiträume von nur wenigen Jahren sind diese Effekte jedoch nur schwer nachzuweisen, da geringe Variationen in den Bewirtschaftungsmethoden (z.B. Pflugtiefenveränderungen) die Suess Effekt bedingten Veränderungen leicht überlagern können.

iv. Entgegen bisheriger Annahmen konnte im Rahmen dieser Studie nachgewiesen werden, dass Lipide in Ackerböden innerhalb weniger Dekaden (20-60 Jahre) umgesetzt werden, wohingegen der Gesamtkohlenstoff deutlich langsamer umgesetzt wird. Die mit Hilfe von stabilen und radioaktiven Kohlenstoffisotopen ermittelten Umsatzraten sind nur schlecht miteinander zu vergleichen, da geringe Verunreinigungen mit fossilem, ^{14}C-freiem Kohlenstoff bei der radiogenen Methode hohe Verweilzeiten vortäuschen. Für den ländlichen Standort Rotthalmünster ergaben sich jedoch zumindest für die Lipidfraktion der Carboxylsäuren ähnliche mittlere Verweilzeiten für stabile (27 Jahre) und radioaktiven Kohlenstoffisotope (42 Jahre), weswegen diese Fraktion als unbelastet einzustufen ist und realistische Umsatzzeiten ergibt. Vor allem die Fraktion der Alkane, mit deren Hilfe auf anderen Versuchsflächen schon Kohlenstoffumsätze berechnet wurden, eignet sich aufgrund von Verunreinigungen und demzufolge erhöhter ^{14}C-Alter nur bedingt für Umsatzratenbestimmungen. Es zeigte sich weiterhin, dass für die Umsatzzeitenbestimmungen von Lipidfraktionen basierend auf stabilen Kohlenstoffisotopen die tatsächlichen Kohlenstoff-Einträge aus unterschiedlichen Pflanzenkompartimenten und deren isotopische Zusammensetzung verifiziert werden müssen, da ansonsten große zeitliche Fehler entstehen können.

v. Die durchgeführten Untersuchungen haben gezeigt, dass Lipide in Ackerböden weniger stabil sind als aufgrund von vorherigen Untersuchungen an torfigen, säurereichen Böden zu erwarten war. Allgemein lassen sie sich dem intermediär stabilen Kohlenstoff-Pool im Boden zuordnen. Wie für Gesamtböden gezeigt werden konnte, ist für Ackerböden der Kohlenstoffeintrag und -umsatz maßgeblich vom Management und dem Biomasseeintrag abhängig. In durchpflügten Ackerböden ist ein hoher Umsatz zu erwarten. Ein hoher Biomasseeintrag in Kombination mit einer schonenden, oberflächennahen Durchpflügung könnte zu einer Lipid- und Kohlenstoffanreicherung in Ackerböden führen.

Allgemein lässt sich resümieren, dass ein diagnostischer Pflanzenbiomarker für die Differenzierung von C3- und C4-Nutzpflanzen gefunden wurde und dass Lipide entgegen vorheriger Erwartungen nicht dem stabilen Teil der organischen Bodensubstanz zuzuordnen sind. Eine schonendere Bewirtschaftung der Ackerböden in Verbindung mit hohem Biomasseeintrag lässt eine erhöhte Fixierung von Kohlenstoff und Lipiden in diesen Böden erwarten. Dagegen führt eine fortwährende intensive Bewirtschaftung der Ackerböden mit nur geringen Biomasseeinträgen zu einer Verarmung an Kohlenstoff, was zu weiteren Nachteile für der Bewirtschaftung führen könnte, wie z.B. eine Verarmung der Bodenfauna und damit einhergehend auch eine verringerte Nährstoffzufuhr für die Nutzpflanzen.

1. Introduction

1.1 Motivation

Soil organic matter (SOM) affects all soil functions and is a central element in the global and especially the terrestrial carbon cycle (Kögel-Knabner, 2002). Approximately 81% of the terrestrial carbon participating in the active carbon cycle is bound in soils, while only 19% are bound in the vegetation (IGBP, 1998). The turnover of carbon during biomass formation (primary production) and decomposition leads to the release or binding of the greenhouse gas CO_2. In addition to anthropogenic fossil fuel burning, as one main cause of CO_2 enrichment in the atmosphere, type and intensity of land use exert significant influence on the global carbon cycle and thus may affect global climate (Janssens *et al*., 2003). These processes could be regulated by human activity, e.g. carbon storage in soils could be intensified, thereby counteracting the global warming process. The fundamental understanding for a sustainable management of the carbon budget has to be established, while at the same time maintaining or even improving major soil functions is needed. As a consequence of the Kyoto Protocol this question got more interesting and several soil organic matter research programs were established worldwide. In Germany the priority program '*Soils as Sources and Sinks for CO_2*', funded by the German Research Foundation, was established to analyse the mechanisms of organic matter fixation in soils. This research program predominantly used long-term field experiments with a well-documented cropping history to analyse short-term and long-term effects of different cropping procedures on soil organic carbon dynamics. Within this project several teams worked on a molecular level, to comprehend how single compound fractions are incorporated into soils, how stable those fractions are and if there are any possibilities to force fixation of CO_2 in soils via one of those fractions. Most teams worked on long-term field experiments, where a C3-plant (e.g. rye or wheat) monoculture was replaced on a part of the plot by a modern C4-plant (e.g. maize) monoculture, because a naturally stable carbon ($\delta^{13}C$) isotopic labelling was performed by the new plant with a heavier isotopic composition.

For SOM management, knowledge about the quantitative relationship between formation and decomposition of stable organic matter is indispensable (IGBP, 1998). At present, a prognosis about the development of the different carbon pools in soils under changing environmental conditions and land use management is not possible. This is due to a lack in understanding the basic mechanisms of SOM stabilisation and consequently, insufficient information about the regulating factors of stabilisation processes. These gaps in knowledge lead to insecurity when simulation models of carbon turnover are applied to the soil system. With further development of more specific analyses more detailed results could be obtained and hence new questions arose, which mechanisms force SOM stabilization. The main

objective of the priority program is to elucidate the major stabilisation mechanisms of organic matter in soils and to delineate a quantitative understanding of SOM regulation. Thereby the fundamental knowledge necessary to manage the carbon budget in soils will be established. This will provide a basis to improve the predictive tools for a prognosis about the impact of environmental change and land management on SOM dynamics. The Cologne research project within the priority program focuses on lipids as a predominantly plant-derived compound class in soils and their turnover in soils. Lipids were assumed to be stable in soils for several hundreds or thousands of years and thus they seemed to be part of the inert organic matter in soils. But previously, there were no systematic investigation on soil lipid turnover in agricultural ecosystems, studying both, plants and corresponding soils from different sites. Within this study a combination of several modern structural chemical and isotopic methods parallel statements should be made about sources and turnover of lipids in agricultural soils.

1.2 Literature review

Increasing amounts of anthropogenic emissions of CO_2 into the atmosphere have led to an intensive debate about potential environmental consequences and initiated activities as defined in the Kyoto Protocol of the United Nations Organization (IGBP, 1998, Prentice *et al.*, 2001). Controversial discussions demonstrate the need for an improved knowledge of the CO_2 sequestration processes. In particular a better quantification of sources and sinks of CO_2 and of turnover times of carbon in the geobiosphere is required. Soils are regarded as one potential sink for atmospheric CO_2 via photosynthetic fixation in plant biomass, which is then transformed into soil organic matter upon soil diagenesis. A simplified scheme of the terrestrial carbon cycle, where the most important natural processes are shown, is given in Figure 1 after Gleixner *et al*. (2001). Fossil fuel burning has led to a significant increase in atmospheric carbon and it is still unknown, how the terrestrial ecosystem acts in detail on these modifications. Soil organic matter studies intend to differentiate whether soils act as sources or sinks of CO_2 and to unravel the incorporation and stabilisation processes of plant biomass (Kögel-Knabner, 2002). Recent studies report controversial results. Nieder & Richter (2000) showed an enrichment of carbon in soils over the last 30 years in Germany as a consequence of applied cropping and fertilization techniques. In contrast Janssens *et al*. (2003) described on a European scale, that agricultural soils export carbon to the atmosphere.

Long-term field experiments are of great value for such analytical approaches, particularly if well-documented crop changes from C3- to C4-monocultures occurred (Balesdent *et al.*, 1988, Gregorich *et al*., 1996a, Liang *et al*., 1998, Collins *et al*., 1999, Fortuna *et al*., 2003). These experiments use the differences in isotope fractionation within C3- and C4-plants during photosynthesis (e.g. O´Leary, 1981, Hayes, 1993).

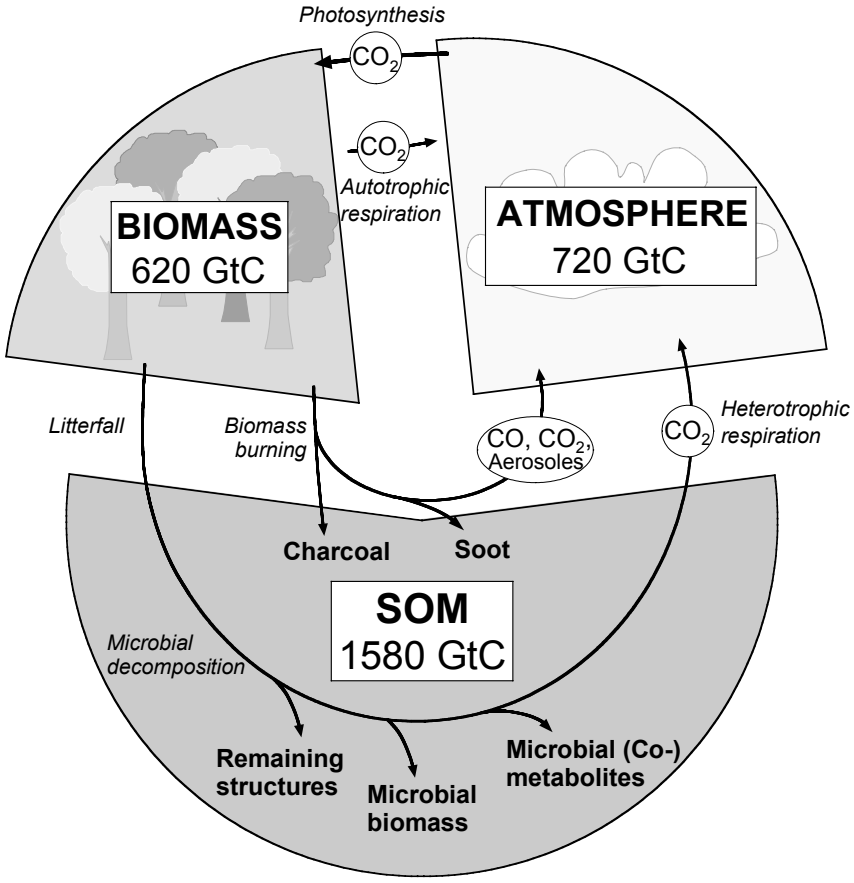


Figure 1. Major processes, pools and fluxes involved in the formation of soil organic matter (SOM) after Gleixner *et al*. (2001). Pool sizes are given in gigatons carbon (GtC).

It is well known that lipids constitute a major part of the organic components of fresh plant materials and soils (e.g. Gregorich *et al*., 1996b). They play an important role in the incorporation of plant material into soil organic carbon (SOC) (Kögel-Knabner, 2002) and contain several diagnostic markers (Bol *et al*. 1996, van Bergen *et al*. 1997, Gleixner *et al*., 2001) for source apportionment and turnover rate determinations. Most molecular studies of agricultural soils utilized single lipid fractions (Lichtfouse *et al*., 1994, 1997a, 1998), or alternatively total lipid extracts (e.g. van Bergen *et al*., 1998). Parallel analyses of several lipid fractions obtained from soil organic matter (SOM) in agricultural soils are still scarce (Stevenson, 1994, Bull *et al*., 1998), whereas numerous studies of molecular composition of SOM and plant litter in forest soils and peats exist (Jambu *et al*., 1991, 1993, Amblès *et al*., 1993, 1994a, Almendros *et al*. 1996, Bol *et al*., 1996, Marseille *et al*., 1999). Furthermore, in the existing studies usually only one plant part (e.g. leaves or stems) was used for analyses of transformation of plant residues into SOM (Lichtfouse *et al*., 1994, van Bergen *et al*., 1998). Combinations or comparisons of several plant organs for turnover rate measurements as performed by Gregorich *et al*. (1996b) are still very scarce. However, such differentiated approaches would be necessary to investigate the effects of different harvesting techniques like silage- and grain-maize cropping.

Lipids are a heterogeneous group of organic substances, operationally defined as being insoluble in water but extractable with non-polar solvents, e.g. hexane, chloroform, benzene or ether (e.g. Dinel *et al.*, 1990). They occur in plants, animals and microorganisms (Harwood & Russel, 1984). In soils they originate almost exclusively from plants and microorganisms (Kögel-Knabner, 2002). Soil lipids represent a relatively stable carbon pool in comparison to other plant-derived organic components like carbohydrates, amino acids, tannins or lignins (Lichtfouse *et al*., 1995b, Kögel-Knabner, 2002). But it is still unknown, how long lipids remain stable in agricultural soils. Additionally, soil lipids can originate from anthropogenic sources, such as petrochemicals, incomplete combustion of fossil fuels or incorporation of coal dust (Lichtfouse *et al*., 1995b).

Lipids range from simple *n*-alkanes, *n*-fatty acids or *n*-alcohols to more complex cyclic terpenoids and steroids. Until recently, the information available on the chemical composition of soil lipids was limited for two reasons. First, it was difficult to extract representative lipid materials from soils, and second, adequate techniques to characterise completely the lipid components of SOM were not available (Dinel *et al.*, 1990). Thus, extraction and separation of soil lipids were complicated and time-consuming. During the last decades, however, the advent of new analytical techniques has fostered SOM research. Work focussed on two fields of research. First, tracing the origin of individual compounds, either from biomass or from anthropogenic pollution (Berset *et al.*, 1999, Bakker *et al*., 2000, Dean & Xiong, 2000, Hubert *et al.*, 2000, Krauss *et al*., 2000, Pörschmann *et al*., 2001). The second aim was to follow SOM transformation and degradation processes, and assess carbon turnover rates (Bol *et al*., 1996, Marseille *et al.*, 1999, Bull *et al*., 2000, Cayet & Lichtfouse, 2001). Gel chromatography was used to separate soil lipids into compound classes defined by their polarity, and became the standard method to obtain more detailed information from soil lipids (Amblès *et al.*, 1993, Lichtfouse *et al*., 1995b, Bull *et al*., 2000). Analytical pyrolysis enabled the analysis of macromolecularly bound lipids and compounds not amenable to gas chromatography (Bull *et al*. 2000, Gobé *et al*., 2000, Nierop, 1998, Nierop *et al*., 2001, van Bergen *et al*., 1997). One prerequisite for reliable structural and isotopic characterisation of lipids in a complex mixture is separation into clean compound classes free of interfering material, i.e. chromatograms with baseline-resolved peaks. Clean compound classes are not only crucial for the correct identification and quantification of single compounds, but also for the proper determination of isotopic signatures (carbon, nitrogen or hydrogen) of individual compounds or compound classes. This accounts especially for components present in low concentrations and/or within complex matrices.

Dynamic analyses of plant-derived lipids in arable soils require the identification of crop-specific individual lipids or groups of lipids. It is well known that production of plant lipids strongly depends on biosynthetic metabolisms, producing the polyester cutin in aboveground

plastids of the epidermis as well as in belowground plastids with the absence of photosynthetic metabolism, producing the polyester suberin (Kolattukudy *et al*., 1976). Fatty acids are predominantly produced directly during biosynthesis, while alkanes can be produced in several ways (Kolattukudy *et al*., 1976). Mainly plant internal alkanes can be derived either by direct decarboxyliation of fatty acids or by degradation of alcohols. Several studies were previously published, where individual lipid compositions especially of epicuticular leaf waxes are shown (e.g. Bianchi & Corbellini, 1977, Bianchi & Bianchi, 1990, Bianchi, 1994). Within those studies only surface waxes, which contain most of the plant lipids, were extracted by dipping the leaves in organic solvents. Significant differences in leaf lipid distributions and isotopies were observed for different sampling times during the growing season (Bianchi, 1994) as well as sun- and shade-leaves (Lockheart *et al*., 1998). However, in most plant lipid studies it is not explained, at which time plants were sampled, and how exposure was. Winkler *et al*. (1978) observed large carbon isotopic ($\delta^{13}C$) variations during the growing season and between several plant parts. However, studies concerning plant internal and/or seasonal variations of several crop lipids from different compound classes (e.g. alkanes and carboxylic acids) were previously not published. Only few studies are available concerning bulk isotopic ($\delta^{13}C$) signatures in different plant parts of several crops (Winkler *et al*., 1978) and the development of crop isotopies during the growing season. Recently, few studies using degradation experiments were published, where large isotopic and molecular differences during decomposition of plant biomass could be determined (e.g. Nguyen Tu *et al*., 2004). Thus, it is incomprehensible, why several studies use isotopies and/or lipid distribution patterns of e.g. leaves of an undefined sampling time to analyse the transfer of lipids or bulk carbon from plant to soil (e.g. Lichtfouse *et al*., 1994, Cayet & Lichtfouse, 2001).

Several characteristic lipid distributions for different plant types were previously described. Bianchi (1994) concluded, that some compounds like β-diketones, hydroxy-β-diketones, alkan-2-ol esters as well as ketones and alcohols with the functional group in the middle of the carbon chain are components of C3-plant waxes only. More simple compounds like *n*-alkanes and the carboxylic acids as main precursors for alkanes (Bianchi, 1994) were only seldom used for plant differentiation, because distribution patterns of different plants are nearly identical and systematic differences were previously not observed. For these compound classes only Lichtfouse *et al*. (1994) observed a crop plant biomarker, where maize and wheat plants and the corresponding monoculture cropped soils could be differed by the C_{27}/C_{29}-ratio. This ratio might not work for other C3-plants than wheat (e.g. rye).

During several decades of farming, modifications of ploughing depth, cropping and fertilization cannot be avoided. Previously, these effects were not mentioned for soil organic carbon pool calculations. Plants fix atmospheric carbon during photosynthesis. Carbon

incorporation and fixation in soil happens on several ways, for example through plant litter, roots and rhizodeposition. Thus, soils react directly to plant biomass input while plant biomass responds to atmospheric carbon changes. This means that soils react indirectly to atmospheric carbon development. Hence, atmospheric changes and their influences on soil carbon have to be mentioned in analyses of soils derived from long-term field experiments. Previous studies showed that CO_2-level in the atmosphere has risen rapidly during the last decades (Amthor, 1995). CO_2 mainly derived from fossil fuel combustion and from biomass destruction has a $\delta^{13}C$ value of ~-25‰ and is thus depleted in ^{13}C (O´Leary, 1981, Friedli *et al*., 1986). This caused a decrease of $\delta^{13}C$ in the heavier atmosphere from ~–7.1 to ~–8.2 ‰ in the last four decades (compared to the rural atmospheric value of ~-6.3‰). In surface waters of marine environments changes in atmospheric carbon caused an annual isotopic $\delta^{13}C$-depletion of 0.1-0.25 ‰ for dissolved inorganic carbon (Freeman, 2001). In the terrestrial environment several investigations focused on plant biomass responses on atmospheric CO_2-changes. Biosynthetic carbon isotope fractionations in tree cellulose as a response on atmospheric CO_2 concentration changes are previously described (Ehleringer & Cerling, 1995, Feng, 1998). Biosynthetic carbon fractionation of C3-plants during photosynthesis causes a shift between –17‰ (Whelan *et al*., 1973) and –20‰ (O´Leary, 1981), resulting in $\delta^{13}C$ values between –25 and –35‰ in C3-plants (e.g. O´Leary, 1981, Ehleringer & Cerling, 1995, Hayes, 2001). The increasing partial pressure of CO_2 and the lighter atmospheric carbon isotopic composition causes a greater production of biomass (Amthor, 1995) and an amplified biosynthetic fractionation in C3-plants (Arens *et al*., 2000, Zhao *et al*., 2001). C4-plants react in a minor magnitude on atmospheric CO_2-changes (Amthor, 1995). Within C4-plants carbon isotope fractionation shows lower values of –2,5 to –3,3‰ (Whelan *et al*., 1973, Marino & McElroy, 1991, Henderson *et al*., 1992), causing plant $\delta^{13}C$ values of –9 to –14‰ (Whelan *et al*., 1973, O´Leary, 1981, Marino & McElroy, 1991, Hayes, 2001). Works concerning soil carbon changes induced by atmospheric CO_2-changes (Torn *et al*., 2002) are still scarce. Thus, exact reactions of soil carbon pools on elevated atmospheric CO_2-concentrations are unknown (Amthor, 1995). Other authors reported greater root growth due to higher atmospheric CO_2 concentrations and thus higher levels of carbon incorporation into soil (Kuzyakov, 2001). However, there are no previous results explaining how soils react on atmospheric carbon isotope changes, by the use of time-series of soil samples.

Within soil profiles and particle-size separates several trends were observed for total carbon and nitrogen as well as stable carbon isotopes ($\delta^{13}C$) as summarized by Christensen (1996). Several alteration and degradation processes of the parent biomass caused changes in stable carbon isotope contents within soil profiles. With increasing soil depth SOC $\delta^{13}C$ increased within most soils by 1-2‰ (e.g. Desjardin *et al*. 1994). These trends have been

observed when vegetation followed the C3-photosynthetic pathway (e.g. most plants of temperate climate in forests, grassland and agriculture, Desjardins *et al*., 1994, Balesdent & Mariotti, 1996, Boutton *et al*., 1998). However, the opposite trend has also been observed, when C4-plants with high $\delta^{13}C$ replaced C3-vegetation, heavy carbon replaced the old biomass to depth stepwise (e.g. Balesdent & Mariotti, 1996). Additionally, the same trend was observed, where a mixed culture of C3- and C4-plants grows on previously only C3-labelled soils e.g. after clearing of virgin forests (Krull & Skjemstad, 2003). In particle-size separates $\delta^{13}C$ often showed a systematic increase from coarse to fine separates (e.g. Balesdent & Mariotti, 1996, Bird & Pousai, 1997, Boutton *et al*., 1998). Most soil carbon isotopic analyses for particle-size separates were done on soils under pasture, and forest (e.g. Desjardins *et al*., 1994, Bird & Pousai, 1997, Bird *et al*., 2003). There exists only one study using a ploughed horizon under agriculture (Cayet & Lichtfouse, 2001), and no studies on agricultural subsoils.

With increasing particle-size and soil depth individual compounds have shown an increasing degree of chemical alteration, including polysaccharides, carbohydrates and lignin in agricultural soils (e.g. Amelung, 1997, Guggenberger *et al*., 1995). Investigations on soil lipids, however, are scarce for particle-size separates and profiles of agricultural soils, while several analyses exist concerning forest soils (e.g. Marseille *et al*., 1999). Most molecular studies of agricultural soils focused on the distribution patterns of lipid fraction, like e.g. *n*-alkanes (Lichtfouse *et al*., 1994, 1998, Wiesenberg *et al*., 2004a, 2004b), or distribution patterns of bulk lipid extracts (e.g. van Bergen *et al*., 1998). Masses of bulk lipid extract yields have been discussed rarely, except for the study of Amblès *et al*. (1994b). Similarly, distribution patterns of lipid fractions or single lipids have been seldom analysed in particle-size separates of ploughed horizons from arable soils (Cayet & Lichtfouse, 2001), and never for deeper soil horizons.

Soil colour strongly depends on mineral assemblages and soil lightness can reflect SOC content (Schulze *et al*., 1993). Thus, soil lightness might be used as additional parameter for soil characterisation. There were no systematic studies available concerning soil colour determinations of particle-size fractions within arable soil profiles.

Recently, stable carbon (^{13}C) isotope analyses have been used in combination with lipid analyses. First it was used as bulk isotopic measurements of total SOC and thereafter as compound-specific isotopic analyses of individual lipids (Lichtfouse & Budzinski, 1995, Cayet & Lichtfouse, 2001). This combination allows for the differentiation between several compound sources, to assess how fast they are incorporated into soils and to calculate their residence times in the pedosphere. It could be expected, that stability of different compounds might be significantly different (Figure 2b), while bulk carbon isotopic composition reflects a mixture of all compounds in the dynamic soil system (Balesdent & Mariotti, 1996).

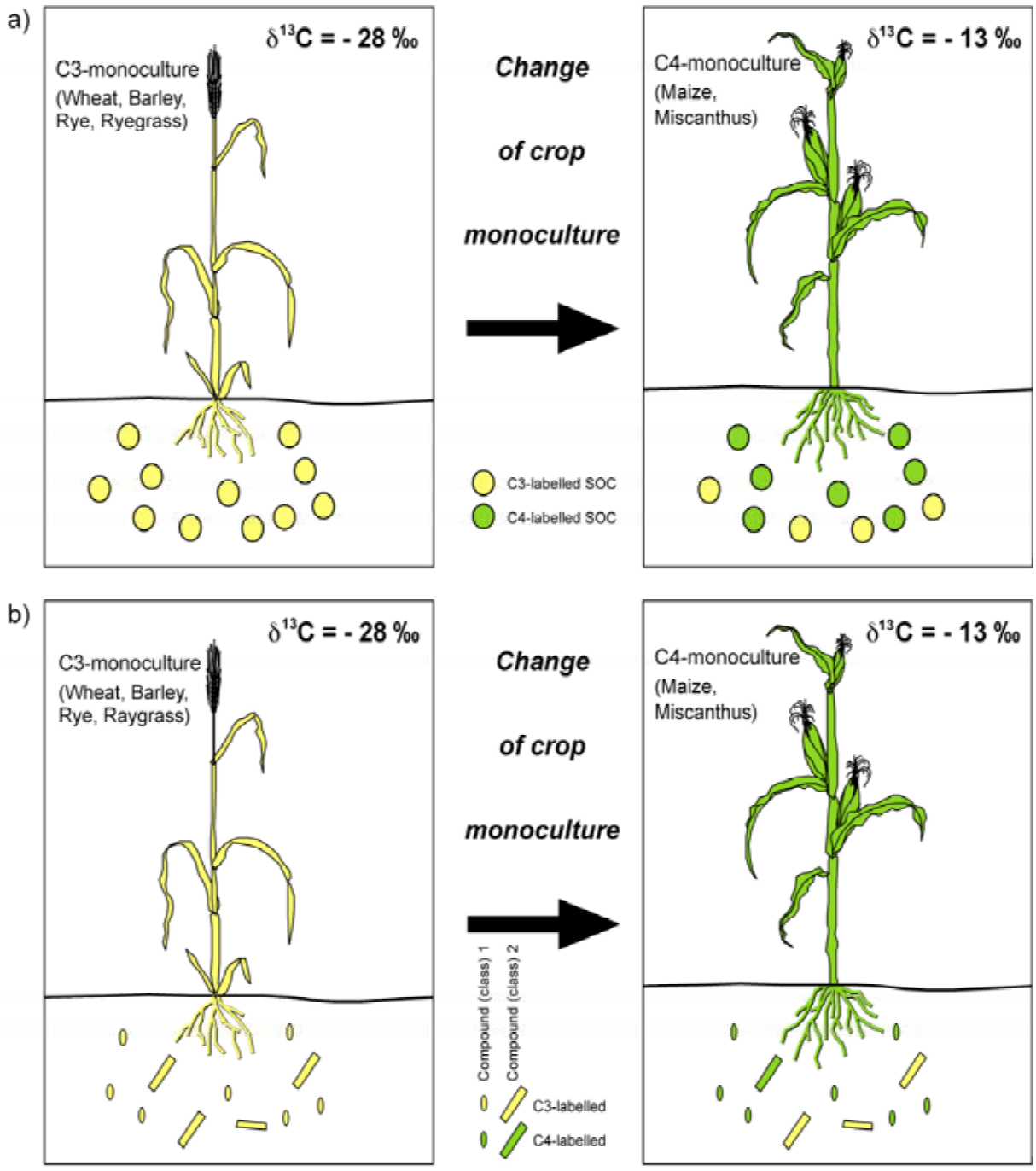


Figure 2. Effects of a C4-monoculture introduction on a previously C3-cropped soil. Parts of the soil organic matter isotopically labelled by C3-plants are coloured yellow and by C4-plants coloured green. A) For bulk carbon (shown as circles) a mixture of C3- and C4-derived carbon in soils could be determined after a defined time, leading to mixed carbon isotopic signal. B) For individual compounds or compound classes, visualised as oval and rectangular signs, different turnover times could be determined at the same time. This heterogeneous turnover can be determined by e.g. compound-specific isotope analyses (CSIA). The figure was modified after e.g. Balesdent & Mariotti (1996) and Gleixner *et al*. (unpublished).

Until now comparisons between different sites as well as different harvesting techniques were not available. Different harvesting techniques, like silage- and grain-cropping for maize, lead to variable proportions of shoot versus root biomass incorporation into soils (Anderson, 1988, Bolinder *et al*., 1997). Molecular and isotopic signatures may allow to discriminate between shoot and root biomass. For a better understanding of SOC-stabilisation molecular and compound-specific isotope data are thus of crucial importance because they allow for an assessment how different cropping methods may affect CO_2 sequestration rates and soil carbon fluxes.

Radiocarbon measurements have been helpful to classify bulk SOM separated into physically or chemically defined organic matter pools (O'Brien, 1986, Balesdent, 1987, Trumbore *et al.,* 1990, Trumbore, 1993, Trumbore & Zheng, 1996). ^{14}C concentrations of SOM pools represent the mean residence time and thus the stability of the organic matter (Scharpenseel & Becker-Heidmann, 1992, Trumbore, 1996). However, most physical and chemical SOM fractions still consist of a complex mixture of organic molecules with different origin and decomposability. Additionally, SOM properties may be influenced by the contribution of anthropogenic pollutants such as fossil fuel-derived carbon, which complicates the interpretation of the ^{14}C data (Rumpel *et al*., 2003, Rethemeyer *et al*., 2004a, 2004b).

Compound-specific ^{14}C-AMS analysis is a new technique to exclude contamination by fossil carbon sources, and to obtain information on origin and biodegradability of organic matter in soils and sediments (Eglinton *et al*., 1996, 1997, Uchida *et al*., 2000). Different 'biomarker' compounds that can be attributed to specific sources have been used to study pathways of organic carbon in soils and sediments (Hedges, 1991, Eglinton *et al*., 1997, Lichtfouse *et al.,* 1997a).

1.3 Aims

In the first part of this study, it was taken advantage of existing organic geochemical separation methods developed for lipid extraction from crude oils, petroleum source rocks, coals and sediments and adopted those for soil lipid fractionation. Soil lipids were extracted using accelerated solvent extraction (ASE). Up to 24 soil samples per day, up to 40 g each, could be extracted automatically and simultaneously at high temperatures and pressures. Compared to Soxhlet extraction (Bull *et al.*, 2000, Almendros *et al.*, 1996) or ultrasonic extraction (Lichtfouse *et al.*, 1994) ASE is much faster and of high extraction efficiency (Berset *et al*., 1999, Hubert *et al*., 2000). Additionally, there are further advantages of ASE, such as easy handling of the automated extraction and consumption of less solvent (Berset *et al.*, 1999). ASE was combined with a commercially available, automated, preparative, hetero-compound medium-pressure liquid chromatography (H-MPLC), yielding six compound

classes of increasing polarity (Willsch *et al*., 1997). The low-polarity fraction obtained during this first fractionation step was subjected to a second medium-pressure liquid chromatography (MPLC) treatment (Radke *et al*., 1980) to separate aliphatic and aromatic hydrocarbons as well as aliphatic ketones. Within a week, the automated extraction and separation procedures can yield eight compound fractions for each soil sample. So far, these modern, automated methods have not been applied to soil lipid analysis. Hence, i) mass recoveries for six individual lipid fractions, ii) reproducibility by comparing compound patterns for alkanes and carboxylic acids extracted in duplicate or triplicate, and iii) purity of individual compound classes by gas chromatography-mass spectrometry analysis (GC/MS) were evaluated.

In comparison to previous studies concerning plant lipid analyses, within relatively simple compounds like *n*-alkanes and the carboxylic acids as main precursors for alkanes (Bianchi, 1994) characteristic plant biomarkers should be found. These simple compounds were assumed to be more common in soil organic matter. A generalized biomarker was needed to differentiate between C3- and C4-plants and cropped soils. It was one main aim of this study to find a diagnostic molecular lipid biomarker to differentiate between several cereal crops. Furthermore plant lipid evolution during the growing season within crops and the transfer of lipids from crops to soil using i) distribution patterns and molecular markers and ii) isotopic signatures ($\delta^{13}C$) should be determined.

Additional to a check up of standard soil organic carbon parameters like carbon and nitrogen concentrations as well as stable carbon isotopes, soil profiles and particle-size separates were subjected to further analyses. First, total soil lipid distributions in soil profiles and size separates are studied in order to analyse, if soil lipid yields follow similar decomposition trend with soil depth, and particle-size, as observed for other chemical compound classes. In the soil samples studied here, soil colour, and especially lightness, seemed to be different on visual inspection between soils from several sites, within soil profiles. Lightness seemed to change after lipid extraction and differed between corresponding soil horizons, and particle-size separates. Systematically analyses of soil lightness in comparison with lipid carbon contents were previously not performed on arable soils. These should be determined within this study. Additionally, it should be answered, if lightness reflects carbon contents in soil profiles and particle-size separates.

Archived samples of four decades of ‘Eternal Rye’ plot with parallel cropping of C3- and C4-plants facilitate i) analysis of the parallel development of different plant species on the same rural soil and ii) a direct comparison over a period of several decades between different cropped soils. The direct comparison of the different cropped soils for each year gives the chance to avoid mistakes caused by different microclimatic changes like e.g. water

stress and times of sampling. Disregarding ploughing modifications and atmospheric changes may cause mistakes in soil carbon analyses and predictions of carbon budgets.

In this study plant-derived lipids, typical for all and individual cereal crops, respective plants following different photosynthetic pathways are identified and expressed as a diagnostic ratio. Dynamics of plant lipids and their isotopic composition as well as distributions within distinct plant parts are shown. This leads to the recommendation for sampling and analysing complete plants directly before harvesting in order to get realistic signatures of plant-derived lipids that are incorporated into soils. Turnover of soil carbon is determined for stable (^{13}C) and radioactive (^{14}C) carbon isotopes for both, bulk soils and individual lipids respective lipid fractions. These results give new insights into molecular carbon turnover in arable soils.

In addition to extensive plant analyses, bulk carbon, nitrogen and stable isotope analyses of archived samples and profiles give new insights into soil carbon dynamics. Within arable soils, atmospheric changes might cause significant long-term changes, which could be covered by soil management modifications (e.g. ploughing depth modifications). Plant-derived lipids can be found in soils in different amounts, strongly dependent on duration of monoculture cropping and harvesting techniques applied. It is demonstrated, that pollution with fossil carbon might lead to large overestimations of turnover times, derived from ^{13}C and ^{14}C analyses. Thus, it is recommended to practise turnover rate determinations with care. With the combination of several methods (GC/MS and ^{14}C-AMS) detailed informations on ages and sources of individual classes may be obtained. The results of this study confirm the state of the art that careful, lasting arable soil management like no-tillage and/or grain-harvesting or converting to grassland or forest might lead to higher carbon sequestration in soils.

Briefly, the aims of this study are: First, an adequate, affordable, fast, effective and reproducible extraction and separation scheme was needed to obtain several interference-free lipid fractions of well-defined contents, which could be used for further detailed analyses. Second, new diagnostic biomarkers within the plant lipids should be found, to differentiate between several plant types like maize vs. rye or wheat and the soils under these monocultures. Third, it should be analysed, how SOM might reflect the atmospheric Suess effect, using archived soil samples of the same plot from several decades. Fourth, turnover of total soil organic carbon and, as a new method, of different soil lipids should be obtained by measuring stable ($\delta^{13}C$) and radioactive (^{14}C) carbon isotopes. Finally, the relevance of soil lipids for the terrestrial carbon cycle should be defined, resulting in the answer, whether soil lipids are part of the stable carbon pool in soils and which role soil lipids play for CO_2 fixation or release in soils.

2. Sampling sites

For the analyses of soil organic carbon, lipid dynamics, and plant-derived contributions in agricultural soils, sampling sites with well-documented cropping history were needed. Additionally, crop changes from continuous C3- to continuous C4-cropping must be established on the sampling sites, because those crops have similar physiological and molecular properties, except for photosynthesis mechanisms, leading to another biosynthetic isotopic carbon fractionations in plants. Thus, soils get naturally labelled and isotopic differences facilitate turnover time determinations based on stable carbon isotope analyses. To exclude contributions of organic fertilizers like manure, leading to uncertain changes especially in lipid distribution of soils, only long-term field experiments with mineral fertilization (e.g. N or NPK) were chosen. Only few long-term agricultural trials were available in Central Europe and especially in Germany, which fulfilled all prerequisites.

Except for Boigneville site (Bo), which is located in France 70 km south of Paris, all sampling sites are situated in Germany (Figure 3). Several sites are situated in the heavy industrialized surrounding area of Halle/Seeben (Beuna, Seeben) or directly in the city of Halle ('Eternal Rye' trial, Halle). Contrastingly, sampling sites of Scheßlitz, which is located between Bamberg and Bayreuth, and Rotthalmünster, which is situated near Passau, represented rural areas. Thus, in addition to crop changes, different contributions of fossil carbon due to pollution could be expected in soils of different sites. For characterisation of possible pollution of soils from the Halle area, brown coal samples from Beuna were analysed. Sample types derived from each site and climatic as well as soil properties of the sampling sites are shown in Table 1.

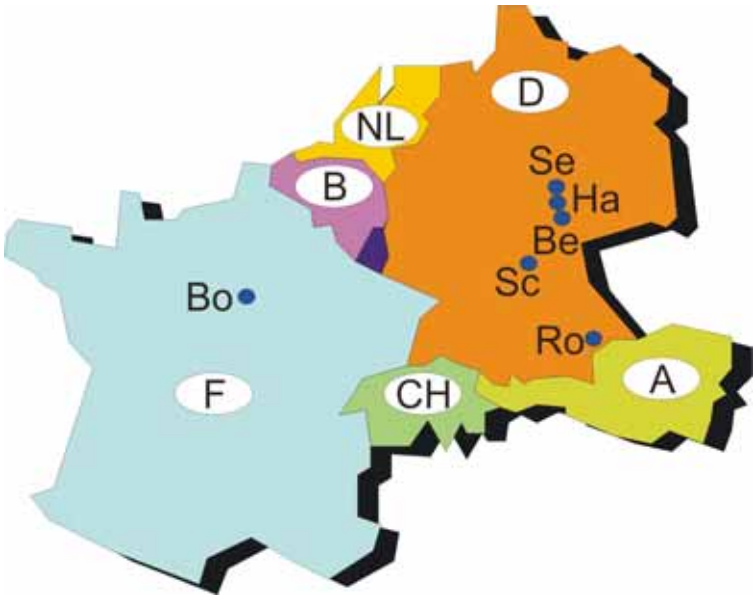


Figure 3. Sampling sites: Be = Beuna, Bo = Boigneville, Ha = Halle, Ro = Rotthalmünster, Sc = Scheßlitz, Se = Seeben.

Table 1. Sampling sites.

	Soil type[a]	Mean annual temperature [°C]	Mean annual precipitation [mm]	Soil pH	Samples[b]
Beuna $R^{44}97450\ H^{55}68663$	-	-	-	-	B
Boigneville $R^{00}37500\ H^{53}71500$	Dystric Cambisol	10.1	640	5.8	S
Halle $R^{44}99750\ H^{57}06800$	Haplic Phaeozem	9.2	465	5.7	P, S
Rotthalmünster $R^{45}88950\ H^{53}58760$	Stagnic Luvisol	8.7	886	6.7-7.1	P, S
Scheßlitz $R^{44}31450\ H^{55}38550$	n.d.[c]	8.2-8.8	633-724	n.d.	P
Seeben $R^{44}98888\ H^{57}10363$	Haplic Phaeozem	9.0	480	5.9	S

[a] According to FAO-UNESCO (1994).
[b] Samples taken at sampling site: B = Brown coal, P = Plants, S = Soil.
[c] Not determined.

Halle site was one of the most important sites for this study. Samples were derived from the Julius-Kühn-Field of the University of Halle-Wittenberg. The field was established in 1866 and the 'Eternal Rye' plot (70.6m x 85.2m) was introduced in 1878. Detailed descriptions of the sampling site and the history of the field are published e.g. by Stumpe *et al*. (1990), Merbach *et al*. (1999, 2000), Schmidt *et al*. (2000). Only a short description of the site is given here. Since 1878 soils were cropped with rye monoculture and on six different strips different fertilizers were applied. In 1961 the plot was subdivided into three parts of identical size with i) further rye monoculture cropping, ii) rye-potato crop rotation, and iii) silage-maize monoculture cropping. The fertilization experiments were then practised on each part of the plot. During silage-maize cropping, most of the aboveground biomass is removed, leaving only the lowermost parts of stems (up to 15 cm height) and all of the root biomass on the field. Alternatively, during grain-maize cropping only cobs are harvested, leaving most of the aboveground biomass and all of the root biomass on the field. A part of fallow land (1.5m x 25m), directly situated beneath the silage-maize plot was converted to grain-maize monoculture cropping in 2001. It was assumed, that this plot was cropped with rye monoculture until 1961 and then converted to fallow land. All samples from Halle site were derived from rye and silage-maize monoculture cropping with mineral NPK (nitrogen, phosphorous, potassium) fertilizer or from the grain-maize plot without fertilization.

The experimental station of the Agriculture School in Rotthalmünster was established in 1960. In this year the whole plot was converted to grassland. In 1969 a part of the grassland was converted to winter wheat monoculture cropping. 1979 grain-maize monoculture cropping was introduced on another part of the grassland plot. The introduced crops are

used for several experiments including herbicide and fungicide experiments as well as long-term influences of monoculture cropping on yields during harvest. Soils were not available for this site. The fact, that wheat was not cropped on the same site prior to conversion to maize, possible differences could be expected between wheat and maize cropped soils, because of lateral soil heterogeneities. Nevertheless, both soils were predominantly marked by C3-grasses, prior to conversion into wheat and maize cropping. On both soils all biomass, except for ears respective cobs, is left on fields during harvest, leading to high biomass incorporation into soils. Thus, similar soil properties and high labelling by the individual biomass satisfied the use for e.g. turnover time determinations.

The experimental field of Boigneville was previously described in detail by Balabane & Balesdent (1992). In 1970 a plot, previously cropped with C3-plants was converted to wheat and grain-maize monoculture cropping. The long-term field experiment was finished in 1993. Thus, only archived soil samples from 1993 were available and younger soil samples as well as plant samples were not available. A lot of studies were published from this site and experiment, concerning lipid dynamics in soils (e.g. Lichtfouse *et al*., 1994, 1995b, Lichtfouse, 1997, Cayet & Lichtfouse, 2001). As a result of the well-documented properties of the soils and the lipid components therein, the soils of this site provided ideal conditions, to compare the results to those obtained from soils of Halle and Rotthalmünster sites. Additionally, the effectiveness of extraction procedures and the reliability of analyses like e.g. compound-specific isotope analyses could be checked.

Brown coal samples of Beuna site were either derived from the main seam (named brown coal) or the briquette from a nearby briquette factory. Seeben soil sample was taken randomly on an agricultural plot, where a Haplic Phaezem could be determined, which was assumed to be unpolluted (Schmidt, 1998). All samples were taken during the 1990s. Seeben sample was only used for the method evaluation, because information about crop changes and cropping techniques applied were not available. From Scheßlitz site only one maize plant was collected on a randomly chosen field, to compare maize plants grown on agricultural trials with plants grown on other fields. Thus, no soil and climate conditions were available. The data in Table 1 for this site represent means of data derived of weather stations from Bamberg and Bayreuth, because the site is situated between both cities.

3. Materials

As described above, preferentially long-term experimental trials with C4-monoculture cropping on a previously C3-cropped soil and a parallel reference C3-monoculture cropping were chosen as sampling sites. In Central Europe several C3-plants are cropped as monocultures, whereas usually maize is introduced as C4-monoculture plant. Other C4-monoculture crops are very scarce in this region. Only Miscanthus was found as alternative crop to maize, which is subject of ongoing studies and thus not mentioned here.

3.1 Plants

Several plants were taken from Rotthalmünster and Halle sites at different times during the growing season to analyse seasonal variations within plant parts (Table 2). From Scheßlitz only one single plant was taken at one sampling time. Generally, plants were separated into different parts like roots, stems and leaves after sampling and drying.

Table 2. Plant samples.

Sampling site	Plant	Sampling year	Sampling month	Roots	Stems	Leaves
Halle	Rye (*Secale cereale* (L.))	2001	March		X[b]	X[b]
		2002	May	X	X	X
			June	X	X	X
			July	X	X	X
	Maize (*Zea mays* (L.))	2001	March	X[a]	X[a]	
		2002	June	X	X	X
			July	X	X	X
			August	X	X	X
			September	X	X	X
Rotthalmünster	Wheat (*Triticum aestivum* (L.))	2002	May	X	X	X
			June	X	X	X
			September		X[abc]	X[abc]
	Maize (*Zea mays* (L.))	2002	May	X	X[b]	X[b]
			June	X	X	X
			August	X	X	X
			September	X	X	X
Scheßlitz	Maize (*Zea mays* (L.))	2001	July	X[d]	X	X

[a] Degraded biomass collected on the agricultural plot.
[b] Stems and leaves could not be differentiated and thus prepared together.
[c] Straw.
[d] Coarse and fine roots analysed separately.

Maize (*Zea mays* (L.)) and wheat (*Triticum aestivum* (L.)) plants from Rotthalmünster site were sampled during growing season in 2002 (Table 1). Complete wheat plants were only available from May and June. Harvesting was practised on this plot in July. Wheat straw was left on the field after harvesting and a straw sample was collected in September 2002 on the same plot. Maize plants were sampled in May, June, August and September 2002. Plants from May were sampled only few weeks after shooting and were only ~30cm of height. Hence, stems and leaves could not be differentiated. Therefore, they were prepared and analysed together for this sampling time. The last sample of September 2002 was taken two weeks before harvesting.

'Eternal Rye' trial was sampled for rye (*Secale cereale* (L.)) and maize (*Zea mays* (L.)) at several times during growing season 2002. Rye plants were sampled in May, June and July 2002. Additionally, leaves of very young shoots were sampled in March 2001. Samples from maize were taken in June, July, August and September in 2002. Latest samples were taken only one week before harvesting. Degraded stems and roots that were left on the field after harvesting were collected on the plot in March 2001.

Only one maize plant was sampled in July 2001 on an agricultural plot in Scheßlitz to analyse the diversity of maize plants, independent of sampling site. Additionally to the differentiation between plant parts like leaves, stems and roots for this plant, roots were divided into coarse roots, situated directly at the stem and several mm in diameter, and fine roots, growing at the end of the coarse roots and less than 1 mm in diameter.

Within most figures in this study, where plant parts from several growth stages were shown, they were numbered in the order of the sampling month corresponding to Table 2 (e.g. 3 = March, 5 = May, and so on).

3.2 Soils

Soils from different sampling sites were collected for different objectives of studies. From Boigneville only ploughed horizons of wheat and grain-maize plots were available, because the trial was closed in 1993 as described above and no further samples were stored. Contrastingly, Rotthalmünster and Halle experimental sites have been managed until the present and thus different soil horizons of C3- and the corresponding C4-plots were available (Table 3). From Halle site rye, silage- and grain-maize cropped soils were taken and archived soil samples from rye and silage-maize sites were available. Rotthalmünster site was sampled for wheat and grain-maize cropped soils. From Seeben only the ploughed horizon of an agricultural plot with a mixed culture of several C3-plants and maize was sampled.

Table 3. Soil samples.

Sampling site	Soil type [a]	Monoculture	Sampling year	Years of maize cropping	Horizon	Depth [cm]
Boigneville	Dystric Cambisol	Wheat	1993	0	Axp	0-20
		Grain-maize	1993	23	Axp	0-20
Halle	Haplic Phaeozem	Rye	1958	0	Axp	0-20
			1961[b]		Axp	0-20
			1961[c]		Axp	0-20
			1963		Axp	0-20
			1965		Axp	0-20
			1967		Axp	0-20
			1969		Axp	0-25
			1974		Axp	0-25
			1977		Axp	0-25
			1990		Axp	0-25
			1995		Axp	0-30
			2000		Axp	0-30
			2001		Axp	0-30
					Axh	30-60
					Bv	60-70
		Silage-maize	1961[c]	0	Axp	0-20
			1963	2	Axp	0-20
			1965	4	Axp	0-20
			1967	6	Axp	0-20
			1969	8	Axp	0-25
			1974	13	Axp	0-25
			1977	16	Axp	0-25
			1990	29	Axp	0-25
			1995	34	Axp	0-30
			2000	39	Axp	0-30
			2001	40	Axp	0-30
					Axh	30-50
					Bv	50-70
		Grain-maize	2001	0	Axp	0-30
					Axh	30-49
					Bv	49-69
			2002	1	Axp	0-30
					Axh	30-50
Rotthalmünster	Stagnic Luvisol	Wheat	2002	0	Axp1	0-30
					Axp2	30-40
					Sw-M	40-55
					BtSd	55-75
		Grain-maize	2002	23	Axp1	0-30
					Axp2	30-45
					Sw-M	45-64
					BtSd	64-103
Seeben	Haplic Phaeozem	Various crops	1993	n.d.[d]	Axp	0-20

[a] According to FAO-UNESCO (1994).

[b] Before subdivision of 'Eternal Rye' trial.

[c] After subdivision of 'Eternal Rye' trial.

[d] Not determined.

Wheat and grain-maize cropped soils of Rotthalmünster site were sampled in September 2002 after 23 years of continuous maize cropping on a previously C3-labelled soil. Four horizons (Axp1, Axp2, Sw-M, BtSd) could be separated from each plot. Varying sampling depth caused different depths of the BtSd horizons of both plots.

From ‘Eternal Rye’ trial in Halle archived soil samples of the ploughed horizons were available since 1958 for the rye plot and since the introduction of continuous silage-maize cropping (1961) parallel samples were available for rye and silage-maize plots. In contrast to the Rotthalmünster site silage-maize cropping was practised. While grain-maize harvesting leaves most of the biomass on the field and only cobs are removed, during silage-maize harvesting most aboveground biomass is removed and only lowermost parts of stems and roots remain on the plot. Additionally, Prof. Merbach and Dr. Schmidt provided the possibility to introduce grain-maize cropping on a part of the ‘Eternal Rye’ trial. It was introduced in 2001 on fallow land directly beneath the silage-maize plot. Perhaps, the fallow land was slightly C4-labelled by root biomass or plant litter of the nearby silage-maize plot. Fresh soil samples of the three soil horizons (Axp, Ah, Bv) were taken in March 2001 from the rye and the silage-maize cropped plots and in September 2001 from the grain-maize plot. The soil samples of the year 2002 were also taken in September.

From Boigneville and Seeben sites samples were taken of the ploughed horizons during former studies in 1993. In Boigneville grain-maize and wheat cropping were practised similar to the Rotthalmünster site on neighboured plots for 23 years. On the soil from Seeben crop rotation was practised, thus it was cropped with various C3-plants and additionally with maize in some undefined years. This soil was only comparatively analysed for method establishment in addition to the soils with monoculture cropping of the other trials.

3.3 Brown coal

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

4. Methods

Fresh plant and soil samples were stored in a freezer (-27°C) until further treatment or were air-dried directly after sampling on the experimental trials. Frozen samples were freeze-dried (Steris Lyovac GT-2). During crushing of coarse soil aggregates with a pestle and mortar macroscopically visible plant fragments and black particles were removed from soil samples. Thereafter soil samples were dry-sieved over a 2 mm sieve. Plant samples were fractionated into small pieces using a chaffcutter or secateurs.

4.1 Photo-spectrometry

Aliquots of soil samples before and after lipid extraction, except for the silt-size fraction of the Axh horizon from rye cropped soil before extraction from 'Eternal Rye' trial in Halle, were fine ground with pestle and mortar. Non-destructive colour measurements were performed using a Minolta CM-2002 photo-spectrometer. Colours were measured in the L*a*b*-System, with L* as the lightness of a colour, a* as the red-green proportion and b* as the yellow-blue proportion. The results were converted to the Munsell system, which is most familiar to soil scientists (Schulze *et al*., 1993). The colour system based on three attributes: (i) hue (an attribute of colour perception), (ii) value (lightness), and (iii) chroma (difference from black-white or neutral colour). In this study means of triplicate determinations for each sample of Munsell values are presented. Standard deviations of Munsell values were lower than 0.05 for all samples and thus smaller than symbol size in diagrams. Munsell values before and after lipid extraction of 35 samples were tested for statistically significant differences. Differences were not normally distributed and thus, the Wilcoxon rank sum test was selected, a non-parametric test comparing differences for paired samples by calculating differences (Δ) of absolute values as

$$\Delta x = x_{before} - x_{after} \quad (1)$$

where x stands for the Munsell value before, respectively after lipid extraction. As a result, differences were statistically significant (P = 0.0001).

4.2 Particle-size separation

Soils from the Axp, Axh and Bv horizons from rye and silage-maize plots of Halle ‚Eternal Rye' trial were preparatively separated into three particle-size separates (clay, silt, sand), by combining wet sieving and sedimentation after sequential ultrasound dispersion. The ultrasonic energy of a Branson ultrasonic titanium probe with a diameter of 12.7mm was calibrated. According to Ludwig *et al*. (2003) 60J ml^{-1} of suspension were applied to destroy macroaggregates (63-2000µm). Sand (63-2000µm) was separated from the suspension by wet sieving. To disrupt micro- and mesoaggregates, ultrasonic energy of 440J ml^{-1} of

suspension was applied. Coarse silt (20-63µm) was also removed from the suspension by wet sieving. Remaining clay and silt were separated by gravity sedimentation, and clay (0.45-2µm) was recovered by pressurized filtration (cellulose nitrate, 0.45µm). The soluble fraction (<0.45µm) was not recovered. All separates were oven-dried at 40°C. Preparative particle-size separation was repeated at least ten times for each soil horizon, and separates of each horizon were combined and homogenized.

4.3 Elemental analyses

All samples were measured for total organic carbon content (TOC) after decarbonatisation with HCl (10% v/v) using a Leco CS-225 analyser. Additionally, all samples were measured for total carbon (TC) and total nitrogen (TN) contents using a Heraeus Vario EL analyser.

All samples were analysed for isotopic carbon composition on the CO_2 obtained by combustion in a continuous flow sample preparation system (Robo Prep-CN). The purified CO_2 from the Robo Prep-CN was analysed with a Europa Scientific Tracer Mass Classic mass-spectrometer. Carbon isotope compositions are expressed in permil relative to the Vienna Pee Dee Belemnite standard:

$$\delta^{13}C = [(^{13}C_{sample} / {}^{12}C_{sample}) \times (^{13}C_{standard} / {}^{12}C_{standard}) - 1] \times 10^3 \quad (2)$$

where $^{13}C \times {}^{12}C_{standard}^{-1} = 0.0112372$.

4.4 Lipid extraction

For the extraction of free soil lipids, an accelerated solvent extractor (Dionex ASE 200) was used (Figure 4). Stainless-steel extraction vessels were filled with up to 30g of dried soil or plant samples, with a plug of pre-extracted glass wool between two cellulose filters applied at both ends. Free lipids were extracted with dichloromethane/methanol (93/7; v/v) at 5×10^6 Pa and a temperature of 75°C. The heating phase required five minutes and static extraction time was 20 minutes. Extraction was repeated under identical conditions except for a higher temperature (140°C), which required a heating phase of seven minutes. Both extracts were combined. For most soil samples lipid extract contents of only one filled ASE vessel were too low for detailed analyses. Thus, up to six vessels per soil sample were extracted and extracts were combined. Extract yields were determined gravimetrically.

Additionally to free soil lipids, bound soil lipids were extracted from ASE extraction residue by saponification with methanolic KOH. Bound lipid extract yields were approximately twice as high as free lipid extract yields. Predominantly short-chain compounds were derived during this extraction procedure. Only very low amounts of plant-derived long-chain

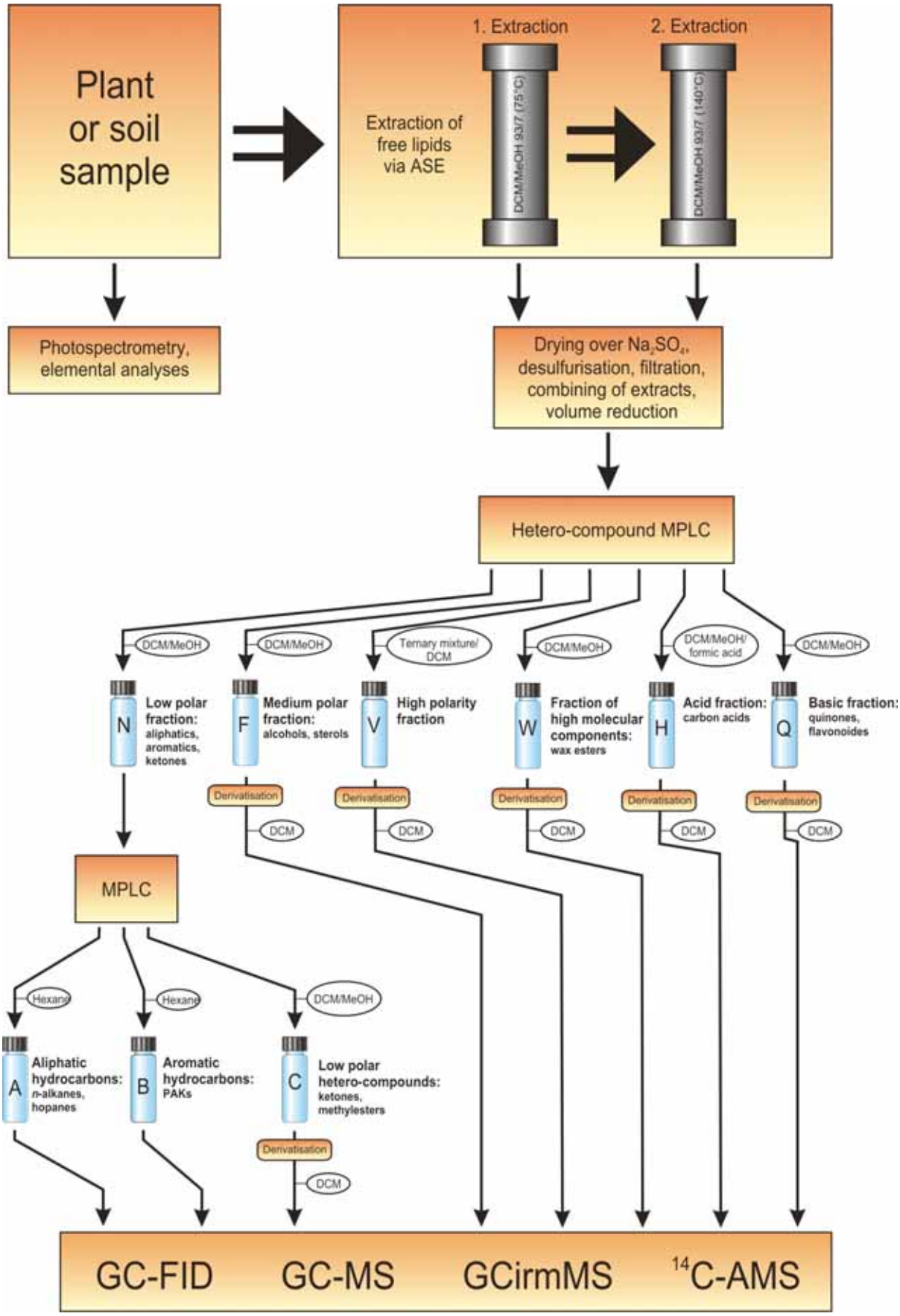


Figure 4. Analytical flow-chart of free lipid extraction and separation procedure (modified after Lüniger, 2002).

compounds of alkanes and carboxylic acids were extracted, without a significant predominance of odd or even compounds in contrast to free lipids. Recovery of bound lipid extracts and reproducibility of bound lipid distribution patterns were very low. Thus, bound lipids were only extracted and separated exemplarily for a few soil and plant samples, where no possible diagnostic lipid marker could be obtained. These facts led to the consequence that only few samples were extracted and analysed for bound lipid composition. The results were not discussed in detail in this study, because of their insignificance.

4.5 Separation of lipid fractions

Total lipids were sequentially separated into eight fractions of different polarity according to Wiesenberg *et al*. (2004a), as shown in Figure 4. A hetero-compound medium-pressure liquid chromatography separation (H-MPLC) described by Willsch *et al*. (1997) yielded six fractions. The low polarity fraction was then re-chromatographed using the medium-pressure liquid chromatography separation scheme (MPLC) described by Radke *et al*. (1980) to yield three additional fractions.

In the first separation step, extracts were dissolved in dichloromethane, injected onto the H-MPLC and passed through three pre-columns. The first pre-column was filled with neutral silica gel, the second and third were filled with KOH- and HCl-modified silica gels, respectively. Highly polar and high molecular weight (HMW) compounds (e.g. carbohydrates, proteins) remained on the neutral pre-column. The HCl-modified pre-column retained the basic compounds and the KOH-modified pre-column retained organic acids as their intermediate salts. Low and intermediate polarity compound classes were separated on a main column filled with activated silica gel using defined dichloromethane elution volumes.

This separation scheme produces six fractions: i) a low polarity fraction containing aliphatic and aromatic hydrocarbons as well as acyclic ketones; ii) an intermediate polarity fraction comprising straight-chain and branched alcohols and sterols; iii) a carboxylic acid fraction; iv) a fraction of organic bases; v) a high polarity and/or HMW fraction containing very long-chain wax esters and vi) a polar fraction of still undefined content. All fractions were volume reduced using rotary evaporation. Thus, the separation yielded polarity-defined lipid fractions, with each fraction corresponding to a specific chemical compound class. Separation was achieved either by different modifications of silica gel in pre-columns, while low and intermediate polarity fractions were separated by defined solvent elution volumes.

The low polarity fraction was further separated using the MPLC-procedure described by Radke *et al*. (1980). The fraction was dissolved in hexane and injected on a pre-column filled

with deactivated silica gel. Low polar hetero-compounds, like ketones, are retained on the pre-column. After disconnecting the pre-column they were eluted with DCM/MeOH (93/7; v/v). Aliphatic and aromatic hydrocarbons were transferred onto the main column. Here aliphatic hydrocarbons were collected after passing through the column, whereas aromatic hydrocarbons were recovered by back-flushing. Volume reduction was performed via a turbo vaporiser (Zymark). Fraction yields were determined gravimetrically.

4.6 Gas-chromatography and mass-spectrometry

For identification and quantification, defined amounts of various deuterated standards (d_{50}-*n*-C_{24} alkane, d_{10}-anthracene, 1,1'-binaphthalene, d_4-cholestane, d_{37}-*n*-C_{18} alcohol, d_{39}-*n*-C_{20} carboxylic acid) were added to the corresponding MPLC and H-MPLC fractions. Compound identification was performed on a HP 5890 Series II gas-chromatograph (GC) coupled with a HP 5989A mass-spectrometer (MS). For quantification, a HP 5890 Series II GC equipped with a flame ionisation detector (FID) was used. Gas-chromatographs were equipped with different columns (50m x 0.25 mm ID, 0.25mm FT): Agilent DB1-HT (for aliphatic hydrocarbon analysis), DB5-MS and HP5-TA (for analysis of aromatic hydrocarbon, ketones, alcohols, carboxylic acids and total lipid extract). Sample injection was done via a HP 7673 autosampler in splitless mode at 50°C. Temperature was held constant for two minutes and then ramped to 140°C at 10°C per minute. Aliphatic hydrocarbons were then ramped to 350°C at 3°C per minute; all other fractions were programmed to 320°C at 3°C per minute and held at final temperature for 20 minutes. While aliphatic hydrocarbons, aromatic hydrocarbons and ketones were measured unmodified, selected lipid fractions (alcohols, carboxylic acids) and total lipid extracts were derivatised with BSTFA (N,O-bis(trimethylsilyl)trifluoracetamide) and compounds were detected as TMS (trimethylsilyl) derivates. High polarity and HMW-fractions (bases, HMW wax esters, high polarity fraction of undefined content) were not completely amenable to standard GC/MS analyses.

Compound-specific isotope analyses (CSIA) of individual aliphatic hydrocarbons and carboxylic acids were carried out under a continuous helium flow using an Agilent 6890 gas-chromatograph coupled to a Finnigan GC combustion unit and a Finnigan DeltaPlusXL mass-spectrometer. Ion currents were monitored continuously (*m*/*z* = 44, 45 and 46). Carboxylic acids were derivatised with BF_3-Methanol (10%, w/w) and corrected for the isotopic signature of the introduced methyl group. All measurements were done at least in triplicate. When reporting weighted averages of CSIA of several compounds the mean of the three most abundant compounds (δ_M) normalised to the proportion of each compound was calculated as follows:

$$\delta_M = (A \times \delta_A) + (B \times \delta_B) + (C \times \delta_C) \quad (3)$$

with A, B, C as the relative proportions of three most abundant compounds and δ_A, δ_B, δ_C as the $\delta^{13}C$ isotopies of the most abundant compounds. All CSIA were carried out at the Institute of Geology and Geochemistry of Petroleum and Coal of the RWTH Aachen.

4.7 Radiocarbon dating

The lipid compound classes were dissolved again using dichloromethane and methanol, depending on the polarity of the fraction. Lipid classes were pipetted into pre-combusted (4h, 900°C) quartz combustion tubes. The solvent was removed by evaporation over night and in case of incomplete removal under a gentle N_2 stream. Samples with carbon contents of >500µg were combusted with 450mg CuO and 150mg silver wool. For combustion of small samples containing <500µg of carbon, reduced portions of 75mg CuO and 30mg silver wool were added. The tubes were evacuated to a pressure of ca. 10^{-4} mbar while immersed in dry-ice/ethanol to avoid possible loss of highly volatile compounds and subsequently flame sealed. Samples were combusted at 900°C for four hours. The produced CO_2 was collected in a cold trap with liquid nitrogen and subsequently reduced to graphite with a 10% excess of hydrogen at 600°C over an iron catalyst (Nadeau *et al.*, 1997, 1998). ^{14}C data are expressed as percent modern carbon (pMC) as described by Stuiver & Polach (1977) with 1‰ measurement uncertainty. The measurement precision was in the range of 0.3pMC for modern, standard-sized (ca. 1mg carbon) samples (Nadeau *et al.*, 1998). The AMS measurements were carried out at the Leibniz Laboratory in Kiel (Germany).

4.8 Calculation of new plant-derived carbon and turnover times in soils

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

$$F_{C4} = (\delta_{C4soil} - \delta_{C3soil}) / (\delta_{C4plant} - \delta_{C3plant}) \quad (4)$$

where $\delta_{C4plant}$ and $\delta_{C3plant}$ are the isotopic signatures of C4- and C3-plants. δ_{C4soil} and δ_{C3soil} are the isotopic signatures of the C4-cropped soil and the original C3-cropped soil. Alternatively, δ_{C3soil} is taken equivalent to a reference site kept under the initial vegetation (Balesdent &

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

$$F_{C3} = F_{C3\,t0} - F_{C4} \quad (5)$$

with $F_{C3\,t0}$ as the fraction of C3-carbon at the time of conversion (t0) to C4-cropping. Because all plots presented in this study were managed for several decades with C3-crops prior to continuous corn cropping, it could be assumed that SOC was completely C3-labelled (Balesdent & Mariotti, 1996) and thus $F_{C3\,t0} = 1$.

Calculations of new C4-carbon proportions based on natural stable isotope labelling in soils are widely used to calculate total soil carbon budgets (e.g. Balesdent *et al*., 1987, Collins *et al*., 1999, Cayet & Lichtfouse, 2001). Simple assumptions are based on bulk soil carbon turnover with only one carbon pool of uniform turnover (e.g. Balesdent *et al*., 1987). Contrastingly models with several carbon pools of different turnover exist (e.g. Jenkinson & Rayner, 1977, Huggins *et al*., 1998, Paul *et al*., 2001). SOC decomposition is assumed in most models to follow first order kinetics at steady state conditions (Balesdent & Mariotti, 1996, Huggins *et al*., 1998). The decomposition of SOC per time unit was introduced as turnover rate equivalent to the decay rate or decomposition rate (k) and can be calculated (Huggins *et al*., 1998, Collins *et al*., 1999) as:

$$k = \ln (F_{C3} / F_{C3\,t0}) / (t - t0) \quad (6)$$

with the remaining F_{C3} in the soil at time t. Based on this calculation the turnover time (T), which is used synonymously to the mean residence time (MRT) of organic carbon in soils can be calculated (Huggins *et al*., 1998, Collins *et al*., 1999) as:

$$T = 1/k \quad (7)$$

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

5 Results and discussion

In the following chapter results obtained during different analyses of plant and soil samples from several sampling sites are shown and discussed.

• First, the adoption and evaluation of lipid extraction and fractionation procedures from rock, oil and sediment samples to soil samples is discussed. It is also demonstrated, how reproducible this method is, by showing recoveries of selected lipid fractions and their distribution patterns. The establishment of an automated, high reproducible, short and inexpensive extraction and separation procedure into soil lipid analyses was needed to i) obtain clean, interference-free fractions for e.g. compound-specific isotope analyses and ii) preparing and analysing numerous samples in a short time.

• Second, lipid contents, distribution patterns and stable carbon isotopic compositions of several plant parts and their development during the growing season are analysed and discussed, to obtain diagnostic biomarkers for the differentiation between several crops as well as molecular and isotopic end-member for the incorporation of biomass into soils. For turnover time calculations of plant biomass into soils adequate compounds were needed to find, which were derived mainly by recent plants and not by other organisms, as e.g. fungi and bacteria. Recently, no typical molecular biomarkers were known, characteristic for distinct crops, facilitating a differentiation of several crops in soils. Thus, extensive analyses of different plant parts are shown here for selected plants, which grew on the long-term field trials subjected to soil lipid analyses.

• Third, soil carbon and lipid dynamics over four decades are discussed, to analyse, how soils developed over several decades and which developments are expected, when regarding probable influences of ploughing depth modifications or atmospheric Suess effect. Previously, no studies were published, regarding the parameters shown here over several decades, using archived soil samples. Thus, it is unknown, how soil carbon dynamics (carbon distribution and isotopes) are influenced by the atmospheric CO_2 enrichment and stable carbon isotopic (^{13}C) depletion as a result of fossil fuel burning (Suess effect).

• Fourth, detailed analyses of soil profiles and particle-size fractions are shown. Bulk parameters are discussed in the beginning, to characterize soils and particle-size fractions. Thereafter lipid distribution patterns are presented and diagnostic molecular plant biomarker are discussed for the soils, leading to an estimation of C3- and C4-plant-derived proportions in the soils. Finally in this chapter, results of compound-specific carbon isotope analyses of the same compounds are discussed, which were previously obtained as typical for distinct plants.

• Fifth, several plant parts have different lipid distribution patterns and stable carbon isotope contents. Here it is shown, which effects are caused, when new carbon proportions and turnover times for bulk carbon or for different lipids are calculated only with one plant part e.g. leaves. It is demonstrated that especially for CSIA derived turnover time calculation a reliable estimation of plant input is needed to calculate realistic turnover times.

• Sixth, results of radiocarbon analyses of soils and lipid fractions are shown. In contrast to stable carbon isotope analyses, the radiocarbon analyses facilitate a direct differentiation between samples of rural or urban sites with distinct amounts of fossil, ^{14}C depleted carbon. Hence, with radiocarbon analyses of lipid fractions a distinction between more or less contributions of fossil carbon is possible. In combination with lipid distribution patterns of these fractions, a differentiation between several recent or fossil sources is shown. Additionally, informations are obtained from these analyses, which lipid fractions contain nearly no fossil carbon and are thus best suitable for turnover time calculations based on ^{14}C or ^{13}C analyses.

• Finally, new results of this study are shortly concluded and it is summarized, which additional information resulted from this study to the terrestrial carbon cycle.

5.1 Method evaluation

The results shown in the following chapter were previously published in the paper Wiesenberg *et al*. (2004a). Automated extraction and separation procedures, established for organic geochemical analyses of e.g. sediments, oil and rock samples, were adopted for soil samples and checked for reproducibility.

Methods were evaluated, using the ploughed A horizons (Axp) of five arable soils (Table 4). Two soil samples were derived from Boigneville (B); one permanently cropped with wheat (w), the other with maize (m) for 23 years. All other soils were derived from the area around Halle/Saale, Germany. A Haplic Phaeozem cultivated with various crops (v) was taken in 1993 from an agricultural plot north of Halle/Saale near Seeben (S). Two soils, also classified as Haplic Phaeozems, were sampled in 2000 from the 'Eternal Rye' trial near the centre of Halle/Saale (H). One plot was permanently cropped with maize (m) and the other with rye (r) for 39 years. Soils from experimental plots Boigneville and Halle were previously described in detail (Balabane & Balesdent, 1992, Merbach *et al*., 1999, 2000, Flessa *et al*., 2000). Fresh soil samples were stored in a freezer (-27°C) until further treatment. After freeze-drying (Steris Lyovac GT-2), samples were crushed with a pestle and mortar, and dry-sieved over a 2 mm sieve.

To test for reproducibility, soils from each sampling site were extracted and separated in triplicate; named sample set 1, 2, and 3. Sample sets 1 and 2 each correspond to three ASE vessels (90 g extracted soil), whose extracts were combined for each sample set. In contrast, sample set 3 corresponds only to one filled and extracted ASE vessel (30 g extracted soil).

Table 4. Soil characteristics.

Locality	Soil type [a]	Crop	Lab code	Horizon	Depth [cm]	Particle size distribution			TOC[e] [g kg^{-1}]	TN[f] [g kg^{-1}]
						clay[b] [%]	silt[c] [%]	sand[d] [%]		
Seeben	Haplic Phaeozem	Various	Sv	Ap	0-20	19.6	47.5	32.8	17.5	2.1
Halle	Haplic Phaeozem	Maize [g]	Hm	Ap	0-25	10.5	22.1	67.4	11.6	1.4
Halle	Haplic Phaeozem	Rye [g]	Hr	Ap	0-25	9.2	20.1	70.7	12.4	1.1
Boigneville	Dystric Cambisol	Maize [g]	Bm	Ap	0-20	24.9	31.6	38.7	9.8	0.9
Boigneville	Dystric Cambisol	Wheat [g]	Bw	Ap	0-20	28.8	32.7	35.3	11.6	1.0

[a] According to FAO-UNESCO (1994)

[b] 0.45-2 µm

[c] 2-20 µm

[d] 20-2000 µm

[e] Total organic carbon

[f] Total nitrogen

[g] Monoculture

5.1.1 Mass recovery: Organic matter composition and lipid extraction yields

After free lipid extraction, total organic carbon (TOC) concentrations (Figure 5) varied between 8 and 20 g kg^{-1} dry weight. Variation in triplicate analysis of samples Sv and Hm were probably caused by sample heterogeneity. TOC-normalised lipid extract yields varied between 28 and 38 mg lipid extract g^{-1} organic carbon for individual soils (Table 5). Differences in extract yield generally agree with variations in TOC concentrations (Figure 5). Minor variations between multiple extract yield determinations can be attributed to sample heterogeneity and weighing errors of lipid extract. Compared with other soil lipid studies (Amblès *et al.*, 1994b, Lichtfouse *et al.*, 1995b) the extract yields are almost similar. Variations in extraction methods applied, i.e. temperatures, pressures and solvents, are assumed to have caused minor differences in extract yields. The higher efficiency of ASE is mainly due to elevated temperatures and pressures that may force solvent into the fine pores of soil aggregates and particulate organic matter, beyond that achieved by ‘cold’ ultrasonic and unpressurized Soxhlet methods. Sequential ASE of samples may have additionally increased extract yields in comparison to standard Soxhlet methods.

Table 5. Extractable lipid yields [mg gOC^{-1}] [a] of bulk soils as a proportion of total organic carbon.

Site	Sv			Hm			Hr			Bm			Bw		
Sample	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Extracted lipids [mg gOC^{-1}]	28	29	29	32	35	34	35	33	32	37	36	37	35	34	38
Standard deviation	0.6			1.5			1.5			0.6			2.1		

[a] Organic carbon

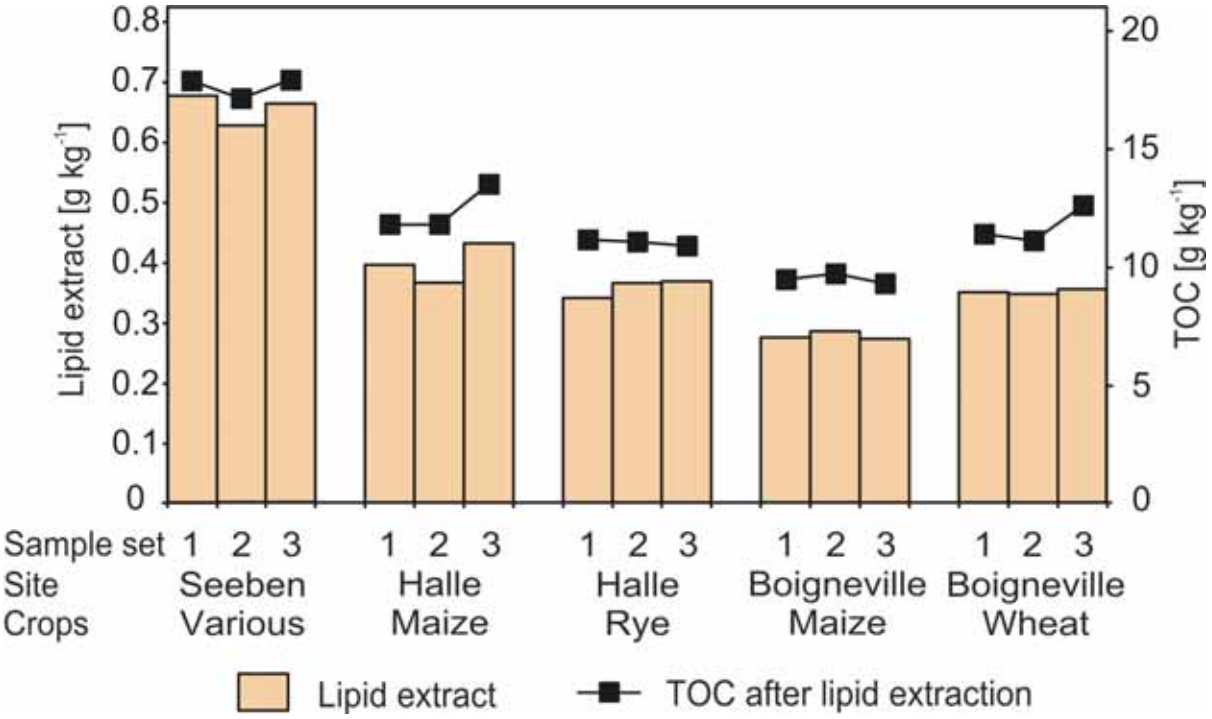


Figure 5. Total organic carbon (TOC) concentrations after lipid extraction, and extractable lipid concentrations of five different arable soils from three sampling sites (Seeben and Halle, Germany; Boigneville, France). To test for reproducibility sample sets 1 and 2 were extracted in triplicate, whereas sample set 3 represents a single extraction.

5.1.2 Reproducibility: Separation of lipids into compound classes

Proportions of lipid fractions after H-MPLC separation were consistent and reproducible (Figure 6). Low polarity compounds were most abundant followed by highly molecular compounds, carboxylic acids, intermediate polarity compounds, the high polarity compound class and finally the basic components. A small proportion (<15% of the total extract) remained insoluble in dichloromethane or could not be recovered from the MPLC-columns. The variation between sample sets 1 and 2 (combined triplicate extracts) is very small.

Differences in sample sets 3 (single extract) are considerably higher and can probably be attributed to soil heterogeneity and weighing errors of fraction vials depending on the smaller amounts of sample extracted. Fine grinding of soil samples may reduce soil heterogeneity. Thus, good recovery rates (>90%) and low variability between compound class yields are characteristic for the automated, fast, and highly reproducible separation method applied in this study. To investigate consistency and reproducibility further, the molecular composition of individual lipid classes was determined by quantitative GC-FID and GC/MS analysis.

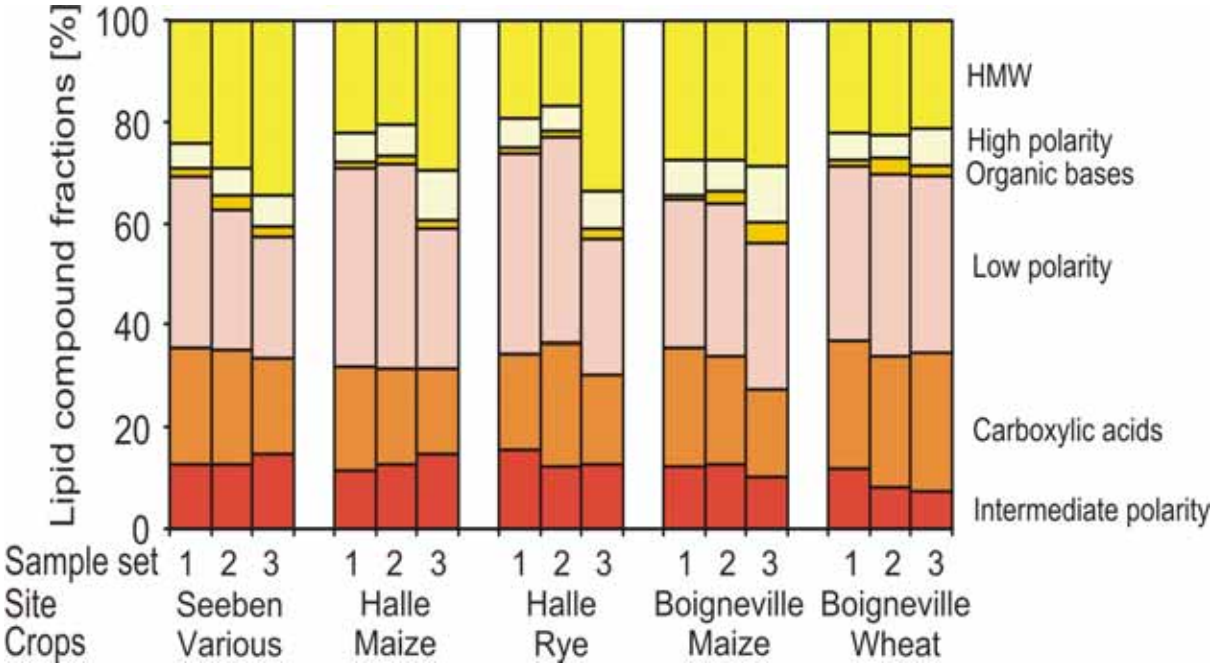


Figure 6. Hetero-compound medium-pressure liquid chromatography (H-MPLC) fraction yields of extracted lipids. To test for reproducibility sample sets 1 and 2 were extracted in triplicate, whereas sample set 3 represents a single extraction.

5.1.2.1 Aliphatic hydrocarbons

MPLC-separation of low polarity fractions after H-MPLC yielded aliphatic and aromatic hydrocarbon fractions in an approximate ratio of 4:1. The distributions of the most abundant components in the aliphatic hydrocarbon fraction, the *n*-alkanes and isoprenoid-alkanes, are shown in Figure 7. Distribution patterns of *n*-alkane in all samples show a characteristic predominance of long-chain odd carbon numbered alkanes (Figure 8), typical for terrestrial plant biomass input (Lichtfouse *et al*., 1994, Bol *et al*., 1996, Nierop, 1998, Bull *et al*., 2000). Minor variations between *n*-alkane distribution patterns of individual soils are related to the heterogeneity of plant biomass input. Major differences between the *n*-alkane patterns for different sampling sites are caused by soil characteristics and type of microbial assemblage. Absolute amounts and distribution pattern of *n*-alkanes are very consistent for all samples and reproducibility for multiple qualitative and quantitative analysis is excellent.

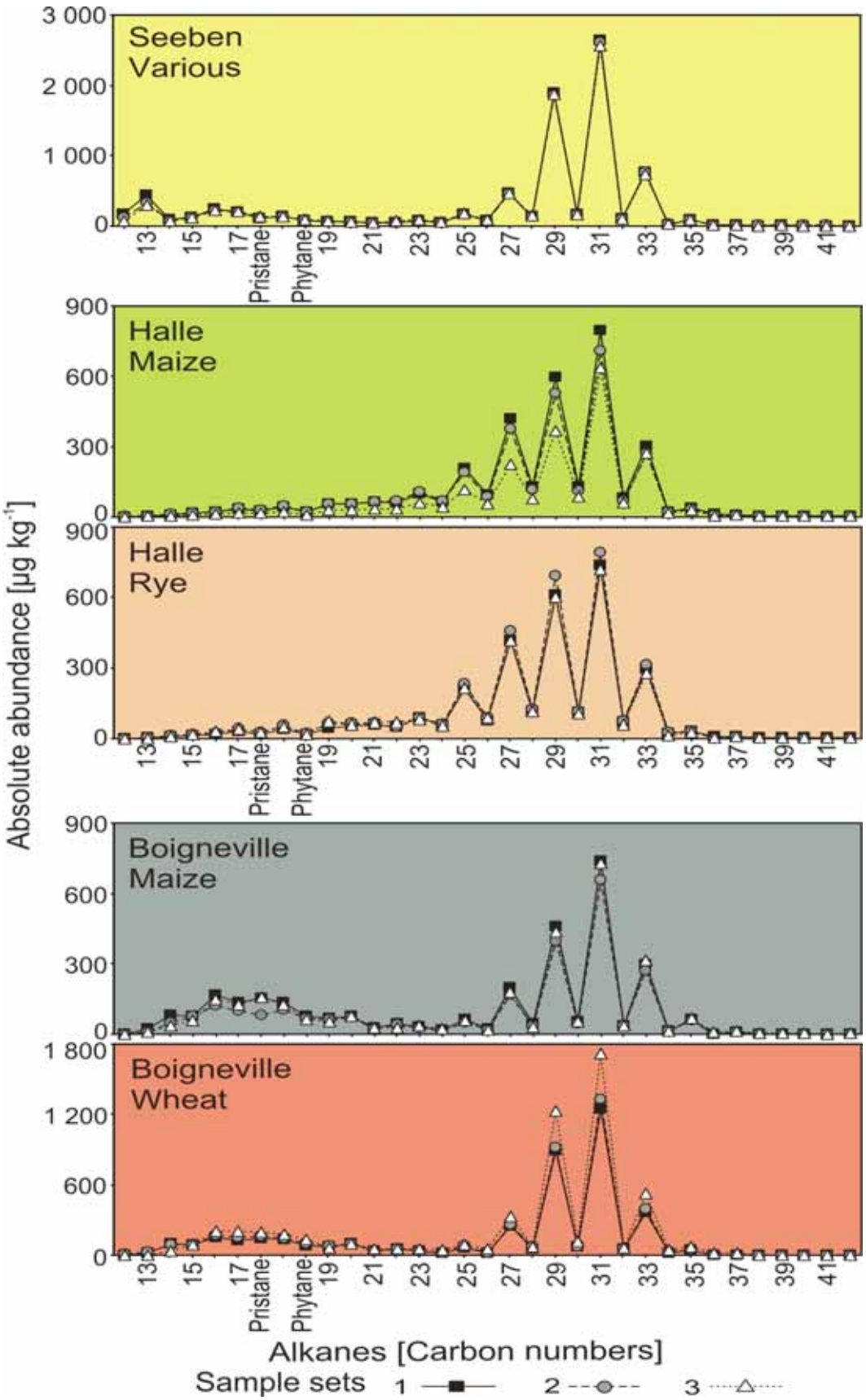


Figure 7. Comparison of aliphatic hydrocarbon distributions (*n*-alkanes and isoprenoid-alkanes) after hetero-compound medium-pressure liquid chromatography (H-MPLC) and medium-pressure liquid chromatography (MPLC) separation of extracted lipids. Note different scales for y-axis.

5.1.2.2 Carboxylic acids

Carboxylic acids (Figure 8) were dominated either by *n*-C_{16}, presumably derived from microbial biomass, or long-chain fatty acids with even carbon numbers (*n*-C_{24}, *n*-C_{26}, *n*-C_{28}) derived from plant biomass (Amblès *et al.*, 1994a). Concentrations of most abundant carboxylic acids varied between 700 and 2 700 μg kg^{-1} dry weight. In general, patterns for carboxylic acids are diagnostic for soil properties and microbial assemblages at the individual sampling sites. Long-chain fatty acids dominate in samples from Halle, whereas in the Boigneville samples short-chain (<C_{24}) carboxylic acids are more abundant. Samples from Seeben reveal a balanced distribution of carboxylic acids with a specific enrichment in the *n*-C_{22-24} acids. Between different soils of each sampling site only minor differences in free carboxylic acid compositions occurred. Nevertheless, the carboxylic acid distribution patterns may still be related to the specific crops grown on the respective plots or to distinctive soil properties. In this study, maize cropped soils are particularly depleted in *n*-C_{22} when compared to the *n*-C_{24} fatty acid, whereas wheat cropped soils were enriched in *n*-C_{22} fatty acid. Rye cropped soil exhibits an intermediate fatty acid signature. Carboxylic acid composition of the maize plant inputs showed a depletion of *n*-C_{22}, equal amounts of *n*-C_{22} and *n*-C_{24} within rye and a depletion of *n*-C_{24} within wheat (discussed later in this study). This is consistent with the observations within the soils. In fact, the Seeben plot cropped with various plant species may thus represent a wheat signature superimposed on a maize and rye pattern. Quantification of carboxylic acids shows that there were only minor differences between the most abundant compounds within individual soils. Variations in Boigneville maize samples are due to single, as compared to composite, extracts and are attributed to greater sample heterogeneity as described above.

5.1.3 Separation effectiveness

A multi-step separation scheme was employed in order to obtain well-defined fractions containing chemically distinct structural classes (e.g. aliphatic and aromatic hydrocarbons, ketones, alcohols, carboxylic acids). This is regarded as crucial for reliable quantification of minor components in individual compound classes. It is also a pre-requisite for further compound specific isotope studies, where baseline separation of individual peaks is mandatory. Total lipid extract as shown in Figure 9f consists of an extensive mixture of various lipid classes. To identify and quantify not only the main components, gel chromatography separation procedures were required. For polar compound classes, very clean fractions with little interferences were obtained during the H-MPLC treatment. Separation effectiveness was achieved by using modified stationary phases as described by Willsch *et al*. (1997).

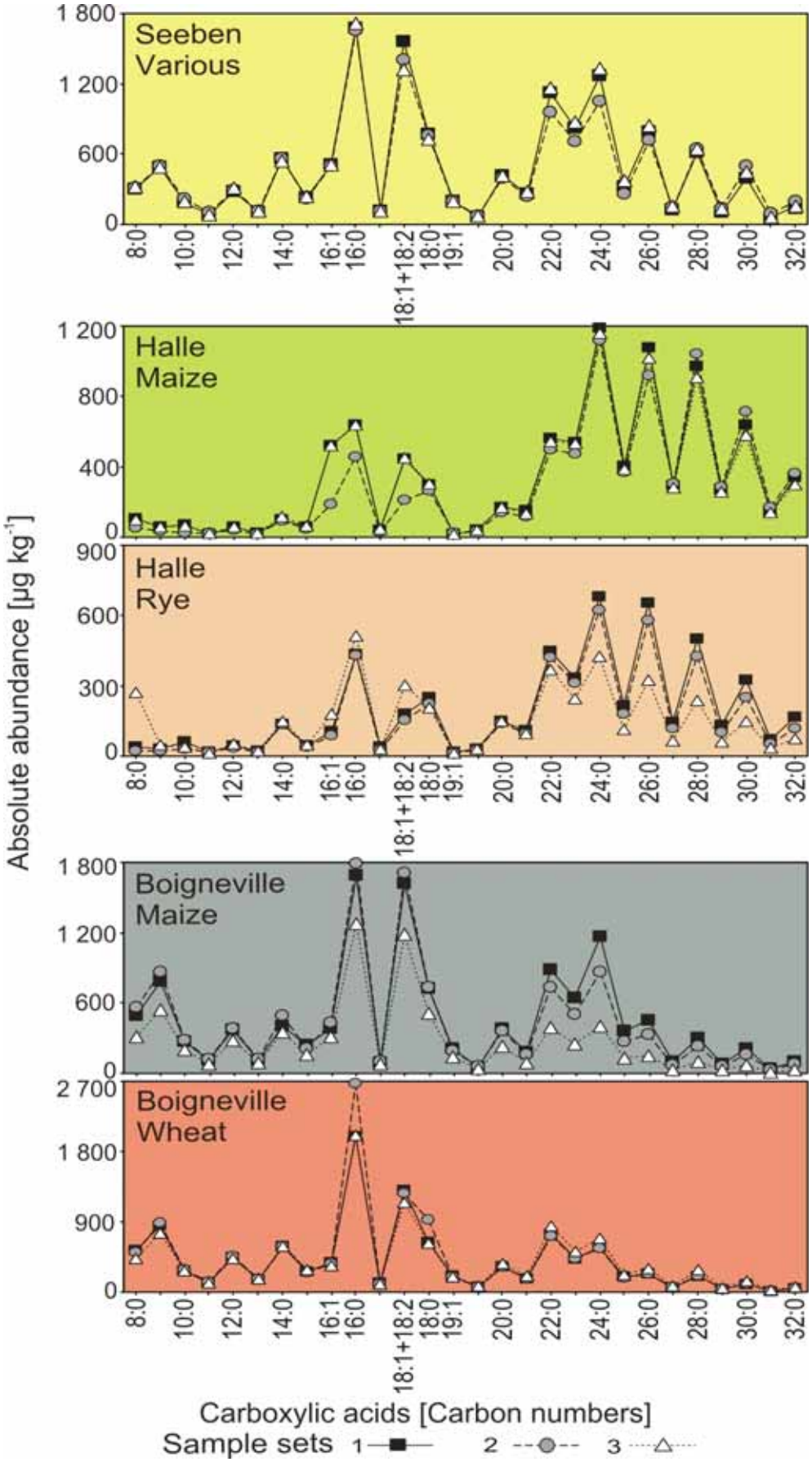
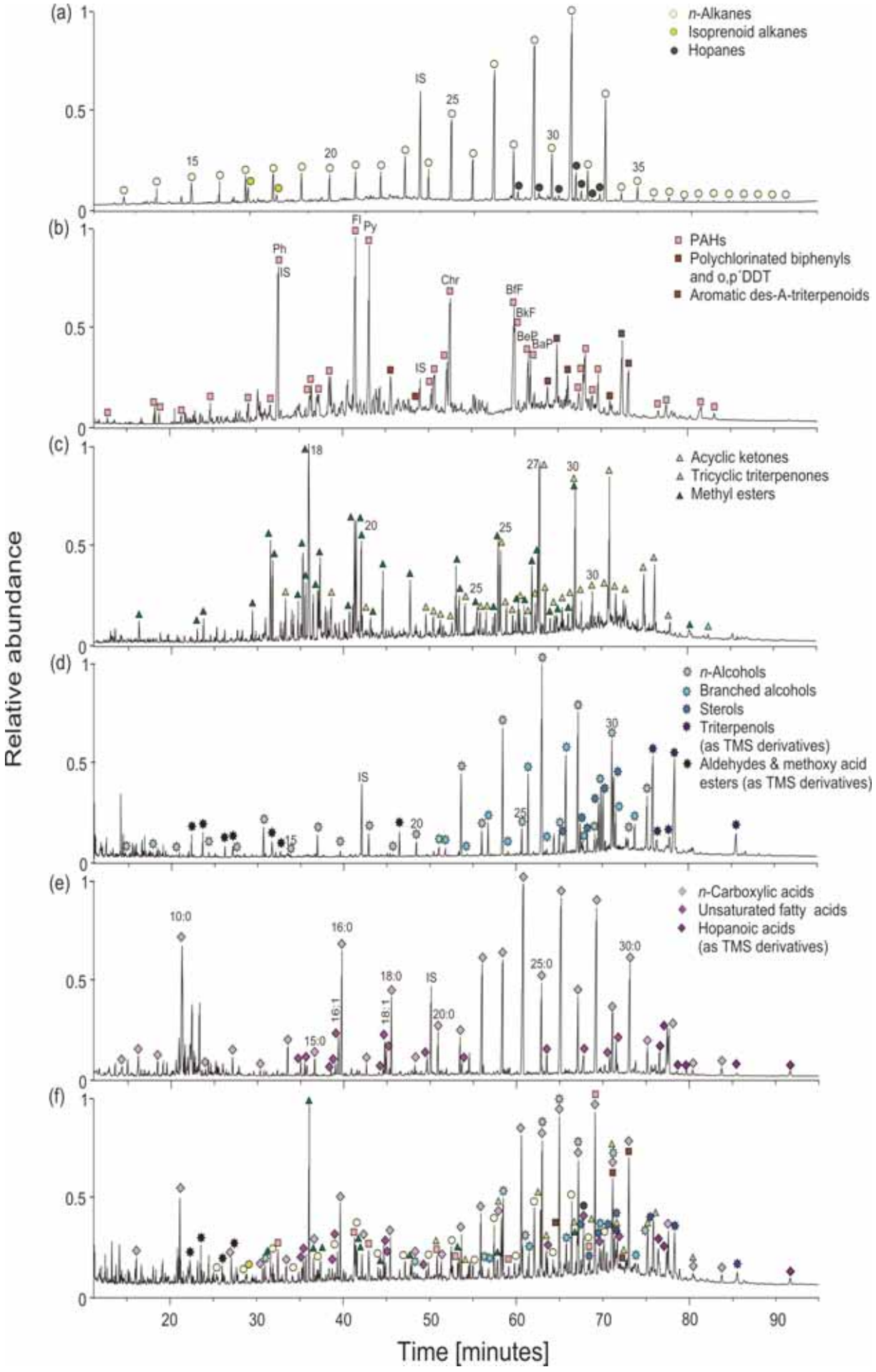


Figure 8. Comparison of fatty acid compositions after hetero-compound medium-pressure liquid chromatography (H-MPLC) separation. Note different scales for y-axis.

Subsequent MPLC-treatment of the heterogeneous low polarity fraction obtained upon H-MPLC yielded three additional discrete fractions, the aliphatic and aromatic hydrocarbons, and the ketones plus low molecular weight methyl ester fraction, respectively. In Figure 9a-e, gas chromatograms of those fractions most important for soil lipid studies are shown. The chromatograms of the operationally defined compound classes comprise the aliphatic and aromatic hydrocarbons, low polar hetero-compounds (acyclic ketones, triterpenoid ketones, esters), intermediate polarity compounds (alcohols, sterols, triterpenols), and carboxylic acids. The high polarity and HMW-fractions (bases, wax esters and high polarity fraction of undefined content) were not completely amenable to GC/MS analysis under standard conditions. Analysis of HMW-compounds may require high-temperature-GC-analysis with sophisticated sample injection techniques or application of high-resolution HPLC-analysis combined with high performance detector systems. Analysis of highly polar fractions would require application of analytical HPLC or GC-separation using very polar stationary phases. Without the preceding gel chromatography separation procedure, target compound co-elution and interference could not have been avoided upon GC-analysis. Clean-cut fractions, as obtained by the procedure described, are directly amenable to compound-specific isotope analysis.

Summarizing, a fast, reproducible, inexpensive extraction and separation procedure could be adopted and optimized for soil lipid analyses. The obtained clean-cut fractions are best suitable for e.g. GC/MS or ^{14}C-AMS analyses. Additionally, from the obtained interference-free lipid fractions individual compounds can be separated by preparative gas chromatographic separation, which can be subjected to detailed molecular analyses as described e.g. by Rethemeyer *et al*. (2004a, 2004b).

Figure 9. (On the following page.) Gas chromatograms of soil lipid fractions after separation of total extract (sample Hm, sample set 1) into compound classes: (a) aliphatic hydrocarbons, (b) aromatic hydrocarbons, (c) low polarity hetero-compounds, (d) intermediate polarity compounds, (e) carboxylic acids, and (f) total lipid extract. Numbers above peaks denote number of carbon atoms in molecule. For carboxylic acids, the number following the colon denotes the number of double bonds in the molecule. IS indicates the internal deuterated standards added to the respective fractions (d_{50}-*n*-C_{24} alkane, d_4-cholestane, d_{10}-anthracene, 1,1'-binaphthalene, d_{37}-*n*-octacosanol, d_{39}-*n*-eicosanoic acid). Abbreviations for polycyclic aromatic hydrocarbons are: phenanthrene (Ph), fluoranthene (Fl), pyrene (Py), chrysene (Chr), benzo[b]fluoranthene (BfF), benzo[k]fluoranthene (BkF), benzo[e]pyrene (BeP), and benzo[a]pyrene (BaP).


(a)
n-Alkanes
Isoprenoid alkanes
Hopanes
IS
15
20
25
30
35
(b)
PAHs
Polychlorinated biphenyls and o,p´DDT
Aromatic des-A-triterpenoids
Ph
IS
Fl
Py
Chr
BfF
BkF
BeP
BaP
IS
(c)
Acyclic ketones
Tricyclic triterpenones
Methyl esters
18
20
25
27
30
(d)
n-Alcohols
Branched alcohols
Sterols
Triterpenols (as TMS derivatives)
Aldehydes & methoxy acid esters (as TMS derivatives)
IS
15
20
25
30
(e)
n-Carboxylic acids
Unsaturated fatty acids
Hopanoic acids (as TMS derivatives)
10:0
15:0
16:0
16:1
18:0
18:1
IS
20:0
25:0
30:0
(f)
Relative abundance
Time [minutes]

5.2 Seasonal plant-internal variations of lipids

In the following chapter 5.2.1, bulk lipid, molecular, and bulk isotopic variations of several crops are shown to obtain trends during the growing season and to establish molecular markers, typical for individual crops. Thereafter (chapter 5.2.2), compound-specific isotopic results are shown to analyse isotopic differences between selected plant-derived alkanes and carboxylic acids of several plants and plant parts, important for plant lipid turnover studies.

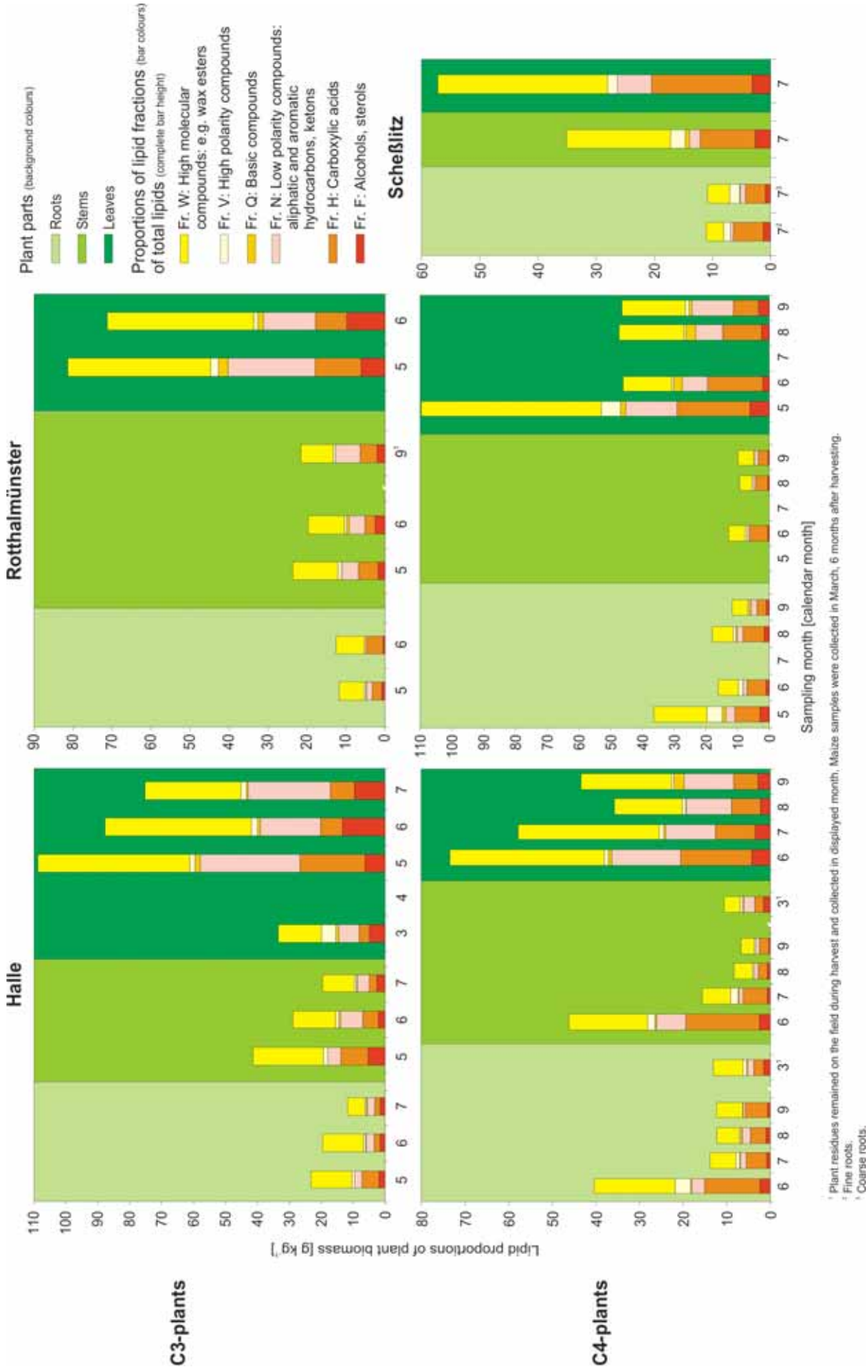
5.2.1 Bulk lipid, molecular and bulk isotopic variations

The analysed plant samples were sampled for investigations of plant biomass incorporation into soil. Thus, samples were taken at one to four different times during growing season. Very young plants as well as degraded plant parts were only sampled on a random basis.

5.2.1.1 Bulk lipid compositions

Proportions of free extractable lipids of plant biomass and the distribution of lipid fractions within the total lipid extract are shown in Figure 10. Generally, analysed C3- and C4-plant parts contained 10-110 g kg^{-1} free extractable lipids. Extract yields were slightly higher for C3- than for C4-plants. Lipid contents decreased significantly with increasing plant growth and maturity during the growing season. Highest contents could be obtained in all plant parts sampled either in May or in June. Similar observations were made for tree leaves, where highest lipid contents were determined in May, which decreased in the following months (Prasad & Gülz, 1990). With plant growth and between several plant parts significant developments of lipid contributions and distributions could be observed for crops analysed in this study. All leaves showed the highest lipid contents. Stems of C3-plants had higher lipid contents than roots. Contrastingly, within C4-plants roots were slightly enriched in lipids when compared to stems, except for plants from Scheßlitz site, where stems had higher lipid contents than roots. The highest lipid contents in leaves and lower amounts in the other plant parts were caused by high contents of lipids in plant leaf surface waxes (e.g. Bianchi, 1994).

Figure 10. (On the following page.) Free extractable lipid proportions of plant biomass (g kg^{-1}) and distribution of separated lipid fractions within the lipid extracts in different plant parts (roots, stems and leaves). Changes of lipid contents and proportions of lipid fractions during the growing season are shown for the plant parts of different sampling months. Different y-axis scales should be noticed.


Plant parts (background colours)
Roots
Stems
Leaves
Proportions of lipid fractions (bar colours) of total lipids (complete bar height)
Fr. W: High molecular compounds: e.g. wax esters
Fr. V: High polarity compounds
Fr. Q: Basic compounds
Fr. N: Low polarity compounds: aliphatic and aromatic hydrocarbons, ketons
Fr. H: Carboxylic acids
Fr. F: Alcohols, sterols
Halle
Rotthalmünster
Scheßlitz
C3-plants
C4-plants
Lipid proportions of plant biomass [g kg⁻¹]
Sampling month [calendar month]
¹ Plant residues remained on the field during harvest and collected in displayed month. Maize samples were collected in March, 6 months after harvesting.
² Fine roots.
³ Coarse roots.

Distribution patterns of plant lipid fractions varied between different plant parts and during growing season (Figure 10). Proportions of lipid fractions showed for C3-plants (rye and wheat) highest contents of high molecular compounds (30-65%), lower amounts of low polarity compounds (5-35%), carboxylic acids (5-35%), alcohols and sterols (5-15%), and lowest amounts of basic and high polarity compounds (both fractions together usually <5%). Generally, maize plants from all sampling sites showed slightly different lipid fraction distributions. High molecular compounds (25-50%) were most abundant, followed by lower proportions of carboxylic acids (15-45%), low polarity compounds (5-30%), intermediate polarity compounds (5-15%), and lowest proportions of basic and high polarity compounds (together <15%). Thus, maize plants had higher proportions of carboxylic acids and lower proportions of high molecular compounds, which might be due to different lipid biosynthesis mechanisms in C3- and C4-plants. Observations made by Bianchi & Bianchi (1990) with C3-plants containing higher proportions of ketones (included in the low polarity fraction) and lower contents of carboxylic acids in comparison to C4-plants could be confirmed in this study. All other lipid fractions showed similar proportions within C3- and C4-plants.

The low polarity fraction was separated into aliphatic hydrocarbons, aromatic hydrocarbons, and low polarity hetero-compounds. Proportions of those three fractions were not derived for all samples, because of probable loss of short-chain compounds during evaporation and low amounts of both hydrocarbon fractions. The low amounts would lead to difficulties in reliable gravimetric determinations. Exemplarily, for a few samples the proportions were determined with highest proportions of low polar hetero-compounds with mainly ketones and methyl esters (60-85%), lower proportions of aliphatic hydrocarbons (15-40%), and trace amounts of aromatic hydrocarbons (<5%). Within all plant parts, high molecular weight compounds with mainly wax esters as well as carboxylic acids decreased significantly during the growing season. Most other compound classes avoided of any significant increasing or decreasing tendencies. The variations of those compound classes during the growing season were usually less than 5%, which could be caused by uncertainties during gravimetric determinations. An increase of alkanes in plant leaves during the growing season as described by Bianchi (1994) could not be observed in this study, because total amounts of alkanes were not determined.

5.2.1.2 Aliphatic hydrocarbons

Distribution patterns of plant-derived aliphatic hydrocarbons and their development during the growing season are shown in Figure 11. Generally, long-chain *n*-alkanes were most abundant in aboveground biomass and especially in leaves, while roots contained more short-chain compounds. Long-chain *n*-alkanes (C_{24}-C_{35}) showed a strong odd/even-predominance, typical for terrestrial plants (Eglinton *et al*., 1962). Aboveground biomass maximised at *n*-C_{31} alkane, except for rye stems from Halle site. Generally, for maize plants

these results confirmed a previously reported *n*-C_{31} maximum for C4-grasses (Boom *et al*., 2002). Short-chain *n*-alkanes (C_{14}-C_{23}) of most plant parts revealed a minor odd/even-predominance.

Lower contents of long-chain components in roots and their minor odd/even-predominance most likely resulted from a minor production of long-chain alkanes, which are common in plant waxes (Bianchi, 1994). On the one hand, microorganisms grown within or on the surface of roots, as discussed by Bonkowski (2004), might have caused those distribution patterns. On the other hand, a major proportion of the short-chain compounds in roots resulted from breakdown of long-chain wax alkanes during biodegradation processes, might have led to a low predominance of odd over even carbon-numbered compounds. Additionally, a part of these compounds might be translocated within the plants from aboveground biomass and was alterated during transport processes. Contrastingly, alkanes in leaves and stems were predominantly in situ produced in cuticles as degradation products of primary carboxylic acids or alcohols (Bianchi, 1994), leading to the a high amount of long-chain components with a strong odd/even-predominance.

Alkanes of leaves showed the lowest heterogeneities in comparison to roots and stems during the growing season. All leaves were enriched in *n*-C_{29}, *n*-C_{31}, and *n*-C_{33} alkanes, except for wheat leaves from Rotthalmünster, which were dominated by the *n*-C_{31} alkane in the June sample. Such a strong predominance of a single *n*-alkane was not shown previously for other crop samples. This samples was extracted and separated in duplicate and showed the same distribution patterns in both replicates. Thus, an artefact during extraction could be excluded and the distribution pattern might be related to unusual sampled plant. A similar *n*-alkane distribution pattern like the one of wheat leaves sampled in May was published for leaf waxes by Tulloch & Hoffman (1973), Bianchi & Corbellini (1977) and Conte *et al*. (2003).

Stems of all plants revealed an enrichment of the same compounds like the leaves. Additionally, higher amounts of odd carbon-numbered homologues with medium chain-length (from *n*-C_{21} to *n*-C_{25}) could be observed early in the growing season, especially for most stem biomass sampled in May or June, and leaf biomass of Rotthalmünster maize, sampled in May. The latter sample could be classified as stem of leaf alternatively, because it was sampled very early, when stem and leaf biomass could not yet fully differentiated.

The C_{27}/C_{29} *n*-alkane ratio introduced by Lichtfouse *et al.* (1994) allows differentiating between wheat and maize plants or cropped soils, with maize plants containing higher proportions of *n*-C_{27} alkanes. In this study, wheat leaves agreed with the observations made by Lichtfouse *et al*. (1994). The other plant parts of wheat showed similar C_{27}/C_{29} *n*-alkane ratios like rye and maize plants and thus, this ratio could not be used to differentiate between C3- and C4-plants and the corresponding monoculture cropped soils.

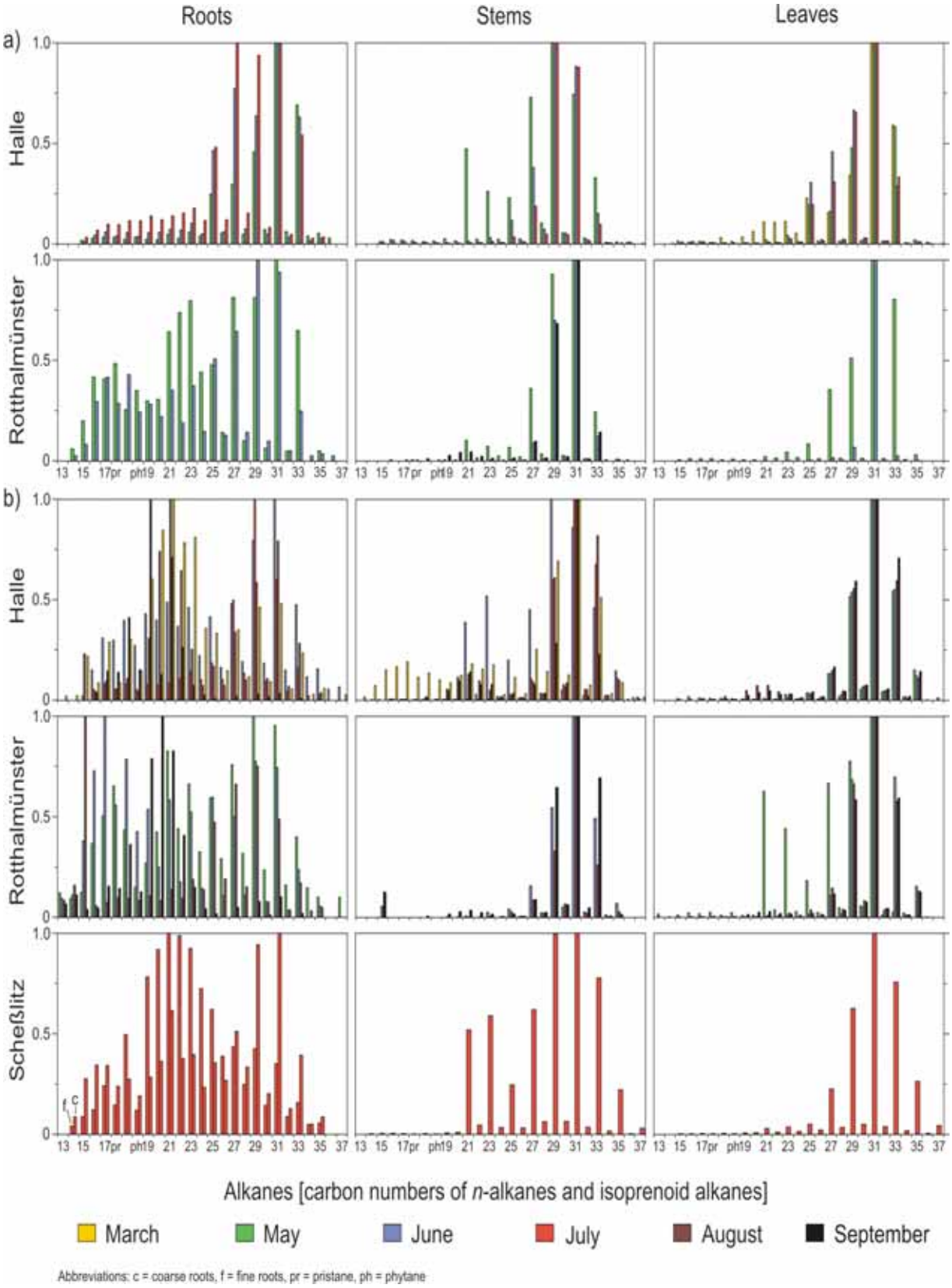


Figure 11. Distribution patterns of most abundant aliphatic hydrocarbons for different plant parts (roots, stems, leaves) of several crops, sampled at distinct months during the growing season: a) C3-plants rye (from Halle site) and wheat (from Rotthalmünster site), and b) maize plants from Halle, Rotthalmünster and Scheßlitz sites.

To analyse differences within the long-chain *n*-alkane distribution patterns between several plant types (following C3- respective C4-photosynthetic pathway) and plant parts, the most abundant compounds (C_{29}, C_{31}, C_{33}) are plotted in a ternary diagram (Figure 12). Generally, all plants contained lowest amounts (<40%) of *n*-C_{33} and higher amounts of *n*-C_{29} and *n*-C_{31} alkanes. C3-plants showed relatively homogeneous compositions of stem biomass with approximately 40-50% of C_{29} and C_{31} *n*-alkanes and lowest proportions of less than 20% C_{33} (Figure 12a). In contrast to stems, wheat and rye roots contained uniform proportions of C_{31} *n*-alkanes. With increasing plant growth, root biomass got depleted in C_{33} and enriched in C_{29} *n*-alkanes. Probably, *n*-C_{29} was more in situ produced in root cell membranes or was faster metabolised during degradation of precursor lipids. Wheat and rye plants had inhomogeneous compositions of leaf biomass (Figure 12a). Exceptional high amounts of *n*-C_{31} alkane of wheat leaves sampled in June could not be explained yet. The inhomogeneous compositions of leaves and the homogenous compositions of stems led to the assumptions that root *n*-alkanes were predominantly built in situ and plant internal transport was less important for *n*-alkane compositions of roots, especially for young plants. Possibly, root derived *n*-alkanes are transported from aboveground biomass to the roots with increasing plant growth, because root alkane composition converged to stem biomass composition during plant growth. Thus, translocation of plant alkanes might be possible from stems to roots. Alkane translocation from stems to leaves or vice versa could not be observed in C3-plants.

Generally, distribution patterns of long-chain *n*-alkanes of C4-plants (Figure 12b) were similar to those of C3-plants with low proportions of *n*-C_{33} alkanes as described above. In contrast to C3-plants, stems of C4-plants showed variable proportions of long-chain *n*-alkanes. Root biomass had similar compositions like C3-plant stem biomass with low proportions of C_{33} and high proportions of C_{29} and C_{31} *n*-alkanes. During the whole growing season the root *n*-alkane proportions remained constant. Maize leaves showed uniform distributions of *n*-alkane during the growing season with 40-50% C_{31}, 25-30% C_{29}, and 25-35% C_{33}. As a result of inhomogeneous stem alkane compositions during the growing season, plant internal translocations from roots to leaves or vice versa, each with constant compositions throughout the growing period, could not be observed from long-chain *n*-alkane compositions of C4-plants.

Distribution patterns of medium chain-length odd carbon-numbered *n*-alkanes (from *n*-C_{21} to *n*-C_{25}, Figure 13) showed large variations for all plant parts of C3- and C4-plants. C3-plants contained predominantly *n*-C_{25}, lower amounts of *n*-C_{21}, and lowest amounts of *n*-C_{23} alkanes (Figure 13a). Stems of wheat and rye plants revealed intermediate compositions of all compounds for samples of May. For samples from the following months a predomination

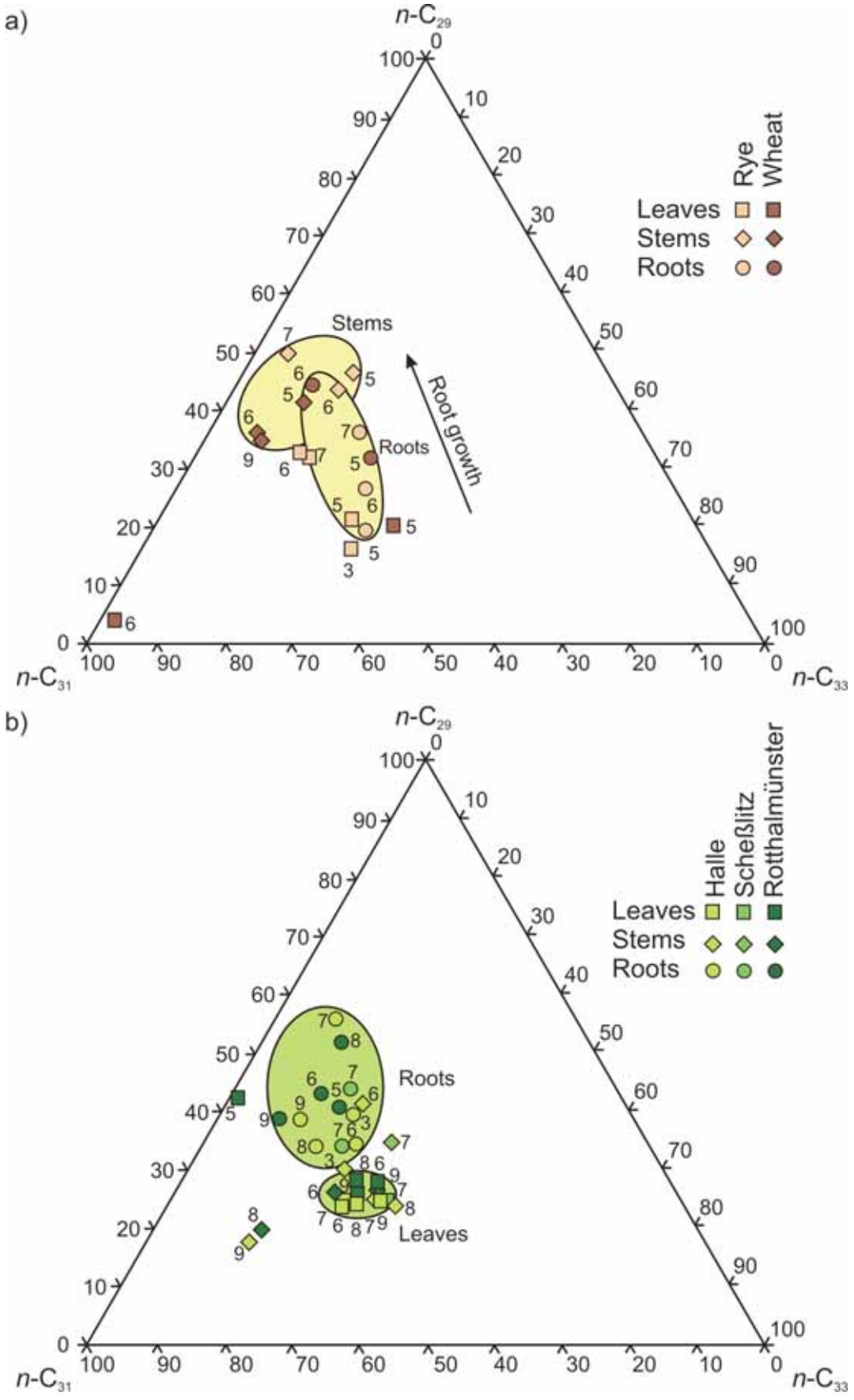


Figure 12. Distributions of most abundant long-chain *n*-alkanes ($C_{29\text{-}33}$) for a) C3-plant parts and b) maize plant parts.

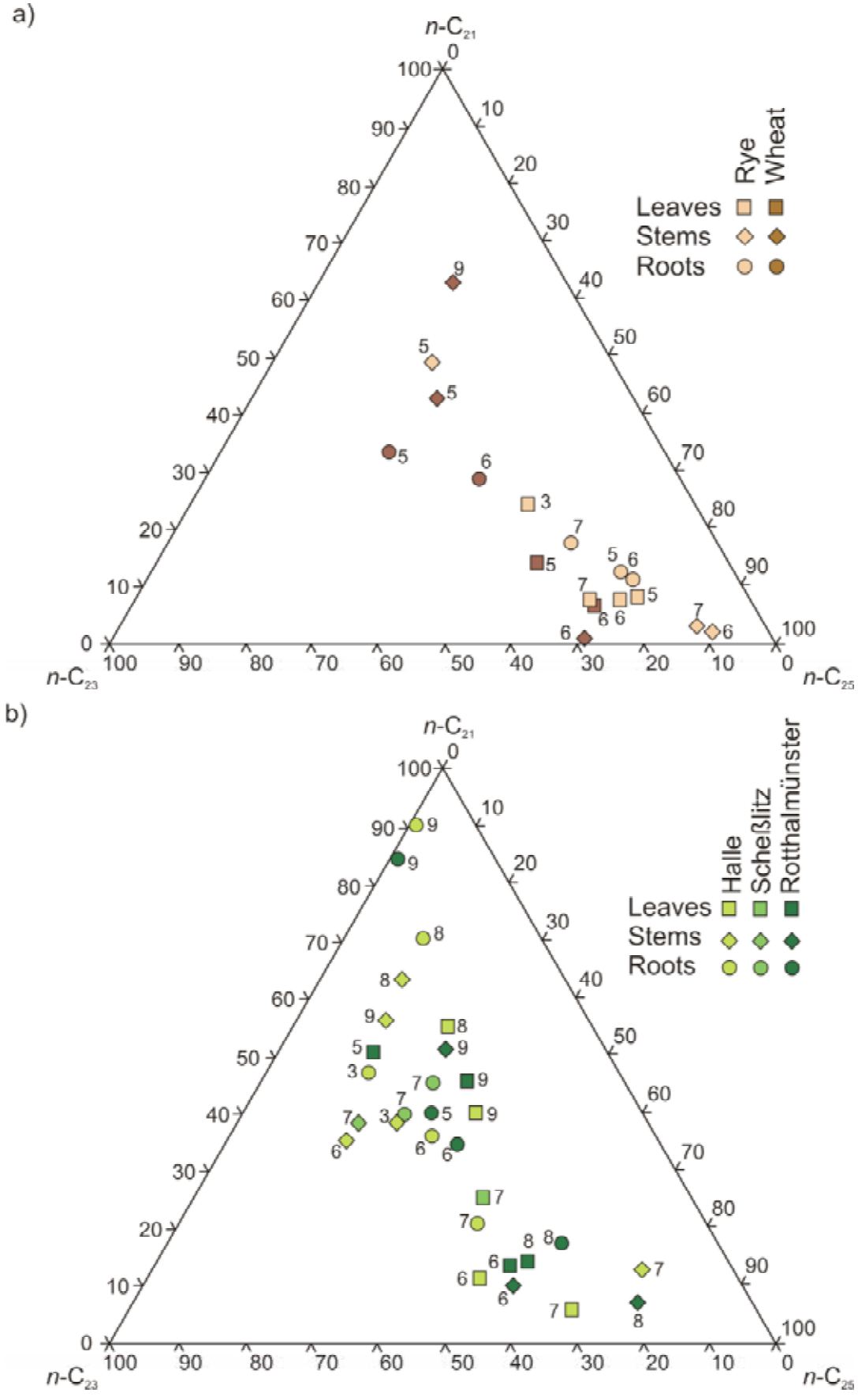


Figure 13. Distributions of medium chain-length odd carbon-numbered *n*-alkanes (C_{21-25}) for a) C3-plant parts and b) maize plant parts.

predomination of the *n*-C_{25} homologue could be observed, probably as an effect of the generation of a broad variety of compounds early in the growing season and a further production of the typical long-chain homologues (C_{29-33}) with increasing plant growth. Contrastingly, wheat straw, picked up on the plot approximately one month after harvest, revealed an intermediate composition of medium chain-length n-alkanes, with a slight enrichment of *n*-C_{23}. Roots of wheat plants showed a similar trend like stems with increasing proportions of *n*-C_{25} alkane with plant growth, but with a minor enrichment of the *n*-C_{25} alkane. Most likely, this was due to a beginning degradation of long-chain components and a selective preservation of medium chain-length *n*-alkanes. Medium chain-length *n*-alkanes of maize plants showed highly variable compositions of all plant parts during the growing season (Figure 13b). Generally, all maize plant parts contained intermediate proportions of *n*-C_{23} alkane (10-50%) and highly variable contents of *n*-C_{21} (5-90%) and *n*-C_{25} alkanes (0-80%). Plant part compositions varied largely between sampling sites and sampling times.

Only maize roots sampled in September showed for all analysed plant parts uniform compositions of C_{21} (85-90%), C_{23} (10-15%), and C_{25} (0-2%) *n*-alkanes (Figure 13b). Most likely, the predominance of the *n*-C_{21} alkane was caused by an interaction with the microbial biomass as supposed in the 'microbial loop', which was summarized by Bonkowski (2004) and discussed later in this study. With increasing plant growth roots got enriched in short and medium chain-length *n*-alkane homologues (Figure 11). Those compounds seemed to be common in microorganisms associated with root biomass.

A general trend to increasing chain-length with plant growth as described by Bianchi (1994) for leaves was not observable for all C3- and C4-plants.

5.2.1.3 Carboxylic acids

The carboxylic acids of all analysed plant parts were strongly dominated by acids with chain length of 16 or 18 carbon atoms (Figure 14), which are main components during lipid biosynthesis (e.g. Bianchi, 1994). The distinct even over odd predominance with a chain length from 14 to 30 carbon atoms is typical for terrestrial plants (Eglinton *et al.*, 1962). During the growing season carboxylic acids got relatively enriched in long-chain compounds when compared to short-chain length acids. This is in perfect agreement with results concluded by Bianchi (1994) for plant leaf lipids of most higher plants. Branched compounds (especially iso- and anteiso-compounds of C_{15} and C_{17} carboxylic acids) were not mentioned here except for iso-C_{14} carboxylic acid, because those compounds were detectable only in trace amounts within most plant parts. The most abundant compounds with 16 and 18 carbon atoms ($C_{16:0}$, $C_{\Sigma 18:1+2}$, $C_{18:0}$) as well as the most abundant long-chain compounds (*n*-C_{22}, *n*-C_{24}, *n*-C_{26}) were plotted in a ternary diagram each to analyze and discuss

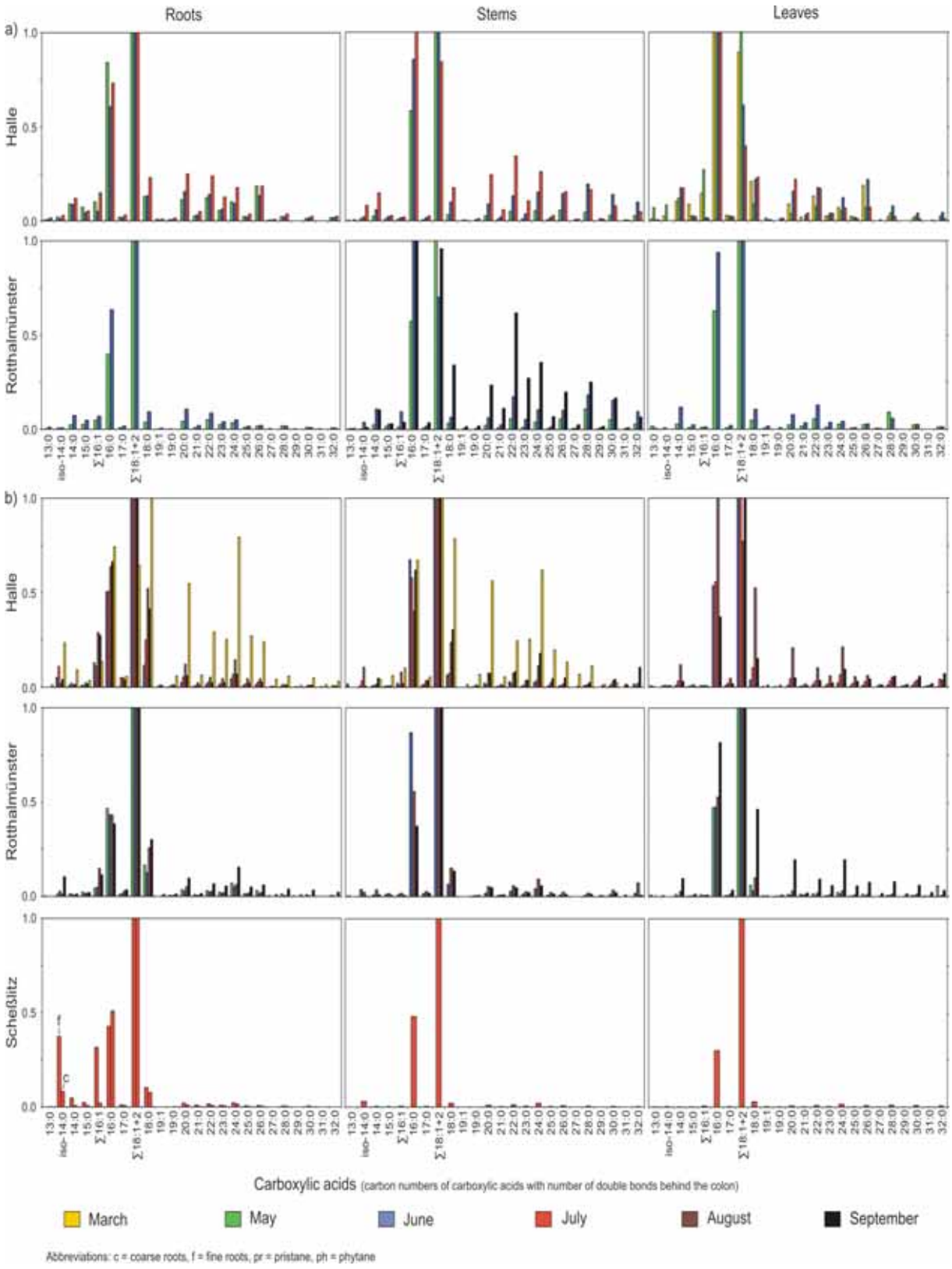


Figure 14. Distribution patterns of most carboxylic acids for different plant parts (roots, stems, leaves) of several crops, sampled at distinct months during the growing season: a) C3-plants rye (from Halle site) and wheat (from Rotthalmünster site), and b) maize plants from Halle, Rotthalmünster and Scheßlitz sites.

differences between C3- and C4-plants and the development of those compound relations during the growing season. Generally, long-chain acids were present in minor amounts with a slight enrichment of even carbon numbers, which is in good agreement with previous studies of plant biomass (Bianchi & Corbellini, 1977, Guil-Guerrero & Rodríguez-García, 1999). In other studies, however, no long-chain carboxylic acids were detected in fresh plant material (e.g. van Bergen *et al*., 1998).

Generally, most plant parts showed low proportions of $C_{18:0}$ (<30%) and higher proportions of $C_{16:0}$ and $C_{\Sigma 18:1+2}$ (20-65% each) within the most abundant compounds (Figure 15). This is in good agreement with general distribution patterns of higher plant tissues as observed by Harwood & Russell (1984).

C3-plants (Figure 15a) had very low amounts of $C_{18:0}$ carboxylic acid (<30%). Roots, stems and leaves showed uniform proportions of short-chain acids. A similar distribution pattern was previously observed in wheat roots by Read *et al.* (2003) with 45% $C_{16:0}$, 51% $C_{\Sigma 18:1-3}$ and 4% $C_{18:0}$. Differences between wheat and rye plants or trends during the growing season of individual plant parts could not be observed. Only rye leaves decreased in unsaturated $C_{\Sigma 18:1+2}$ carboxylic acids with plant growth, except for juvenile leaves of rye. More systematic trends may be observed, when several plants would be analysed during the entire growing season.

Generally, maize plants (Figure 15b) showed uniform proportions of $C_{16:0}$ (around 30%) for all plant parts. Proportions of most plant parts remained constant during the growing season. A similar distribution pattern was previously described by Read *et al.* (2003) with 33% $C_{16:0}$, 65% $C_{\Sigma 18:1-3}$ and 2% $C_{18:0}$. For aboveground biomass increasing proportions of $C_{18:0}$ and decreasing amounts of $C_{\Sigma 18:1+2}$ could be observed with plant growth. Possibly, this trend was an effect of increasing plant maturity, because significant depletions in unsaturated compounds occurred late in the growing season and were determined for degraded biomass.

As demonstrated above, distribution patterns of short-chain carboxylic acids of especially of young C3-plants and C4-plants were not significantly different. Within soils, short-chain carboxylic acids might be derived from several sources including plant biomass, fungi, bacteria, and others (e.g. Harwood & Russell, 1984, Dinel *et al*., 1990). Differences between distinct plants were minor for those compounds. Direct precursors for specific plants could not be found within those compounds. Contrastingly, long-chain carboxylic acids could only be produced in very low amounts by other organisms than plants (Dinel *et al*., 1990) and thus could be related to higher plant precursors. The most abundant even carbon-numbered

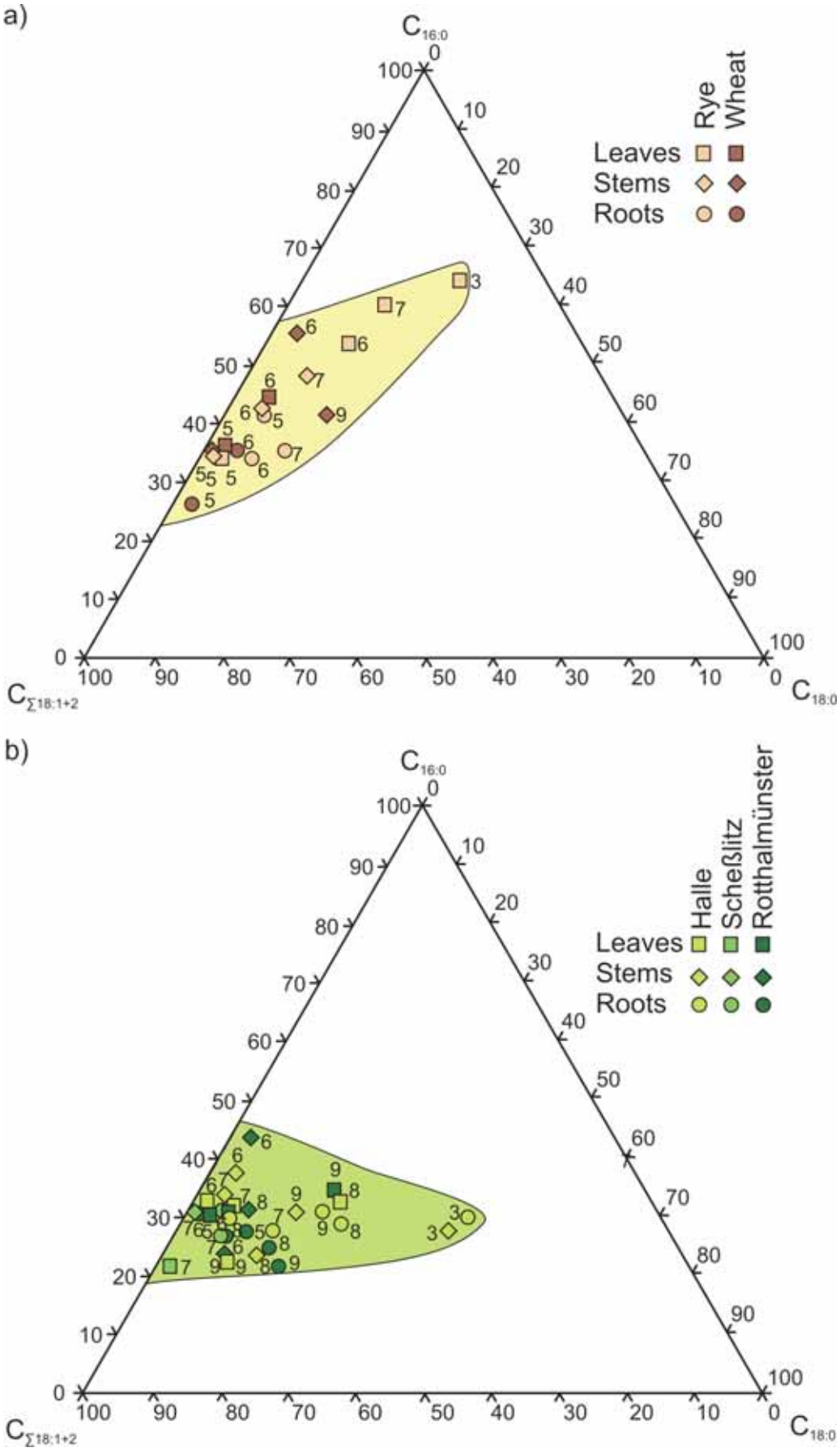


Figure 15. Distributions of most abundant carboxylic acids ($C_{16:0}$, $C_{\Sigma 18:1+2}$, $C_{18:0}$) of a) C3-plant parts and b) maize plant parts.

long-chain compounds ($C_{22:0}$, $C_{24:0}$, $C_{26:0}$) are plotted in ternary diagrams (Figure 16) to study possible differences between the C3- and C4-plants.

All analysed C3-plants are characterized by high proportions of *n*-C_{26} and *n*-C_{22} carboxylic acids (Figure 16a) and relatively low amounts of *n*-C_{24} carboxylic acid (<40%). Rye plants sampled early in the growing season (June and earlier) contained higher proportions of *n*-C_{26} carboxylic acid than rye plants sampled later in the growing season (July). Most likely rye plants got relatively enriched in *n*-C_{22} carboxylic acid or depleted in *n*-C_{26} with plant growth, while *n*-C_{24} remained constant. This trend was observable in all rye plant parts. Root biomass showed intermediate compositions between stem and leaf biomass of the samples taken at distinct times. In comparison to leaf biomass root biomass revealed minor differences throughout the growing season. Minor differences in root biomass and large differences within leaf biomass throughout the growing season could have been caused in the production of different photosynthesis products during the growing season and a plant internal transport of these compounds from the leaves to the roots. This possible reason for the seasonal development of long-chain carboxylic acids should have produced intermediate carboxylic acids compositions of stems between root and leaf biomass, which was not observable in the sample set. Possibly, additional long-chain carboxylic acids were directly generated in roots, leading to a slight enrichment of *n*-C_{22} and *n*-C_{26} in roots in comparison to stems. Additionally, a selective preservation of individual carboxylic acids might play a role during the vertical transport of compounds within plants. Probably, there might be further reasons for the development of carboxylic acids during the growing season and the minor proportions of *n*-C_{24} carboxylic acids within rye plants, which could not be explained yet. Wheat plants were relatively enriched in *n*-C_{22} carboxylic acid in comparison with young rye plants and showed constant proportions of long-chain carboxylic acids during the growing season. Similar distribution patterns of 63-65% *n*-C_{22}, 24-25% *n*-C_{24}, and 11-12% *n*-C_{26} carboxylic acid for acid hydrolysis products of esters were observed for durum wheat by Tulloch & Hoffman (1971) and for the bread wheat *Triticum aestivum* Demar 4 variety by Bianchi & Corbellini (1977). Identical results were reported by Tulloch & Hoffman (1973) for free acids and acid hydrolysis products of esters. Slightly different observations were made by Conte *et al*. (2003), where *Triticum aestivum* contained identical amounts of *n*-C_{22} and *n*-C_{26} carboxylic acids, which were each twice as high as the amounts of *n*-C_{24} carboxylic acid. In contrast to these observations Tulloch & Hoffman (1971) reported a free acid distribution of durum wheat waxes of 29-34% *n*-C_{22}, 31-32% *n*-C_{24}, and 35-39% *n*-C_{26}. The differences between observations made by Tulloch & Hoffman (1971) and results presented here could be most likely related to the extraction with more polar solvents at higher temperatures and pressures in this study, resulting in a combination of free components and a part of the hydrolysis products described by Tulloch & Hoffman (1971). In comparison to mature rye plants the proportions of wheat plant carboxylic acids were in good agreement. Possibly

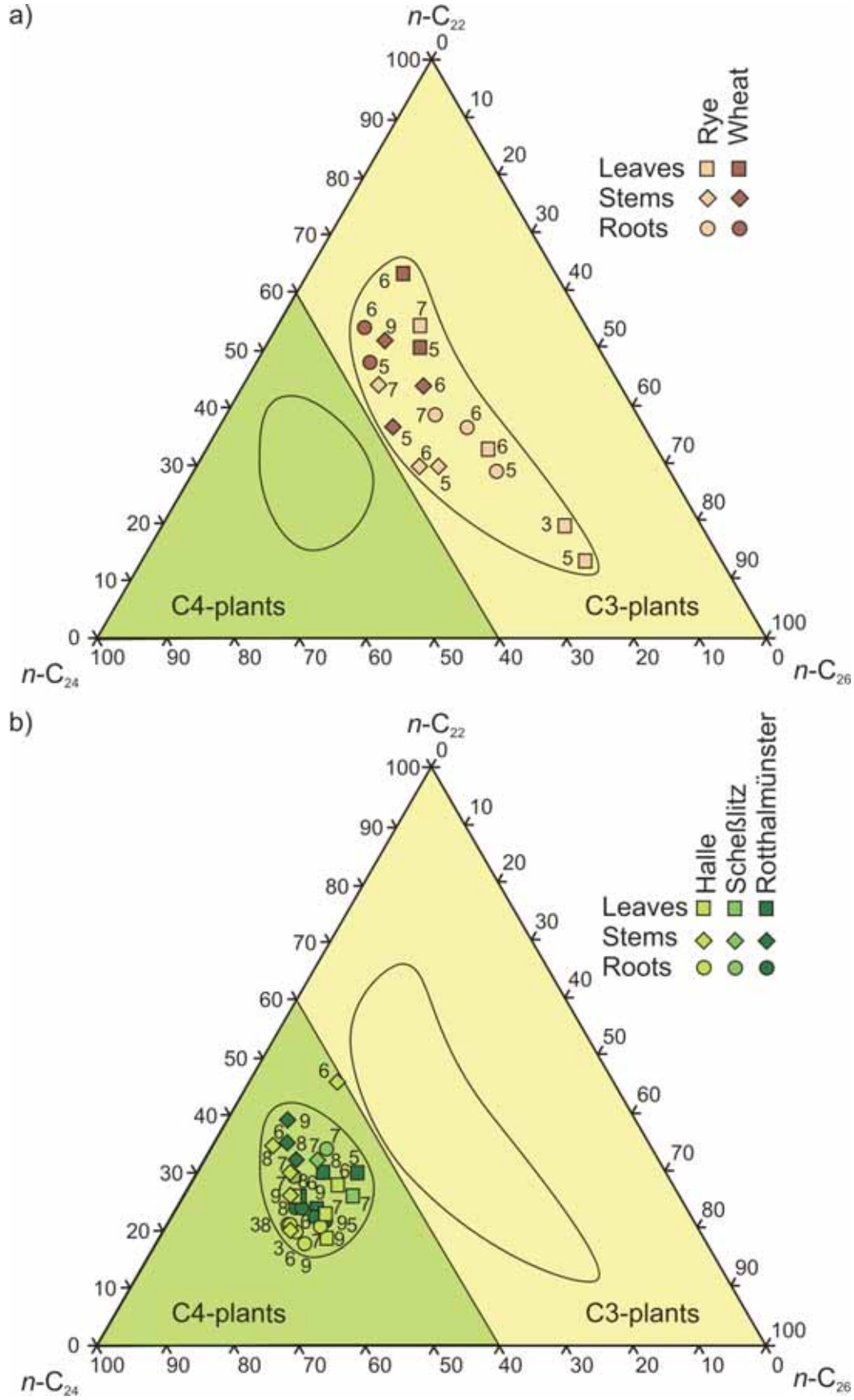


Figure 16. Distributions of most abundant long-chain even carbon-numbered *n*-carboxylic acids ($C_{22\text{-}26}$) for a) C3-plant parts and b) maize plant parts.

analogous developments of carboxylic acid proportions from wheat plants to rye plants could be observed if there were more wheat samples analysed during the growing season.

5.2.1.4 Ratios and bulk isotopy ($\delta^{13}C$)

In comparison to rye and wheat plants, maize plants consisted of major proportions (>40%) of *n*-C_{24} carboxylic acids and minor proportions of *n*-C_{22} and *n*-C_{26} carboxylic acids (Figure 15b). All maize plant parts were characterized by uniform distribution patterns of long-chain carboxylic acids (45-65% *n*-C_{24}, 15-40% *n*-C_{22}, 10-25% *n*-C_{26}), independent of sampling time. These results were in perfect agreement with previous analyses of 'normal' maize genotypes as described by Bianchi *et al*. (1982). Vertical plant internal transport of carboxylic acids within maize plants from leaves to roots or in the opposite direction could neither be indicated nor excluded by the observed results.

The major difference in the proportions of long-chain *n*-carboxylic acids between C3- and C4-crops with low proportions of *n*-C_{24} in C3-crops and high proportions of *n*-C_{24} in C4-crops in comparison to the sum of *n*-C_{22} and *n*-C_{26} carboxylic acids facilitated the introduction of a diagnostic carboxylic acid ratio (CAR) to differentiate between C3- and C4-crops:

$$CAR = n\text{-}C_{24} / (n\text{-}C_{22} + n\text{-}C_{26}) \quad (8)$$

with *n*-C_{22}, *n*-C_{24}, and *n*-C_{26} as the relative proportions or the quantities of the individual *n*-C_{22}, *n*-C_{24}, and *n*-C_{26} carboxylic acids.

C4-plants were characterized by high ratios (>0.67 = >40% *n*-C_{24}), whereas C3-plants are distinguished by low ratios (<0,67 = <40% *n*-C_{24}). To confirm the diagnostic character of the CAR for the differentiation between C3- and C4-plants, the CAR is plotted against the stable carbon isotopic composition (Figure 17). In perfect agreement with literature results like Balesdent *et al*. (1987, 1988), Marino & McElroy (1991), Cayet & Lichtfouse (2001), and Fernandez *et al*. (2003) all maize plants showed $\delta^{13}C$ contents between -10‰ and -15‰ (Table 6, Figure 16). With increasing plant growth and especially after harvest a small isotopic depletion (<2‰) within several Halle maize plant parts could be observed. This was in good agreement with previous observations (Wedin *et al*., 1995), where an isotopic depletion of 1.0-1.5‰ was observed during the maturation and decay of another C4-grass. In contrast to maize plants, rye and wheat plants as C3-crops had isotopic signatures in the range between -26‰ and -33‰, which is characteristic for C3-plants (e.g. Winkler *et al*., 1978). Generally, rye plants showed a larger depletion in $\delta^{13}C$ in comparison to wheat plants. We observed a minor variation of wheat bulk $\delta^{13}C$ isotopy between -26‰ to -30‰ in comparison to Winkler *et al*. (1978), which was most likely related to the less detailed studies

of plant material in this study. The displayed results were in agreement with results shown by Balesdent *et al.* (1988), Lichtfouse *et al*. (1995b), Cayet & Lichtfouse (2001), Zhao *et al.* (2001), and Conte *et al*. (2003), where different wheat plant parts revealed $\delta^{13}C$ isotopic compositions between –27‰ and –30‰. Depending on plant growth stage, Yoneyama *et al*. (1997) reported isotopic compositions between -28‰ and -33‰ for different plant parts, where the plants of the earlier growth stage generally had decreased carbon isotopic compositions up to 2‰. These results could not be confirmed by the results in this study, because a contrasting tendency with a decreasing isotopic composition was observable in the analysed wheat plant parts. This could not be explained yet. The differences between both C3-crops showed a usual natural variation of isotopic signatures between several C3-plants. Similar to previous observations concerning the decay of plant materials (Wedin *et al*., 1995) an isotopic increase (approximately 1‰) could be observed for most plant parts except for stems during the growing season.

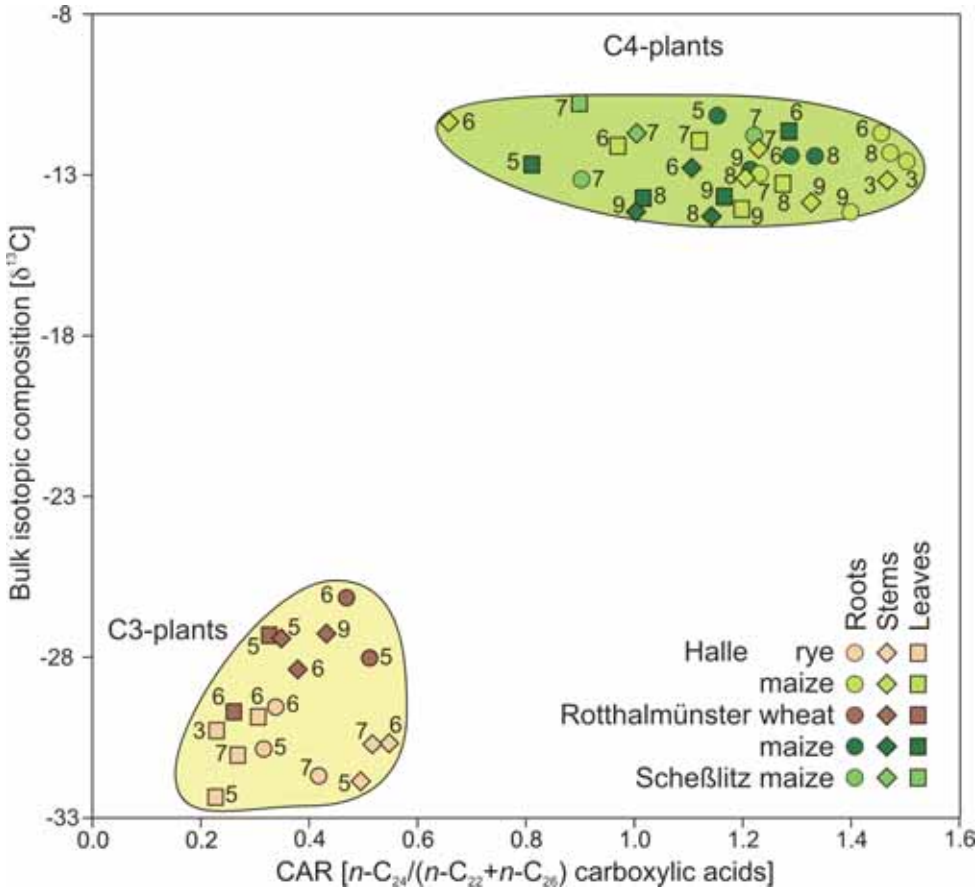


Figure 17. Bulk isotopic composition ($\delta^{13}C$) in relation to the CAR (n-C_{24} / (n-C_{22} + n-C_{26}) carboxylic acid ratio) with definite different populations for C3- and C4-plants.

The stable carbon isotopic signatures, which are diagnostic for the differentiation between C3- and C4-plant types, in combination with the presumed diagnostic CAR marker showed two distinct fields of a) C3-plants with low $\delta^{13}C$-isotopies and low CAR, and b) C4-plants with

high $\delta^{13}C$-isotopies and high CAR. This good agreement between the isotopic signatures and the CAR confirmed the diagnostic character of the newly introduced CAR. This ratio might be used as an additional or alternative tool for the differentiation of C3- or C4-labelled biomass in several matrices like soils and sediments of recent or fossil type. To confirm the diagnostic character of the CAR several further analyses of typical C3- and C4-crops are needed. First results of additional C3-plants like barley, wheat, and other grasses as well as C4-grasses like *Miscanthus X giganteus* (not discussed in detail in this study) and literature results showed results similar to those of plants discussed above and confirmed the diagnostic CAR differentiation between C3- and C4-crops. Otherwise untypical genotypes of some grasses like albino maize (Bianchi *et al*., 1982) seemed to have atypical carboxylic acid distribution patterns. Further analyses are still needed to decide, whether the observed differences between the C3- and C4-plants could be extended to other C3- and C4-plants than grasses. Some implications of the CAR for soil lipid analyses are discussed later in this study.

It is well known, that *n*-alkanes are main transformation products during biosynthesis processes of *n*-carboxylic acids (Kolattukudy *et al*., 1976). Within the decarboxylation reaction the functional carboxyl-group is replaced by hydrogen. Thus, carboxylic acid degradation products within the *n*-alkane fraction contain one carbon atom less, than corresponding precursor acids. Thus, it is required to check, if *n*-alkanes biosynthetic degradation products of the compounds used in the CAR are of a similar diagnostic character like the ratio described above. Similar to the CAR an alkane ratio (AR) could be calculated, where the $n\text{-}C_{23}$ alkane is divided by the sum corresponding odd carbon-numbered neighbours ($n\text{-}C_{21}$ + $n\text{-}C_{25}$):

$$AR = n\text{-}C_{23} / (n\text{-}C_{21} + n\text{-}C_{25}) \quad (9)$$

with $n\text{-}C_{21}$, $n\text{-}C_{23}$, and $n\text{-}C_{25}$ as the relative proportions or the quantities of the individual $n\text{-}C_{21}$, $n\text{-}C_{23}$, and $n\text{-}C_{25}$ alkanes.

The AR was plotted against the CAR in Figure 18 to test the correspondence between both ratios. Except for wheat roots sampled early in the growing season (May), all C3-plants showed homogeneous alkane ratios between 0.1 and 0.45. Wheat plants produced higher ratios than rye plants, paralleling the differences of the bulk isotopy between both C3-plants. In comparison to CAR, AR showed slightly lower values (0.1-0.2). Maize plants showed a large variability for all plant parts, sampling sites and sampling times within the alkane ratio. For maize leaves relatively homogeneous ratios (0.3-0.65) were obtained, while roots and stems showed an AR between 0.1 and 0.9. In contrast to the CAR, the AR could not be used to differentiate between C3- and C4-plant parts. Thus, alkanes especially in maize plants must be derived from other sources like transformation products of alcohols or saturation of

alkenes, leading to a low AR. Within C3-plants the alkanes could be mainly derived directly from decarboxylation of carboxylic acids, because both ratios showed low values. Probably, additional contributions of degraded alcohols caused the lower alkane ratios in comparison to the CAR. It cannot be excluded, that alkanes could be derived from other sources than transformation of alkanes and carboxylic acids. Additional sources for the discussed medium chain-length compounds could be e.g. transformation of alkenes or other compounds like wax esters, but will not be discussed further in this context.

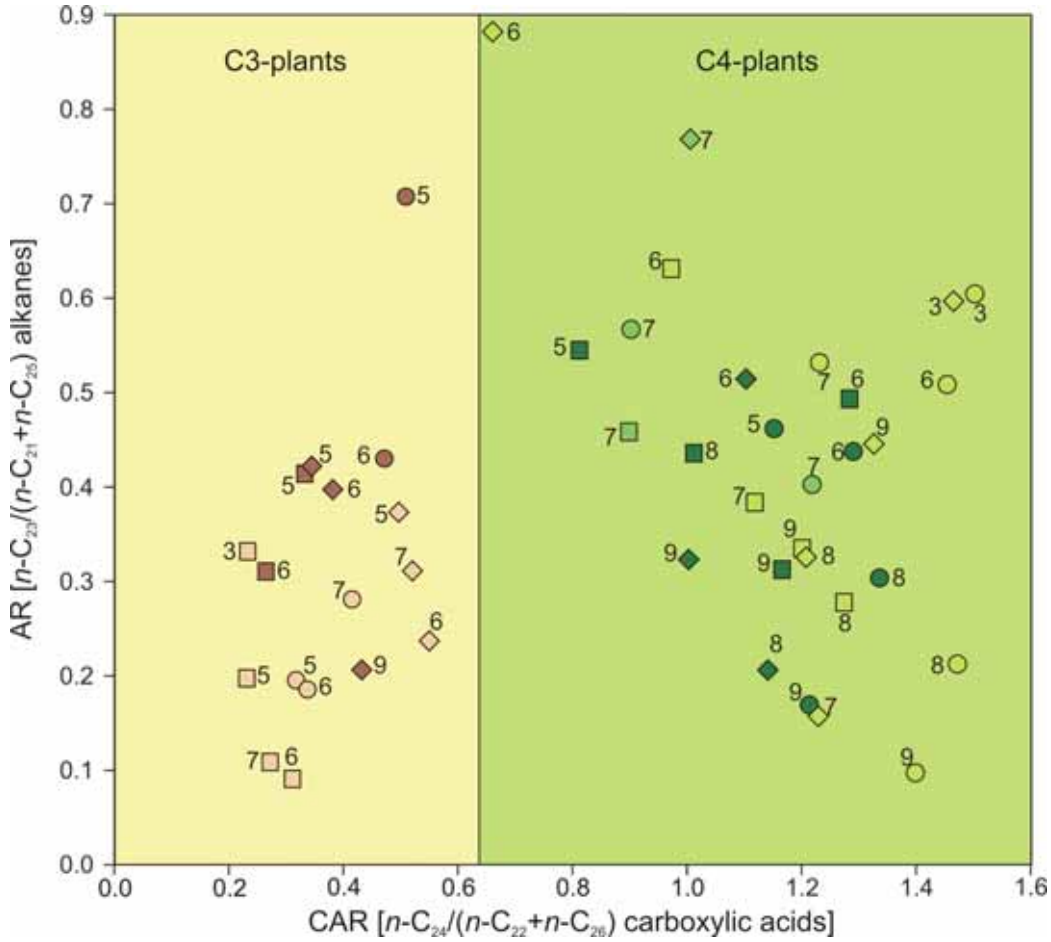


Figure 18. AR (*n*-C_{23} / (*n*-C_{21} + *n*-C_{25}) alkane ratio) in relation to CAR (*n*-C_{24} / (*n*-C_{22} + *n*-C_{26}) carboxylic acid ratio) with definite different populations for C3- and C4-plants. For legend symbols see Figure 17.

5.2.2 Compound-specific isotopic variations ($\delta^{13}C$)

In addition to bulk isotopic data and the CAR, compound-specific isotope ratio measurements ($\delta^{13}C$) were made for aliphatic hydrocarbons and carboxylic acids. In the following section only the most abundant long-chain plant-derived compounds of each lipid fraction were discussed in detail because i) short-chain compounds are often derived from several other sources like bacteria and fungi, and ii) typical plant-derived compounds were a main objective of this study.

5.2.2.1 Aliphatic hydrocarbons vs. bulk isotopy ($\delta^{13}C$)

Generally, most compound-specific isotope data of lipids are depleted by 7-9‰ $\delta^{13}C$ in comparison to bulk isotopic results as an effect of isotope fractionation during lipid generation as previously well documented in other studies (e.g. Winkler *et al.*, 1978, Arens *et al.*, 2000, Hobbie & Werner, 2004). To analyse compound-specific and bulk isotopic relations in detail, compound-specific data were plotted against the bulk isotopy of the individual plant parts (Figure 19). The isotopic variability of single compounds was minimized by producing weighted averages of the isotopic compositions of the three most abundant long-chain homologues of each lipid fraction, as recommended by Arens *et al*. (2000). Thus, weighted isotopic averages of either long-chain *n*-alkanes (C_{29}, C_{31}, C_{33}) or long-chain *n*-carboxylic acids (C_{22}, C_{24}, C_{26}) are calculated and discussed below. In Table 6 the isotopic compositions of those compounds and additionally the isotopic composition of *n*-C_{27} alkanes are given, because the latter alkane is one additional plant-derived compound that is often only slightly less abundant in comparison to e.g. the *n*-C_{33} alkane. Mostly, isotopic results of the individual compounds vary some permil (up to 2‰) because compound-specific isotopic composition depends on several ecological factors, which might lead to significant different isotopic compositions of single compounds (e.g. Arens *et al*., 2000). In a few samples low concentrations of long-chain homologues led to high uncertainties in these results. Thus, weighted averages of compound-isotopic data from long-chain homologues, dependant on the relative proportions of those compounds, are used to avoid uncertainties in compound-specific analyses. In comparison with distribution patterns of alkanes and carboxylic acids not all samples of wheat and rye were available for compound-specific isotope-ratio monitoring, because only a few samples could be selected as a result of time and money consuming analyses.

Alkanes were isotopically ($\delta^{13}C$) depleted between 9 and 10‰ in comparison to bulk isotopy ($\delta^{13}C$) (Table 6), which was in good agreement with previous results (e.g. Collister *et al*., 1994, Conte *et al*., 2003).

For rye and wheat plants the compound-specific isotopic depletion in comparison to bulk isotopy was slightly lower than for maize plants (Table 6, Figure 19). For all analysed C3-plant alkanes the isotopic depletion varied only up to 3‰, resulting in $\delta^{13}C$ isotopies of the alkanes between -37 and -42‰. The larger bulk isotopic depletion of rye plants in comparison to wheat plants, observed previously in this study, could be confirmed in compound-specific isotope data. Only a few rye plant parts sampled late in the growing period showed similar compound-specific isotopic values like wheat plants. During the growing season no significant trends of isotopic depletion or enrichment could be observed for wheat or rye plants. Conte *et al*. (2003) observed a slightly heavier mean isotopic

Table 6. Isotopic ($\delta^{13}C$) signatures of bulk biomass, predominant long-chain *n*-alkanes and *n*-carboxylic acids of different plant parts.

Sampling site / plant and plant part	Sampling month	Bulk sample [‰][a]	*n*-C_{27} alkane [‰]	*n*-C_{29} alkane [‰]	*n*-C_{31} alkane [‰]	*n*-C_{33} alkane [‰]	*n*-C_{22} carboxylic acid [‰]	*n*-C_{24} carboxylic acid [‰]	*n*-C_{26} carboxylic acid [‰]
Rye (Halle)									
Roots	May	-30.8±0.1	-41.7±0.3	-40.9±0.3	-42.2±0.3	-43.4±0.3	-38.9±0.1	-38.3±0.2	-41.0±0.6
	June	-29.5±0.1	-39.0±0.3	-40.9±0.3	-41.3±0.3	-42.4±0.3	-36.8±0.2	-37.7±0.5	-38.0±0.0
	July	-31.8±0.1	-37.0±0.7	-37.6±0.1	-41.5±0.3	-42.3±0.3	-37.5±0.3	-37.0±0.1	-38.1±0.2
Stems	May[b]	-30.7±0.2	-	-	-	-	-	-	-
	June[b]	-30.6±0.1	-	-	-	-	-	-	-
	July	-30.7±0.1	-37.6±0.2	-37.8±0.3	-38.5±0.4	-40.4±0.3	-37.1±0.1	-37.5±0.4	-38.2±0.3
Leaves	March	-30.2±0.2	-39.9±0.3	-40.0±0.6	-39.9±0.1	-39.3±0.0	-37.5±0.1	-37.4±0.5	-38.8±0.3
	May[b]	-30.7±0.2	-	-	-	-	-	-	-
	June[b]	-29.8±0.1	-	-	-	-	-	-	-
	July	-31.0±0.1	-36.5±0.5	-39.8±0.6	-41.6±0.2	-42.1±0.7	-38.6±0.3	-38.4±0.4	-39.3±0.4
Wheat (Rotthalmünster)									
Roots	May	-27.9±0.1	-37.0±0.3	-37.3±0.3	-38.2±0.3	-38.2±0.3	-34.6±0.4	-35.1±0.6	-36.6±1.2
	June	-26.1±0.1	-33.8±0.5	-36.1±0.3	-39.5±1.2	-36.3±1.2	-34.6±0.0	-35.5±0.1	-35.7±0.8
Stems	May[b]	-27.3±0.1	-	-	-	-	-	-	-
	June	-28.3±0.1	-36.7±0.3	-38.0±0.2	-39.0±0.2	-39.7±0.8	-35.9±0.1	-36.8±0.2	-37.0±0.4
	September	-27.2±0.1	-36.7±0.2	-38.3±0.4	-38.7±0.2	-40.0±0.5	-35.9±0.1	-36.0±0.2	-36.7±0.3
Leaves	May[b]	-27.3±0.1	-	-	-	-	-	-	-
	June	-29.7±0.1	-36.6±0.4	-38.6±0.3	-39.5±0.5	-39.0±0.6	-37.9±0.0	-38.0±0.2	-37.5±0.5
Maize (Halle)									
Roots	June	-11.5±0.1	-31.1±0.3	-30.2±0.3	-30.8±0.3	-30.8±0.3	-19.2±0.6	-19.6±0.8	-28.8±1.4
	July	-13.0±0.1	-31.4±0.1	-31.7±0.1	-31.8±0.1	n.d.[c]	-18.8±0.8	-20.6±0.7	-19.7±1.8
	August	-12.3±0.1	-29.9±0.3	-29.1±0.3	-30.3±0.3	-29.6±0.3	-20.5±0.1	-21.1±0.4	-23.6±0.8
	September	-14.1±0.1	-31.0±0.3	-31.3±0.3	-30.6±0.3	-29.6±1.1	-20.6±0.4	-21.8±0.4	-20.2±1.7
	March	-12.4±0.2	-31.6±0.0	-30.3±0.5	-32.1±0.2	-26.7±0.5	-17.7±0.3	-18.7±0.2	-19.8±0.1
Stems	June	-11.3±0.1	-21.5±0.1	-21.9±0.0	-23.5±0.1	-22.6±0.5	-19.1±1.2	-21.7±1.1	-27.5±0.9
	July	-12.1±0.1	-23.2±0.7	-24.5±0.2	-24.7±0.2	-24.6±0.2	-19.4±0.8	-23.5±1.2	-28.2±0.8
	August	-13.1±0.1	-24.6±0.4	-25.9±0.3	-25.4±0.0	-25.4±0.5	-21.3±0.1	-23.1±0.9	-24.0±0.3
	September	-13.9±0.1	-26.5±1.2	-25.5±0.3	-29.7±0.0	-24.7±0.5	-20.7±0.2	-24.6±0.1	-24.1±0.3
	March	-13.1±0.0	-28.5±0.3	-31.2±1.0	-34.1±1.2	-26.8±1.0	-19.0±0.5	-20.0±0.2	-20.9±0.3
Leaves	June	-12.1±0.1	-23.3±0.2	-22.5±0.2	-23.9±0.1	-22.9±0.3	-20.3±0.8	-23.3±0.0	-23.0±0.9
	July	-12.0±0.1	-22.9±0.1	-23.1±0.1	-24.3±0.0	-22.8±0.6	-21.5±0.7	-26.1±0.8	-24.4±0.9
	August	-13.3±0.1	-24.2±1.1	-25.5±0.2	-25.7±0.1	-24.0±0.0	-24.5±0.5	-26.8±0.3	-25.4±0.1
	September	-14.0±0.1	-26.9±0.5	-27.3±0.3	-25.1±0.5	-24.6±0.0	-26.5±0.3	-27.1±0.3	-24.9±0.1
Maize (Rotthalmünster)									
Roots	May	-11.1±0.1	-29.1±0.3	-28.5±0.3	-28.0±0.3	-28.2±0.3	-23.9±0.3	-22.5±0.3	-25.7±0.3
	June	-12.4±0.1	-30.9±0.1	-32.2±0.7	-31.9±1.1	-32.1±0.3	-23.2±1.2	-22.1±0.8	-25.0±1.3
	August	-12.4±0.1	-28.9±0.3	-30.4±0.3	-30.4±0.3	-29.1±0.3	-18.6±0.5	-20.9±0.6	-23.6±0.8
	September	-12.8±0.1	-29.2±0.1	-30.1±0.3	-29.1±0.3	-29.6±0.7	-19.1±0.2	-20.4±0.3	-23.2±0.5
Stems	June	-13.6±0.1	-21.4±0.2	-22.5±0.1	-24.6±0.0	-23.1±0.2	-21.1±0.2	-23.5±0.6	-25.4±1.4
	August	-14.3±0.1	-21.9±0.2	-24.1±0.4	-25.7±0.1	-24.0±0.4	-22.5±0.2	-25.4±0.5	-25.8±0.8
	September	-14,1±0.1	-24.9±0.2	-23.7±0.7	-24.8±0.2	-24.7±0.1	-21.7±0.2	-23.8±0.2	-24.0±0.3
Leaves	May	-12.6±0.1	-21.3±0.0	-22.2±0.2	-25.3±0.0	n.d.[c]	-16.7±0.8	-23.1±1.2	-23.5±0.6
	June	-11.6±0.1	-21.1±0.1	-21.2±0.1	-21.4±0.0	-21.7±0.0	-18.9±0.2	-21.7±0.0	-25.1±1.7
	August	-13,4±0.1	-22.9±0.1	-23.1±0.1	-23.0±0.0	-24.1±0.6	-22.7±0.1	-27.1±0.3	-27.0±0.3
	September	-13.7±0.1	-23.0±0.6	-22.8±0.1	-23.1±0.6	-24.6±0.2	-23.8±0.2	-27.2±0.4	-27.2±0.8
Maize (Scheßlitz)									
Fine roots	July	-13.4±0.3	-27.3±0.4	-30.1±0.4	-30.9±0.4	-31.0±0.2	-24.1±0.6	-22.7±0.5	-24.1±0.7
Coarse roots	July	-11.7±0.1	-29.5±0.4	-32.2±0.2	-33.7±0.3	-33.4±0.7	-24.7±0.2	-23.1±0.5	-24.1±0.2
Stems	July	-11.7±0.1	-19.0±0.3	-19.0±0.2	-20.1±0.1	-20.6±0.1	-18.5±0.4	-20.1±0.3	-19.4±0.4
Leaves	July	-11.1±0.1	-19.9±0.2	-19.8±0.1	-20.4±0.2	-20.1±0.2	-17.7±0.1	-20.4±0.6	-18.9±0.6

[a] For single determinations of bulk isotopic composition a standard deviation of ±0.1‰ was assumed.
[b] Compound-specific isotope analyses were not performed for this sample.
[c] Not detectable.

composition (-35.4‰) of long-chain *n*-alkanes (C_{25-35}) in comparison to values obtained in this study. Probably, this might be related either to distinct sampling times or climatic differences in Central Europe and Canada, leading to varying growth conditions of the analysed plants. Additionally, the difference could be related to significant different isotopic compositions of even carbon-numbered compounds, which might be included in the result reported by Conte *et al.* (2003).

Aboveground biomass of maize generally showed an isotopic depletion of 10‰ $\delta^{13}C$ of alkanes in comparison to bulk isotopy (Figure 19b). The later maize plants were sampled during the growing season, aboveground biomass alkanes became successively isotopically depleted. This trend could not be observed for bulk isotopic data, because all samples from maize roots, stems and leaves showed uniform bulk isotopic compositions between -10‰ and -15‰ $\delta^{13}C$. Similarly, Lockheart *et al*. (1997) observed a general isotopic depletion of single alkanes in several tree leaves from spring to autumn up to 6‰. Fresh root biomass and degraded stems and roots (residues sampled in March after the growing season) showed largest compound-specific isotope depletions. The successive isotopic depletion of aboveground biomass with increasing maturity of the plants most likely might result from a selective preservation of the alkanes with the lighter ^{12}C-isotopes and a selective degradation of the heavier ^{13}C-isotope during the growing season. Otherwise the lighter isotopes might be incorporated in alkanes easier with increasing maturity. A remobilization of stored carbohydrates early in the growing season from roots or stems as discussed by Lockheart *et al.* (1997) for several trees seems to be impossible in the annual crops. Probably, these large variations between different maize plant parts might result from a plant internal biosynthetic fractionation during C-transport and fixation (Hobbie & Werner, 2004). Microbial colonization of plant surfaces may also be considered as a process to alter the isotopic composition of long-chain alkyl lipids. Thus, a selective preservation of the proportions of the *n*-C_{33} alkane and a relative decrease in *n*-C_{31} alkane could not led to the successive isotopic decrease, because the *n*-C_{33} alkane mostly had similar or heavier isotopies than the other homologues as observable in Table 6 and hence led to a reduction of the isotopic depletion caused by *n*-C_{29} and *n*-C_{31} alkanes. If the *n*-C_{33} alkane would not be included in the weighted average calculations the isotopic depletion would have been much larger than observable in Figure 19b. Isotopic compositions of C_{27} and C_{29} *n*-alkanes with –19.1‰ and –18.4‰ of maize leaves observed by Lichtfouse *et al*. (1994) were heavier than all leaf isotopic compositions obtained in this study. Probably, the differences in compound-specific results could be related to different extraction procedures, used by Lichtfouse *et al*. (1994) or different maize genotypes analysed. Contrastingly, the isotopy for a maize plant *n*-C_{31} alkane determined by Collister *et al.* (1994) with -20.5‰ is in good agreement with the results presented in this study for maize leaves from Scheßlitz site. Chikaraishi & Naraoka (2003) presented *n*-alkane

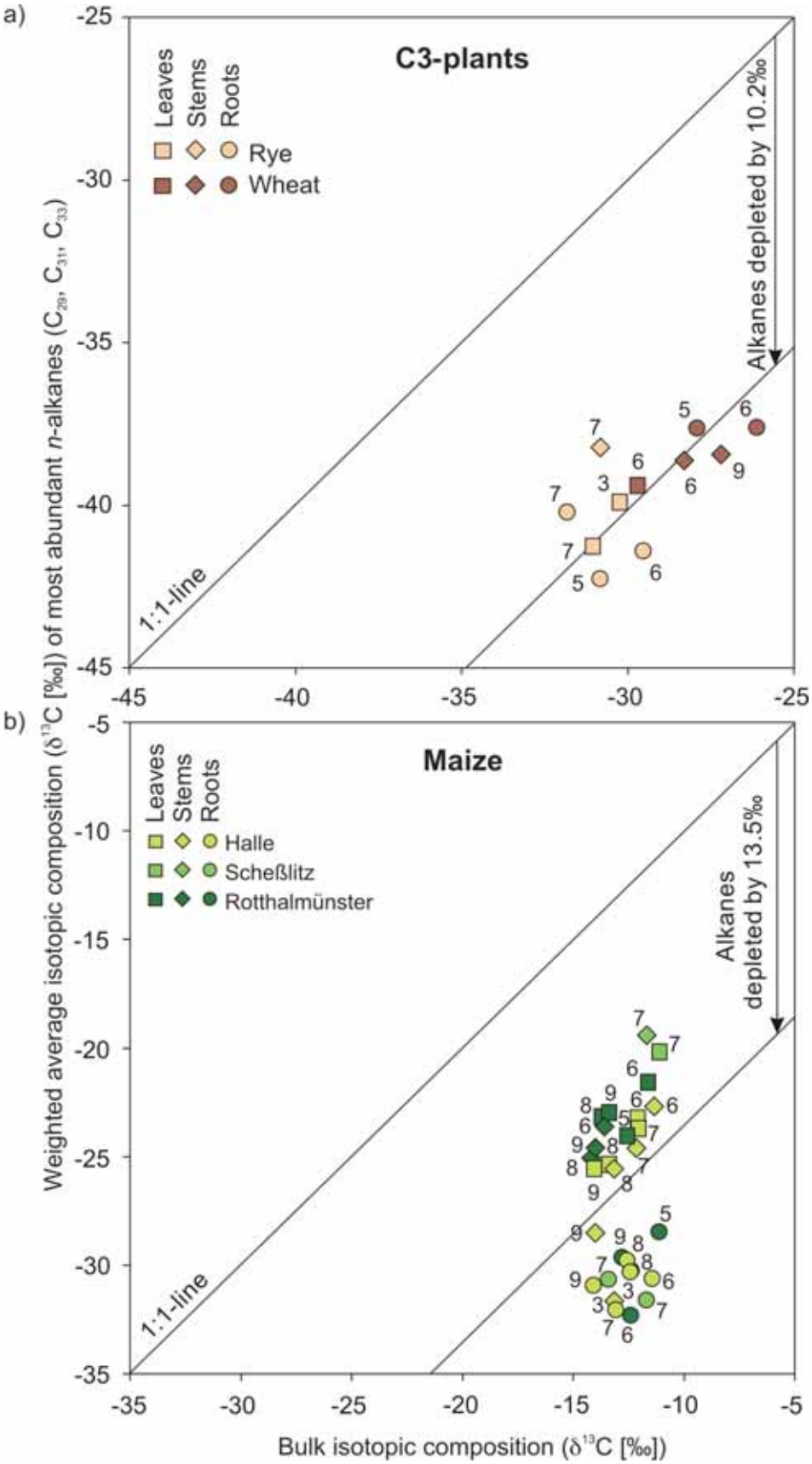


Figure 19. Weighted average compound-specific isotopic composition of most abundant *n*-alkanes (C_{29}, C_{31}, C_{33}) in relation to bulk isotopic compositions for plant parts of a) C3-plants and b) Maize from different sampling sites.

isotopic compositions between –21‰ and -22‰ for maize leaves sampled in July, which corresponded well to our determinations. Summarizing, differences in lipid distributions and compound-specific isotopic results between results reported in the literature and results shown here were most likely caused by different sampling times, climatic differences and/or extraction procedures applied. The very light isotopic compositions of maize roots in comparison to aboveground biomass in this study may be caused by several factors. First, roots probably metabolized alkanes or precursors of alkanes directly in the rhizosphere and thus used other carbon sources for the alkane generation than the aboveground biomass. This could be deduced from the constant compound-specific isotopic signatures of roots throughout the whole growing season, and that they remained stable after plant death, independent of the isotopic development of the aboveground biomass. Second, plant internal transport of substances from stems or even leaves might have led to an isotopic depletion during the vertical transport towards the roots. Third, it is well known that microorganisms play an important role especially in the rhizosphere for the assimilation and uptake of nutrients. The 'microbial loop' means, that nutrient uptake by plants is forced by the presence of microorganisms directly from microorganisms or as a symbiotic effect, while microorganisms themselves degrade SOM and plant material (Bonkowski, 2004). The recycling of plant material by microorganisms and the uptake of microbial biomass into plant biomass possibly have led to a selective preservation of single compounds or compound classes with i) a further discrimination in the abundance of compounds, and ii) a further isotopic discrimination of root compounds in comparison to aboveground biomass. Most likely a combination of all factors led to the different distribution patterns and isotopic compositions of maize root biomass in comparison to aboveground biomass. Reasons for the large differences between plant internal compound-specific isotopies of maize alkanes could not be explained exactly in this study and need to be investigated on a more plant-physiological approach. Probably, seasonal developments within C3-crops like rye and wheat could be observed similar to those of maize, if there were several more samples analysed during the growing period. Otherwise it seemed to be improbable, because all analysed C3-samples showed minor variations in contrast to maize plants.

5.2.2.2 Carboxylic acids vs. bulk isotopy ($\delta^{13}C$)

Compound-specific isotope ratios of carboxylic acids were depleted by 8‰ $\delta^{13}C$ for rye and wheat plants and 10‰ $\delta^{13}C$ for maize plants, when compared to bulk isotopy (Figure 20). Corresponding to bulk and compound-specific isotope data from *n*-alkanes, carboxylic acids of rye plants were slightly more isotopically depleted than those of wheat plants, resulting in -34‰ to -38‰ $\delta^{13}C$ for wheat and -37‰ to -40‰ $\delta^{13}C$ for rye plants (Figure 20a). The results were in perfect agreement with previous observations made by Conte *et al*. (2003), who determined an isotopic composition of –34.4‰ for wheat leaf $C_{24\text{-}34}$ *n*-carboxylic acids. All

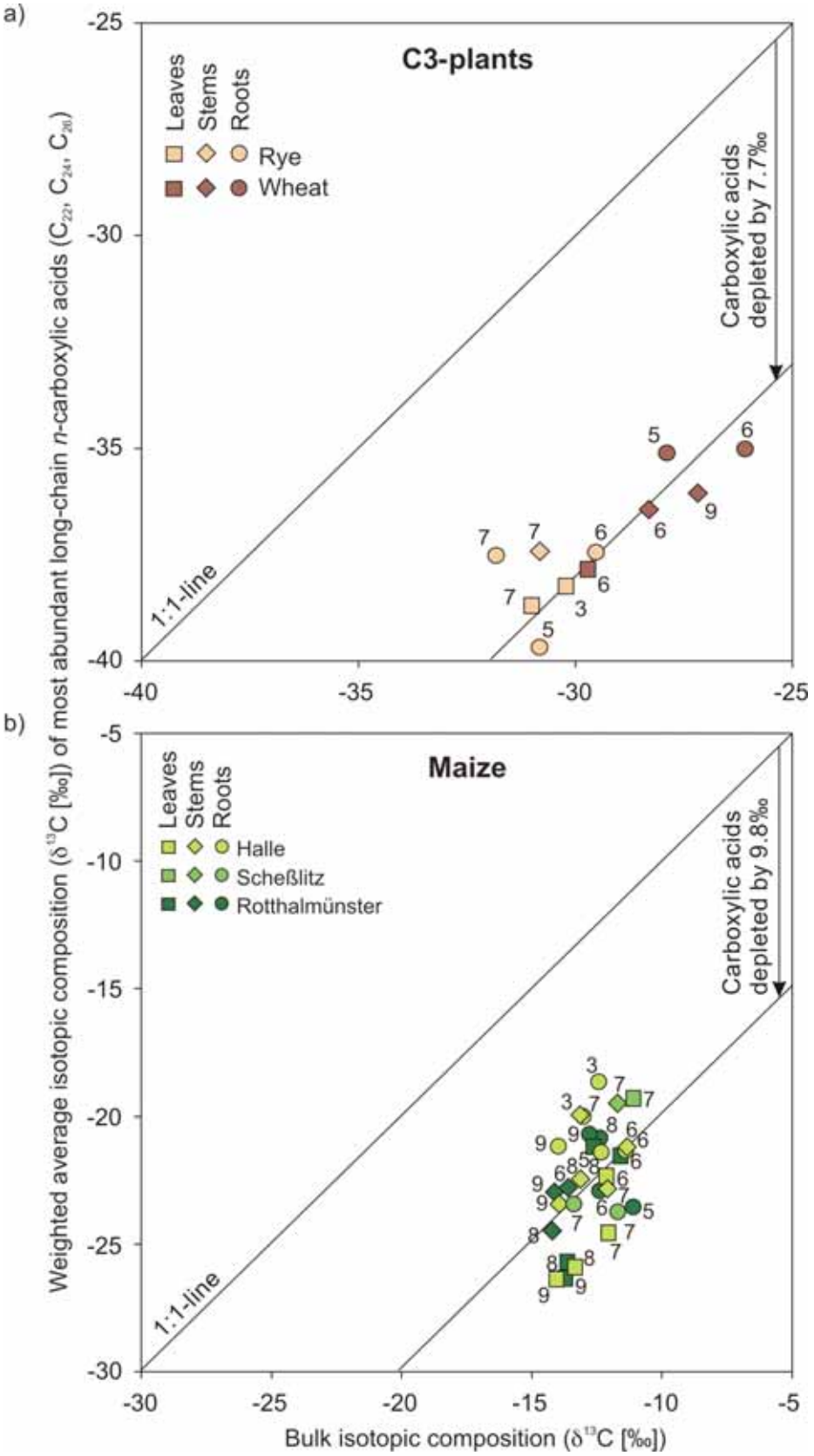


Figure 20. Weighted average compound-specific isotopic composition of most abundant long-chain *n*-carboxylic acids (C_{22}, C_{24}, C_{26}) in relation to bulk isotopic compositions for plant parts of a) C3-plants, and b) maize-plants from different sampling sites.

measured isotopic compositions of C3-plants avoided of any significant trends with increasing plant maturity or vertically within plants.

In contrast to C3-plants, maize plants showed compound-specific isotopic differences for carboxylic acids between distinct plant parts and a variation during the growing season. Roots were depleted in ^{13}C in comparison to aboveground biomass (especially leaves). These isotopic relations between aboveground biomass and roots were contrasting to the isotopic composition of aliphatic hydrocarbons. With increasing plant growth maize leaves got more depleted in ^{13}C, leading to a maximum isotopic depletion of 5‰ of leaves sampled in August and September in comparison to roots. Probably, with increasing maturity of leaves more isotopically light carboxylic acids were incorporated. Maize stems showed similar like roots uniform isotopic compositions during the whole year with a maximum compound-specific isotopic variation of up to 5‰. Degraded stems and roots sampled in March after the growing season showed the heaviest compound-specific isotopic compositions of carboxylic acids, most likely as an effect of preferentially selective degradation of isotopically light compounds. The obtained isotopic results of long-chain carboxylic acids were in perfect agreement with previous determinations made by Chikaraishi *et al*. (2004) of maize leaves sampled in July, where $C_{22\text{-}26}$ *n*-carboxylic acids showed isotopic compositions between –20‰ and -24‰ for free acids and between –23‰ and -26‰ for bound acids.

5.2.2.3 Aliphatic hydrocarbons vs. carboxylic acids

The previous discussions concerned observations made within individual lipid fractions, where differences between aliphatic hydrocarbons and carboxylic acids were determined. To analyse the isotopic differences between both lipid fractions, the weighted averages of the isotopic compositions of the most abundant alkanes were plotted against those of the most abundant carboxylic acids (Figure 21).

Long-chain *n*-alkanes were isotopically depleted in comparison to long-chain *n*-carboxylic acids (2‰ for rye and wheat plant parts). As described above, the isotopic depletion of wheat plants was approximately 2‰ in comparison to rye plants, most likely due to plant differences. For rye plants significant compound-specific isotopic trends were not observable. For wheat plants the isotopic depletion increased from roots over stems with an intermediate depletion to leaves with the largest depletion. Because of the few compound-specific analyses of wheat plants it could not be summarized that the observed plant-internal trend was characteristic for those plants. It could not be excluded, that this trend might be typical for wheat plants. Thus, additional analyses are still needed, to obtain detailed results.

The relations of compound-specific isotopies of long-chain *n*-alkanes and *n*-carboxylic acids for maize plants showed very large variations, which could be expected from

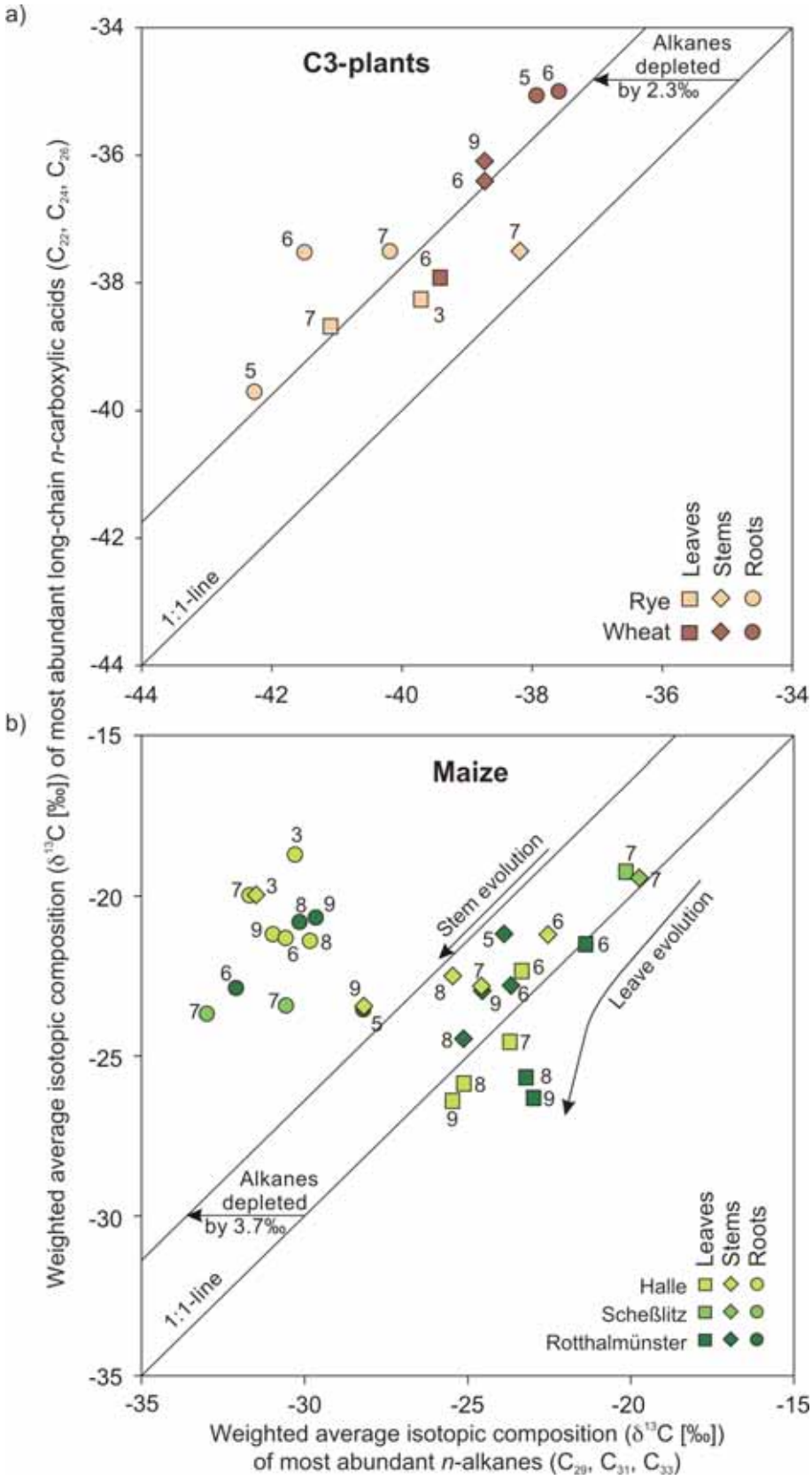


Figure 21. Weighted average compound-specific isotopic composition of most abundant long-chain *n*-carboxylic acids (C_{22}, C_{24}, C_{26}) in relation to weighted average compound-specific isotopic composition of most abundant *n*-alkanes (C_{29}, C_{31}, C_{33}) for plant parts of a) C3-plants and b) maize plants from different sampling sites.

observations made above. For aboveground biomass (stems and leaves) no significant isotopic depletion of alkanes or carboxylic acids could be observed. Roots as well as some stems and leaves sampled between August and March showed significant differences from the 1:1 line. With increasing plant growth leaves got isotopically enriched in both, alkanes and carboxylic acids and late in the growing season leaves from Rotthalmünster site got isotopically depleted exclusively in carboxylic acids, while alkane isotopic compositions remained relatively stable. Early in the growing season stems evolved similar like leaves with a successive isotopic depletion in alkanes and carboxylic acids. For Halle site an isotopic depletion from July over August, September to degraded stems collected in March was recognizable only in alkanes. This was a contrasting trend in comparison to several studies, where *n*-alkanes of fresh and senescent leaves as well as litter of several plants showed an isotopic increase with further degradation (e.g. Nguyen Tu *et al.*, 2004). The comparability of those studies with the results presented here is limited, because previously no plant developments over the growing season or decomposition experiments of grasses were analysed in other studies. The ^{13}C-enrichment of the degraded stem sample from March could not be explained yet, but might be related to selective degradation of isotopically light compounds. The large isotopic depletion of alkanes could not be observed for maize stems of Rotthalmünster site. A good correlation of stems and leaves from both sites until August samples was determined. Maize roots were characterized by a large isotopic depletion of alkanes in comparison to carboxylic acids. First, this might be due to plant internal transport, because stems showed intermediate alkane isotopies between leaves and roots. Second, lipids and especially alkanes in maize roots might be generated from an additional source in contrast to alkanes and carboxylic acids from leaves, because maize roots showed relatively stable isotopic compositions throughout the year and avoid of any significant development similar to stems and leaves. The large heterogeneity of root compound-specific isotopies was most likely due to probable residues of soil and microorganisms on roots or a result of recycling microbial compounds within the roots as a consequence of the ‘microbial loop’, which was previously described in detail in the study and by Bonkowski (2004). This led to a higher contamination in comparison to relatively clean leaves and stems, avoiding any soil and probable low amounts of microorganisms, especially for fresh plant samples. Contrastingly, degraded biomass collected months after harvesting showed a mixed signature of plant biomass and higher contents of e.g. bacteria and fungi, which degraded the plant biomass and grew on and within the plant residues.

Summarizing, long-chain *n*-carboxylic acids were found to be best suitable to differentiate between crops following the C3- and C4-photosynthesis metabolism. Differentiation can be done via the carboxylic acid ratio (CAR = C_{24} / (C_{22} + C_{26}) *n*-carboxylic acids). Within the analysed plants, distribution of lipids and stable carbon isotopes of individual lipids vary between distinct plant parts like roots, stems and leaves, especially for C4-plants.

5.3 Soil evolution during four decades

It is well known, that soil organic carbon usually decreases in soils, where conventional tillage and monoculture cropping is applied over several decades. Most likely, lipids develop similar to total organic carbon in soils, but previously, no studies were published, where lipid contents in soils were described over several decades. Contrastingly, development of isotopic signatures strongly depends on crops used on the individual soils. At 'Eternal Rye' site in Halle simultaneously rye and silage-maize cropping were applied since 1961 on previously exclusively rye cropped soils. Archived samples of ploughed horizons were available since 1958 for rye cropped soil and since 1961 for rye and maize cropped soils. Thus, constant isotopic signatures or a slight isotopic decrease as a result of the Suess effect could be expected for rye cropped soil. For silage-maize cropped soil a progressive isotopic increase could be expected as a result of isotopically heavy maize biomass input.

In the following, dynamics of organic carbon, lipid and carbon isotopic contents during four decades in ploughed horizons of Halle site are discussed. Previously, it was observed, that atmospheric carbon isotopic changes caused significant isotopic changes within plant material (Zhao *et al*., 2001). Contrastingly, a significant Suess effect induced isotopic decrease was not observable in soils over 100 years in the Russian steppe (Torn *et al*., 2002). Probable effects of ploughing depth modifications and Suess effect in soils are discussed in the second part of this chapter.

5.3.1 Carbon and lipid dynamics in soils

Organic carbon content increased slightly since 1958 until 1967 for rye and until 1974 for maize cropped soils. Since these years, carbon values decreased simultaneously in both, rye and silage-maize cropped soils (Figure 22a) during the last two decades, except for samples of the year 1995 with a maximum carbon content of 14g kg^{-1}. An ongoing decrease of carbon contents was estimated for monoculture cropped soils. The increase of carbon contents in the early analysed samples could be most likely related to gentle agricultural managing procedures applied until the late 1960s like horse-ploughing in contrast to tractor-ploughing since the late 1960s or higher contributions of fertilizers. The exceptional high organic carbon content in the year 1995 could not be explained yet. Ploughing depth modifications like the ploughing depth increases in the late 1960s and the early 1990s resulted in significant depletions of organic carbon content within the ploughing horizon of the rye cropped soil as a result of an admixture of the carbon-depleted underlying Axh horizon. Within maize cropped soil only small carbon depletions could be observed simultaneously.

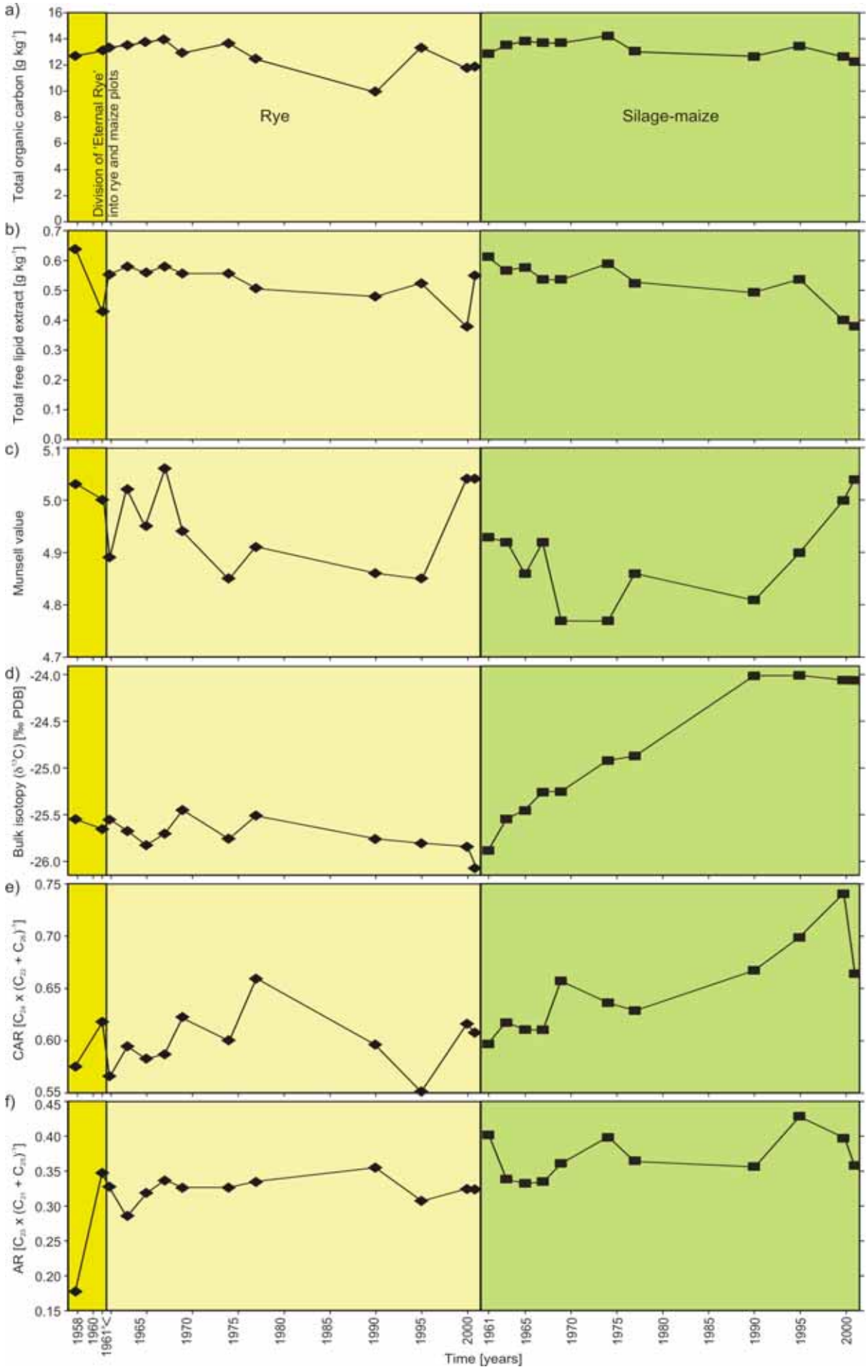
Contents of extractable lipids decreased slightly during the last four decades (Figure 22b). Except for a few samples with large depletions of extractable lipid contents, which were most

likely due to weighing errors of lipid extract or sample heterogeneities, lipid contents generally paralleled organic carbon development. During four decades percentages of lipids varied marginally around 2.5±0.3% of organic carbon content.

Munsell values are shown as an expression of soil lightness (Figure 22c). Within previous studies, soil colour and especially soil lightness has been discussed to coincide with organic carbon contents of soils. Thus, lightness should be lower in samples of higher carbon contents. Especially during the 1960s an increase in carbon contents coincided with decreasing Munsell values, while decreasing carbon contents during the late 1990s until 2001 coincided with an increasing lightness. Some scatter in Munsell values could not be avoided as a result of soil heterogeneity and some minor uncertainties dependant on the measurement procedure.

Isotopic signatures ($\delta^{13}C$) of the analysed soils of 'Eternal Rye' trial developed different dependant on distinct monoculture croppings (Figure 22d). Rye cropped soil showed isotopic variations around –25.5‰. Similar to the TOC, bulk isotopic analyses showed no significant variations over the last four decades for rye cropped soil as also previously reported by others (John *et al*., 2001). Several periods of decreasing ^{13}C-contents like 1961-1965, 1974, 1977-2001 were interrupted by small periods of increasing ^{13}C contents. These small periods were mainly caused by changes in ploughing methods and increasing ploughing depth. Change from horse- to tractor-ploughing in the late 1960s caused an increase from 20 to 25cm (Merbach *et al*., 1999, Schmidt *et al*., 2000). Since the late 1970s tillage modifications were poorly documented and soil samples from the late 1970s to 1990 were not archived. Probably, tillage modifications caused an increase of soil-$\delta^{13}C$. In the early 1990s ploughing depth was increased from 25 to 30cm (personal communication by Dr. L. Schmidt & Dr. W. Merbach, University of Halle). The increase of ploughing depth caused a thinning effect of the ploughed horizon with the underlying horizon with i) lower carbon yields and ii) heavier ($\delta^{13}C$) carbon for rye plot or 'rural' lighter isotopic carbon for maize plot. Generally, an isotopic decrease was observable for rye cropped soil, resulting in –26.0‰ in 2001. An ongoing isotopic depletion was observable since 1977. At hypothesized steady state conditions of the analysed system such depletion could not be explained. Most likely, natural isotopic variations did not cause this depletion, because the isotopic values showed a

Figure 22. (On the following page) Different carbon and lipid parameters of archived soil samples of rye and silage-maize cropped soils from 'Eternal Rye' plot of Halle site. Developments are given for a) total organic carbon content, b) total lipid extract yield, c) Munsell value as parameter for soil lightness, d) carbon isotopic composition ($\delta^{13}C$), e) ratio of long-chain *n*-carboxylic acids (CAR), and f) ratio of long-chain *n*-alkanes (AR).


a)
Total organic carbon [g kg⁻¹]
Rye
Silage-maize
Division of 'Eternal Rye' into rye and maize plots
b)
Total free lipid extract [g kg⁻¹]
c)
Munsell value
d)
Bulk isotopy (δ¹³C) [‰ PDB]
e)
CAR [C21 x (C21 + C23)⁻¹]
f)
AR [C23 x (C21 + C23)⁻¹]
1958
1960
1961<
1965
1970
1975
1980
1985
1990
1995
2000
1961
1965
1970
1975
1980
1985
1990
1995
2000
Time [years]

discrete trend without any variations. Thus, most likely atmospheric Suess effect caused a large isotopic depletion in plants as observed by Zhao *et al*. (2001), leading to a minor isotopic depletion of soil organic carbon. The possible consequences of atmospheric Suess effect for soils of Halle site are discussed in the following chapter. On maize plot $\delta^{13}C$ composition changed significantly from –25.5‰ in the early 1960s to –24‰ in the 1990s (Figure 22d). The same time intervals as described for rye plot could be determined for maize cropped soils. In the late 1960s, the late 1970s and the 1990s until 2001 the maize induced increase of ^{13}C is broken by times of stagnations or slowly increasing ^{13}C contents. Ploughing modifications on the experimental trial have caused these developments. These results were in good agreement with previous determinations of the same plot (John *et al*., 2001, Ludwig *et al.*, 2003). For several long-term periods (1969-1974 and 1977-1990) a C4-plant biomass induced annual increase in $\delta^{13}C$ of 0,066‰ (± 0,000‰) was determined. This corresponds to 0.48% annual input of new maize carbon. After four decades this would have caused a maize-derived carbon proportion of 19.1%. In contrast an isotopic difference between maize and rye cropped soils of 2.3‰ corresponding to only 16.8% maize-derived carbon could be measured (Figure 25). This difference between estimated and measurable maize carbon proportions is caused by several factors like ploughing depth modifications and probably as reactions on atmospheric changes as discussed below.

The newly established *n*-carboxylic acid ratio (CAR calculated with formula (8)) showed differences between rye and maize cropped soils (Figure 22e). For the rye cropped soil of 'Eternal Rye' trial a low ratio of 0.6±0.1 could be observed, which was slightly higher than the ratios of rye plants (Figure 16). The large variations of the rye cropped soil (Figure 20e) during the four decades were most likely due to different amounts of plant fragments and inhomogeneities of soils. Contrastingly, maize cropped soil showed an increasing CAR from 0.6 in 1961 to nearly 0.75 in 2000 as a result of increasing proportions of maize-derived carbon in this soil. Most likely, higher proportions of plant litter were responsible for the increase of the ratio in the year 1969. The depleted ratio of the year 2000 was probably caused by several effects like i) exceptional low litter contents in the analysed sample and/or ii) the different lipid composition of the sample collected in spring, while others were usually taken in autumn. Distributions of other carboxylic acids showed no significant developments during the last decades. Hence, they were not shown here.

The ratio of the decarboxylated *n*-alkane homologues of the carboxylic acid ratio (AR, calculated with formula (9)) showed differences between both soils (Figure 22f). Ratios of rye cropped soil were relatively constant since 1961 and varied around 0.32. For maize cropped soil this ratio showed a larger variability than for rye cropped soil and generally higher values around 0.35. A significant increase in this ratio as a result of higher maize-derived carbon

contents was observable for maize cropped soil. All other long-chain *n*-alkanes showed constant distributions over four decades and no differences between both soils and thus are not shown here.

5.3.2 Suess effect in soils?

During the last decades several studies were published, where effects of the atmospheric Suess effect for plant biomass are studied (e.g. Zhao *et al*., 2001). As a result of the atmospheric CO_2 enrichment due to fossil fuel burning (e.g. Amthor, 1995), fossil fuel derived light carbon isotopes (approximately –25‰) depleted isotopic composition of the atmosphere significantly by nearly 2‰ since the beginning of the industrial revolution (e.g. Friedli *et al*., 1986). Plants following different photosynthetic pathways react significantly different on atmospheric carbon dioxide enrichment and $\delta^{13}C$ depletion. Plants following the C3-photosynthetic pathway reinforce atmospheric isotopic depletion significantly during biosynthetic fixation of carbon, as demonstrated for grasses (Zhao *et al*., 2001). Contrastingly, C4-plants reflect directly atmospheric isotopic depletion during biosynthetic carbon fixation, by a constant isotopic fractionation, independent of atmospheric carbon isotopic composition (Marino & McElroy, 1991). Due to isotopic response of plant biosynthesis it seems to be probably, that soils react on changes of atmospheric composition. Previous studies were very scarce, where soils were analysed for their response on atmospheric and plant compositional changes, except for the work by Torn *et al*. (2002), where no significant response on atmospheric changes was observed, when regarding an archived, 100 year old, soil profile of Russian steppe in comparison with a recent soil. Developments of soils in urban areas were not analysed previously, where an atmospheric carbon dioxide concentration plume as a result of increased fossil fuel burning (Idso *et al*., 2001, 2002) might cause an increasing atmospheric isotopic depletion in this area. Responses of these effects were not mentioned previously in soils. Archived samples from over four decades of the ‘Eternal Rye’ site in the urban Halle area might provide ideal conditions for time series analyses of soil responses on atmospheric carbon development. While e.g. long-term developments of carbon concentrations and isotopic compositions of soils are discussed in the chapter above, in the following hypothetical developments of soil carbon isotopies are calculated and it is postulated, how ploughing modifications and Suess effect might affected the soils of ‘Eternal Rye’ trial.

Under steady state conditions, carbon isotopic composition of rye cropped soil, in the following named C3-cropped soil, should have remained constant during the last 4 decades (Figure 23), expressed as

$$\delta_S = \delta_0 \qquad (10)$$

with δ_S as the carbon isotopic composition [‰] of the soil at the time t_S [a], the year after plot subdivision or ploughing depth modifications. δ_0 is the carbon isotopic composition [‰] at the time t0 [a], the year of the plot subdivision or ploughing depth modifications.

Ploughing depth modifications would have caused an isotopic increase as a result of the slightly isotopically heavier underlying Axh horizon (–25.1‰ $\delta^{13}C$). It was measured an identical isotopic composition of Axh horizons of both, rye and maize cropped soils, with a standard deviation of ±0.1‰. The ploughing depth modifications must have led to an isotopic increase of 0.2‰ for rye cropped soil as a result of a ploughing depth increase from 20 to 30cm in two steps (Figure 23), which could be calculated for the individual steps as follows

$$\delta_S = (\delta_0 D_0 D_S^{-1}) + (\delta_R (D_S - D_0) D_S^{-1}) \quad (11)$$

with D_0 as the ploughing depth [cm] at time t_0 [a] and D_S as the ploughing depth [cm] after modification at time t_S [a] and δ_R as the carbon isotopic composition of the corresponding Axh horizon (-25.1‰). This formula is used for the calculation of the carbon isotopic composition of the Axp horizon directly after the ploughing depth modifications. For the calculation of the carbon isotopic composition of the following years (Figure 23) formula (10) was used with the time t_0 as the year of the ploughing depth modification.

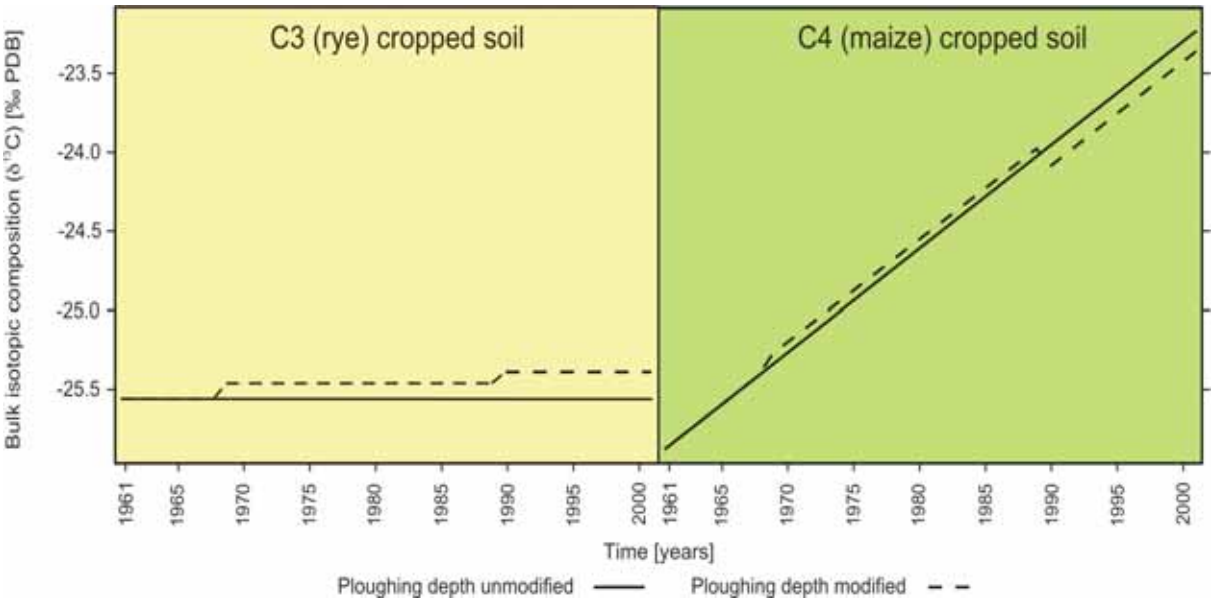


Figure 23. Theoretically expected developments of a C3 (rye) cropped soil and a C4 (maize) cropped soil under steady state conditions, with an annual incorporation of similar amounts of the corresponding biomass (black lines). Theoretical changes of soil carbon isotopic composition as a result of ploughing depth modifications are displayed with interrupted lines, based on calculated data derived from formulas (9) – (11).

In contrast isotopic composition of maize, isotopy of maize cropped soil would have increased with further maize incorporation, because maize with larger $\delta^{13}C$ contents

(–11.7‰) was incorporated on a previously C3-cropped soil. The initial, C3-labelled isotopic composition of the maize plot of the year 1961 was slightly isotopically depleted in comparison to rye cropped soil as could be observed in Figure 22, probable as an effect of soil heterogeneity between both plots. Nevertheless, this slightly depleted soil was taken as the initial basis. As observed for several periods (1969-1974 and 1977-1990) an annual increase of 0.066‰ $\delta^{13}C$ could be measured (Figure 22), calculable as follows:

$$\delta_S = 0.066\ (t_S - t_0) + \delta_0 \qquad (12)$$

A constant annual isotopic increase seemed to be improbable, because the largest changes following an exponential expression would be theoretically expected during the first years. But for a first approximation, a simple linear model seemed to be easier to handle, than exponential expressions, and is thus discussed here. Under steady state conditions this would have caused a $\delta^{13}C$-content of maize cropped soil of ~ –23.2‰ in 2001 (Figure 23). Reported ploughing depth modifications could be calculated with the following formula:

$$\delta_S = ((0.066\ (t_S - t_0) + \delta_0)\ D_0\ D_S^{-1}) + (\delta_R\ (D_S - D_0)\ D_S^{-1}) \qquad (13)$$

Ploughing depth modifications of the maize plot from Halle site led to a $\delta^{13}C$ increase in the late 1960s and an isotopic depletion in the 1990s (Figure 23). The idealised results for a steady state system corrected with ploughing depth modifications would have caused an isotopic difference of 2.3‰ $\delta^{13}C$ between both soils in 2001. This final steady state result for the year 2001 coincided well with the measurable isotopic results as shown in Figure 22. But this model might not explain the following tendencies: i) slightly isotopic depletion of rye cropped soil, ii) isotopic depletion within maize cropped soil in the late 1970s, and iii) stagnation of maize cropped soil isotopic compositions during the 1990s. Possible reasons for these effects are discussed in the following section.

During the last four decades fossil fuel burning caused a depletion in atmospheric $\delta^{13}C$ (δ_A [‰]) of ~1.1‰ (Figure 24a), which was calculated after Feng (1998):

$$\delta_A = -6.429 - 0.0060 \exp (0.0217\ (t_S - 1740)) \qquad (14)$$

The atmospheric carbon isotope shift caused different biosynthetic reactions of different plants. A greater production of biomass (Amthor, 1995) as well as greater root biomass production (Kuzyakov, 2001) on the one hand, and an amplified biosynthetic fractionation in C3-plants (Arens *et al*., 2000, Zhao *et al*., 2001) on the other hand have been observed. Zhao *et al*. (2001) observed biosynthetic evolutions for wheat (Figure 24b) that can be transferred to rye plants, because of identical biosynthetic mechanisms in both plants:

$$\delta_G = -23.24 - 0.00605 \exp (0.0398\ (t_S - 1843)) \qquad (15)$$

$$\delta_W = -25.27 - 0.00038 \exp (0.0571\ (t_S - 1843)) \qquad (16)$$

with δ_G for wheat grain, δ_W for wheat straw and t_S as the year for the calculated isotopy.

In contrast to C3-plants, isotopic fractionation during photosynthesis is significantly lower during C4-plant biosynthesis. Additionally, atmospheric changes like an increase in CO_2 and isotopic carbon changes caused no significant larger isotopic fractionation in maize plants (Marino & McElroy, 1991). Furthermore, maize plants directly reflect atmospheric carbon isotopy, calculable with the following expression after Marino & McElroy (1991), which is also shown in Figure 24b:

$$\delta_M = -3.276 + \delta_A \quad (17)$$

with δ_M for maize $\delta^{13}C$ and δ_A for atmospheric $\delta^{13}C$.

The atmospheric carbon changes as i) an enrichment in CO_2 and ii) a depletion in $\delta^{13}C$ caused an isotopic decrease of ~2.7‰ for rye and 1.1‰ for maize plants during the last four decades, calculated as described above and shown in Figure 24b.

The successive incorporation of lighter carbon isotopes during the last decades is calculated for a steady state system and an annual fixation of 0.48% new carbon for eternal rye plot (Figure 24c). For this annual carbon fixation for rye cropped soil a biosynthetic induced slight depletion of 0.19‰ $\delta^{13}C$ after four decades could be expected. This depletion corresponded to the measured depletion for the rye cropped soil but in a minor magnitude, because a depletion of 0.5‰ was determined after four decades. Probably, changes in rhizodeposition and greater root biomass production caused by atmospheric CO_2 changes (Kuzyakov, 2001) as well as higher biomass input produced a larger reflection of atmospheric and biosynthetic fractionation processes, than estimated. Other explanations could be i) the continued heavier fractionation during microbial processes, and/or ii) the natural heterogeneity of soils, leading to uncertainties in the obtained results, depending on sampling time or sampling treatment.

For maize plants a slight decrease of 0.08‰ caused by atmospheric changes could be estimated during the last four decades, if 0.48% new maize carbon was incorporated per year. Hence, a rise in atmospheric CO_2 must have caused isotopic effects in soils of long-term field experiments, although the Suess effect induced changes in soils must have been low due to an annual incorporation of only 0.48% new carbon. The annual carbon incorporation calculated here represented the minimum carbon input as a result of silage harvest. Grain cropping would have led to higher biomass input and thus, a larger carbon isotopic shift, resulting in heavier isotopies, but otherwise a larger isotopic depletion as a result of the atmospheric Suess effect.

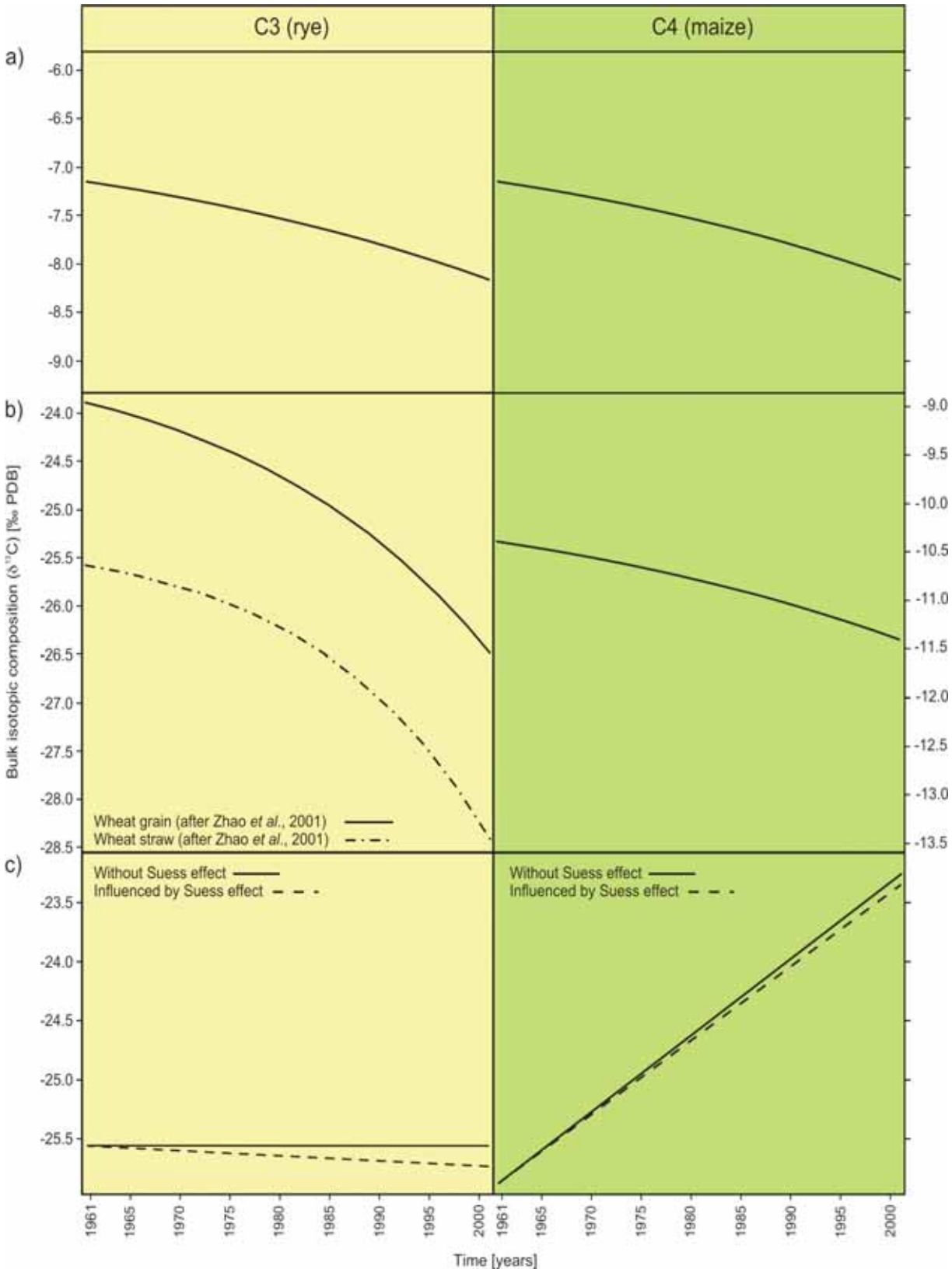


Figure 24. Influence of the Suess effect on the carbon isotopic composition ($\delta^{13}C$) in several ecosystems over the last four decades: a) development of the atmospheric composition after Feng (1998), b) plant material development after Zhao *et al*. (2001) for C3-plants and after Marino & McElroy (1991) for C4-plants, c) theoretical Suess effect postulated for Halle soils, without any ploughing modifications and for steady state conditions.

An isolated contemplation of carbon results of individual maize plots without regarding the corresponding reference plots for each year might have caused some mistakes, because the obtained results depended on several factors like water stress or sampling date. Thus, it would be helpful to analyse the development of the isotopic difference between rye and maize cropped soil over the last four decades. Only regarding the measured isotopic differences between both plots, a difference of 2.3‰ could be observed (Figure 25). But as discussed above, disregarding of ploughing depth elevation, atmospheric and biosynthetic fractionations have led to underestimations in soil carbon fixation processes. Correction of ploughing depth modifications for the isotopic difference would have led to an isotopic difference of 3.3‰, corresponding to 23.7% maize-derived carbon after four decades of maize cropping. The annual increase of the isotopic difference between both soils (0.085‰) without regarding periods of decreasing isotopic carbon differences led to a similar result (3.39‰ difference and 24.5% maize derived carbon). These results seemed to reflect realistic developments of eternal rye plot without ploughing modifications. Hence, ploughing modifications have led to a significant reduction and underestimation of carbon fixation. To predict future trends adequate corrections as discussed above are needed. A more progressive tendency is also shown in Figure 25 that reflects only the mean of the increasing isotopic periods and corresponded to an annual increase of 0.137‰. This model could be negotiated because it seems that obtained results can be overestimated at a great degree for silage-maize cropping. The discussed models could not explain the reduction of the isotopic difference, observed for 1977 compared to 1974. Probably, the ploughing depth was slightly (e.g. 5cm) elevated for a short time or perhaps sampling was practised less carefully or at different times during the growing season. If there was another ploughing depth modification, it had to be corrected, leading to higher proportions of new maize-derived carbon. There were no documented modifications and hence, any exact recalculations were impossible. Another inexplicable effect was observed during the 1990s until 2001 where an isotopic stagnation or a slight decrease of the isotopic difference was measured. It was assumed that ploughing depth was elevated 1990, but a successive ploughing depth elevation over several years could not be excluded. However this cannot explain the slight isotopic decrease observed between 1995 and 2000. Probably, maize biomass input was reduced during the last decade or other processes have led to this effect.

Summarizing, soil development and evolution of stable carbon isotopes depended on a lot of factors, but were strongly influenced by ploughing and harvesting techniques applied. Thus, probable corrections of these effects were very difficult and reliable informations about ploughing and harvesting method modifications are needed. If such informations were not available, recalculations might contain large uncertainties. Hence, the measurable isotopic difference of 2.3‰ for Halle soils represented only a minimum of the new maize-derived

carbon proportion after four decades of different cropping. Higher proportions and thus lower turnover times could be estimated, probably. These results can be transferred to other long-term arable field experiments, because for field experiments it often cannot be avoided that ploughing and cropping modifications have occurred.

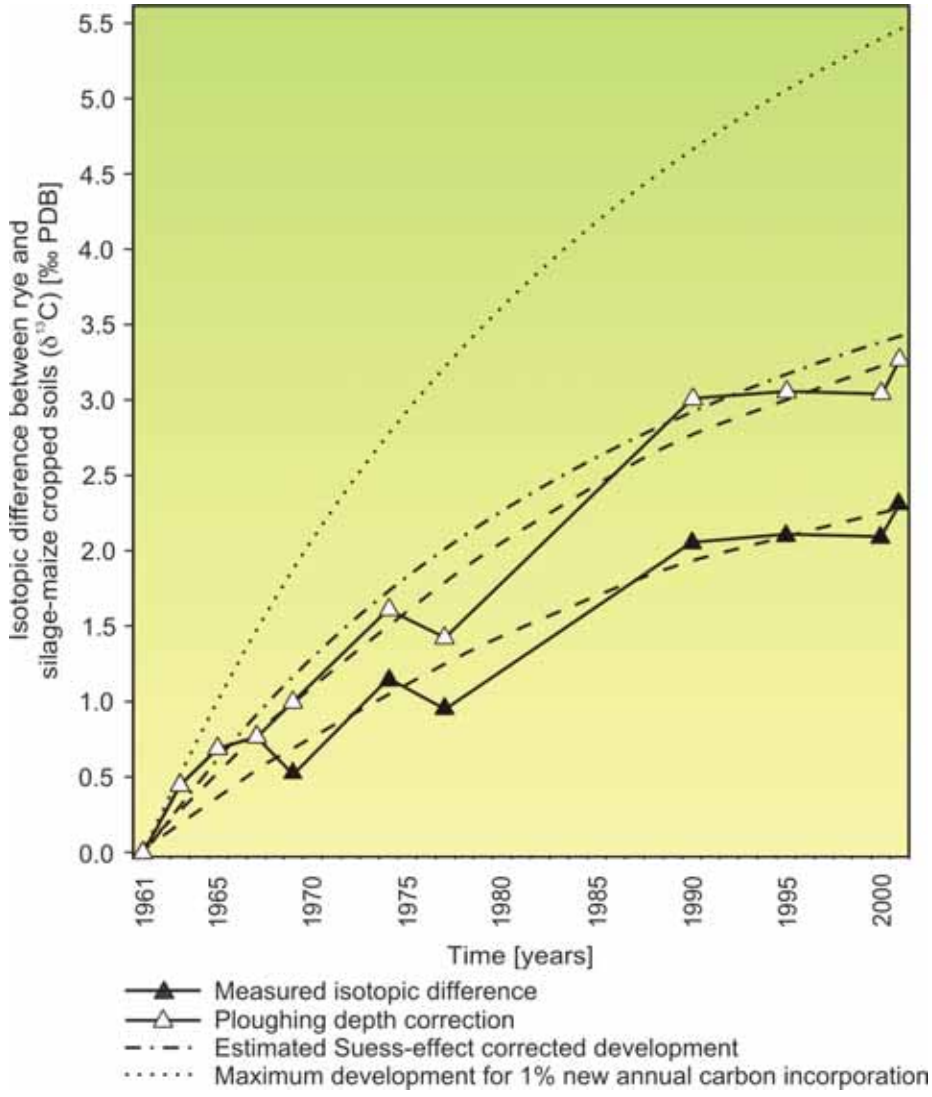


Figure 25. Measured and calculated developments of the isotopic difference between rye and silage-maize cropped soils from 'Eternal Rye' plot. The measured development of the isotopic difference was significantly lower than those obtained after recalculation of ploughing depth modifications and Suess effect changes. Additionally, an annual incorporation of 1% new maize carbon is shown.

Summarizing, time-series of archived arable soil samples from Halle site reveal significant changes as a result of several ploughing depth increases. Nevertheless, a small isotopic ($\delta^{13}C$) decrease over four decades in Halle rye cropped soil indicates atmospheric Suess effect induced changes of soil organic matter. As a result of observations and calculations on archived soil samples, significant carbon (mass and isotopic) changes can be estimated for arable soils, due to atmospheric Suess effect and resulting plant carbon changes.

5.4 Organic carbon and lipid distribution in soil profiles

In this chapter, first, results of soil textures, carbon and nitrogen distributions as well as stable carbon isotopic ($\delta^{13}C$) compositions in particle-size separates and bulk soils are shown, followed by analyses of lipid extract yields and lightness of corresponding samples. Here, predominantly samples of Halle site are discussed, because as a result of limited time only samples of three profiles with different crops from Halle site could be preparatively separated into several particle-sizes. Additionally, some results of samples from other sites are discussed. Thereafter, lipid distribution patterns of bulk soils from the ploughed horizons of several sites (Halle, Rotthalmünster, Boigneville) are revealed, ending with the discussion of compound-specific isotopies of the most abundant plant-derived long-chain lipids.

5.4.1 Bulk analyses of bulk soils and particle-size separates

Most results discussed in the following chapter are part of the study Wiesenberg *et al*. (2004c).

5.4.1.1 Soil texture, organic carbon and nitrogen distribution

Soil texture

The preparative particle-size separation recovered most of the bulk material (>99%) for all samples from Halle site (Table 7). Sand dominated the textures of all soils and horizons (64-75 mass %), with maize cropped soils containing 3-8% less sand than the rye cropped soil, consistent with previous results (e.g. Stumpe *et al*., 1990, Merbach *et al*., 2000, Ludwig *et al*., 2003). In the maize cropped soils sand concentrations were uniform throughout the profile, but increased with depth in the rye cropped soil. For all soil profiles clay contents were lowest in the ploughed horizons. As additional information, pH increased with soil in rye cropped soil from 5.7 to 6.2 and in maize cropped soil from 5.7 to 7.1 (Ludwig *et al*., 2003). The different soil textures and pHs for rye and maize cropped soils confirmed previous observations made by Stumpe *et al.* (1990).

Carbon and nitrogen concentrations and distributions

Carbon and nitrogen concentrations (Table 7) were similar in all soil profiles from Halle site, typical for Haplic Phaeozems (Batjes, 1996). In bulk soils, carbon and nitrogen concentrations systematically decreased with increasing soil depth (Axp>Axh>Bv), typical for most soils (e.g. Boutton *et al*., 1998). Carbon and nitrogen concentrations generally were larger in the maize cropped soils than in the rye cropped soil, except for carbon in the Axp horizon of the silage-maize cropped soil and nitrogen in the Bv horizon of the grain-maize cropped soil. In contrast to the decrease of carbon and nitrogen concentrations of grain-maize and rye cropped soils, the concentrations remained constant in the Axh and Bv horizons of silage-maize cropped soil.

Table 7. Soil properties of bulk soils and size-separates from Halle soil profiles.

Horizon	Size separate	Mass±SD [a]	Organic C		$\delta^{13}C$	N		C:N	Lipid extract yield	
	[μm]	[% of bulk] [b]	[g kg^{-1}] [c]	[% of bulk]	[‰] [d]	[g kg^{-1}] [c]	[% of bulk]		[g kg^{-1}] [c]	[g kg^{-1} OC] [e]
Rye cropped soil										
Axp	Sand 63-2000	70.3±0.4	3.0	18	-24.5	0.1	9	26	0.03	83
	Silt 2-63	19.9±0.7	21.7	36	-25.6	0.4	23	21	1.37	63
	Clay 0.45-2	9.2±0.3	47.5	37	-25.7	4.0	52	9	1.56	33
	% recovery	*99.4*		*92*			*84*			
	Bulk soil		11.8		-25.7	0.7		13	0.55	47
Axh	Sand 63-2000	73.3±0.6	0.6	13	-23.8	< 0.1	10	- [f]	0.20	313
	Silt 2-63	17.6±0.3	4.5	22	-25.7	0.4	16	12	0.44	97
	Clay 0.45-2	8.6±0.2	26.1	62	-25.4	3.7	74	7	1.44	55
	% recovery	*99.5*		*96*			*100*			
	Bulk soil		3.6		-25.0	0.4		10	0.35	96
Bv	Sand 63-2000	75.1±0.5	0.3	11	-25.3	< 0.1	11	- [f]	0.01	33
	Silt 2-63	16.4±0.0	2.0	15	-25.8	0.3	16	6	0.05	23
	Clay 0.45-2	7.6±0.2	21.5	74	-24.8	3.1	68	7	0.37	17
	% recovery	*99.1*		*99*			*95*			
	Bulk soil		2.2		-25.1	0.3		6	0.02	9
Silage-maize cropped soil										
Axp	Sand 63-2000	67.0±0.7	3.1	18	-22.9	0.1	9	28	0.11	37
	Silt 2-63	22.0±0.5	19.5	37	-24.2	0.9	25	21	0.60	31
	Clay 0.45-2	10.5±0.2	41.8	38	-23.9	4.6	58	9	1.41	34
	% recovery	*99.5*		*93*			*93*			
	Bulk soil		11.6		-24.0	0.8		14	0.38	33
Axh	Sand 63-2000	67.0±1.7	0.5	5	-25.0	< 0.1	5	- [f]	0.03	53
	Silt 2-63	20.8±1.0	5.6	16	-24.9	0.6	16	10	0.23	41
	Clay 0.45-2	12.1±0.4	34.9	56	-25.1	4.4	72	8	0.81	23
	% recovery	*99.9*		*76*			*93*			
	Bulk soil		7.5		-25.1	0.8		10	0.13	17
Bv	Sand 63-2000	66.6±1.3	0.4	5	-20.1	< 0.1	6	- [f]	0.03	86
	Silt 2-63	20.8±0.1	3.7	14	-24.8	0.4	13	9	0.08	21
	Clay 0.45-2	12.5±0.0	34.7	75	-25.4	4.0	76	9	0.57	17
	% recovery	*99.9*		*93*			*95*			
	Bulk soil		5.8		-24.8	0.7		9	0.09	15
Grain-maize cropped soil										
Axp	Sand 63-2000	64.3±0.5	3.3	17	-24.3	0.1	7	26	0.08	23
	Silt 2-63	23.7±0.9	18.9	36	-25.2	0.9	20	20	0.62	33
	Clay 0.45-2	11.3±0.2	48.6	45	-25.1	4.9	49	10	1.71	35
	% recovery	*99.3*		*98*			*76*			
	Bulk soil		12.3		-24.6	1.1		11	0.35	28
Axh	Sand 63-2000	63.7±0.9	0.8	6	-24.2	< 0.1	4	- [f]	0.02	29
	Silt 2-63	22.4±0.7	6.9	20	-25.7	0.5	15	14	0.14	20
	Clay 0.45-2	13.0±0.6	39.3	67	-25.3	4.3	76	9	0.89	23
	% recovery	*99.1*		*98*			*96*			
	Bulk soil		7.7		-25.7	0.7		10	0.13	17
Bv	Sand 63-2000	65.0±1.1	0.3	5	-26.0	< 0.1	5	- [f]	0.01	34
	Silt 2-63	21.5±0.6	2.5	15	-26.3	0.3	14	9	0.05	19
	Clay 0.45-2	12.7±0.6	22.9	80	-25.5	2.8	78	9	0.39	17
	% recovery	*99.2*		*100*			*96*			
	Bulk soil		3.6		-25.8	0.4		8	0.04	11

[a] Standard deviation. Number of analyses varied between 10 and 13.
[b] Particle-size separates obtained after ultrasonic dispersion. For analytical details see chapter 4.2.
[c] Expressed as g kg^{-1} size separate.
[d] Carbon isotopic composition ($\delta^{13}C$) expressed as ‰ PDB.
[e] Expressed as g kg^{-1} organic carbon size separate or bulk soil.
[f] Nitrogen concentrations too small to calculate.

In the particle-size separates carbon and nitrogen concentrations consistently decreased with increasing particle-size (clay > silt > sand) (Table 7) (Christensen, 1996). To discuss the distribution of carbon and nitrogen between the particle-size separates, the percentages of the total organic carbon or nitrogen are calculated (Table 7), respectively. The calculated recoveries generally were large (≥92%) for carbon and nitrogen, with only one exception for carbon and two exceptions for nitrogen (76-84%). Distributions of carbon and nitrogen were identical in all horizons, i.e. clay-size separates contributed the largest proportions to soil organic carbon (≥37%) and nitrogen (≥49%), followed by silt- and sand-size separates. Proportions of carbon and nitrogen stored in the clay-size separates increased consistently with soil depth, on the expenses of the silt- and sand-size separates. Compared to other soils, the contributions of the clay-size separates to total organic carbon were lower than those observed for other arable soils of similar textures (Christensen, 1996). The lower contributions of the clay-size separates were most likely due to larger contents of carbon enriched brown coal particles in the coarse separates, leading to higher contributions of sand- and silt-size separates to total carbon.

C:N ratios were similar for all soils of Halle site (Table 7), but slightly less for grain-maize cropped soil, most likely due to low biomass input during the last four decades prior to conversion to grain-maize cropping. During these decades existing carbon was probably degraded, while microorganisms mineralized nitrogen, preferentially. The C:N ratios of the bulk soils decreased with soil depth, i.e. from the Axp horizon (11-14) down to Bv horizon (6-8) – a typical trend for agricultural Phaeosems (Marseille *et al*., 1999, Christensen, 1996). With decreasing particle-size C:N ratios decreased. C:N ratios in the Axp horizons were large for sand-size (26-28) and silt-size (20-21) separates – typical for plant fragments, whereas in they clay-size separates C:N ratios were small (7-9) – typical for microbial biomass (Cayet & Lichtfouse, 2001, Gleixner *et al*., 2001). For sand-size separates of Axh and Bv horizons nitrogen concentrations were too low to calculate reliable C:N ratios.

5.4.1.2 Bulk isotopic composition ($\delta^{13}C$)

In the Halle plots, the stable carbon isotope ratios of the plant biomass differed due to their distinct photosynthetic pathways. Rye (using the C3-pathway) had smaller ^{13}C contents (–31‰) than maize (–13‰) with a C4-pathway (Wiesenberg *et al*., 2004b). Thus, due to these different cropping histories of the individual plots, isotopic ratios of bulk soils and particle-size separates did not reveal those uniform trends, which we found for carbon and nitrogen concentrations (Table 7, Figure 26).

Bulk soils of the ploughed (Axp) horizons of both maize cropped soils had larger ^{13}C contents (–24.0‰ to –24.6‰), while subsoil horizons (Axh and Bv) contained smaller ^{13}C contents (–24.8‰ to –25.8‰) (Figure 26). With soil depth two opposite isotope trends were observed. For the rye cropped soil isotopic ratios increased with soil depth, typical for C3-cropped soils. Contrastingly, the ^{13}C content decreased in maize cropped soils with increasing soil depth as a result of decreasing C4-biomass input in the deeper soil horizons (e.g. Balesdent & Mariotti, 1996). For the subsoil horizons (Axh and Bv) of the rye and grain-maize plots similar isotopic contents were expected because the grain-maize plot was cropped with rye, then (1961) converted to fallow land and converted to grain-maize in the year (2001) of sampling. However, ^{13}C contents were smaller by 0.7‰, either reflecting small scale differences of soil isotope properties or due to the fact that for 40 years no new root biomass entered this fallow land soil and present carbon was probably degraded by microorganisms, leading to smaller ^{13}C contents.

Silt- and clay-sized separates had relatively uniform ^{13}C contents, only differing up to 1.5‰, whereas ratios for sand-sized separates were more diverse (Table 7, Figure. 26). Bird & Pousai (1997) reported similar trends for particle-size separates of forest soils. The largest ^{13}C contents (-20.1‰) occurred in the sand-size separate from a subsoil (Bv) of the silage-maize plot, and probably indicated the presence of particles from maize roots developed in the year of sampling. Generally, particle-size separates of individual horizons followed the pattern, that silt-size separates had smaller ^{13}C contents than sand- and clay-sized separates (except for the Axh horizon of the silage-maize cropped soil).

Summarizing, the data set from Halle plots did not support the postulated general trend of larger ^{13}C contents with increasing depth or decreasing particle-size, as described for most soils of temperate climate under forests, grassland and agriculture (Desjardins *et al*., 1994, Balesdent & Mariotti, 1996, Boutton *et al*., 1998). One obvious explanation, which holds for the maize plots, is that the input of isotopically different parent biomass will change the isotope pattern. However, there are more potential explanations for isotope trends with depth. Recently, Krull & Skjemstad (2003) and Bird *et al*. (2003) summarized the major potential processes, which can alter the ^{13}C contents for soil organic carbon with depth and decreasing particle-size. The processes include: i) preferential microbial degradation and mineralization of nutrient and energy rich compounds with large ^{13}C contents (carbohydrates, sugars, proteins) compared to less decomposable compounds with small ^{13}C contents (lignin, lipids), ii) kinetic fractionation accompanying microbial metabolism favours the partitioning of ^{13}C into the microbial biomass with the preferential respiration of ^{12}C, yielding larger ^{13}C contents for the remaining organic carbon, iii) translocation of less decomposed (^{13}C-rich) soluble compounds downwards the profile, and iv) the Suess effect, a ^{13}C depletion in

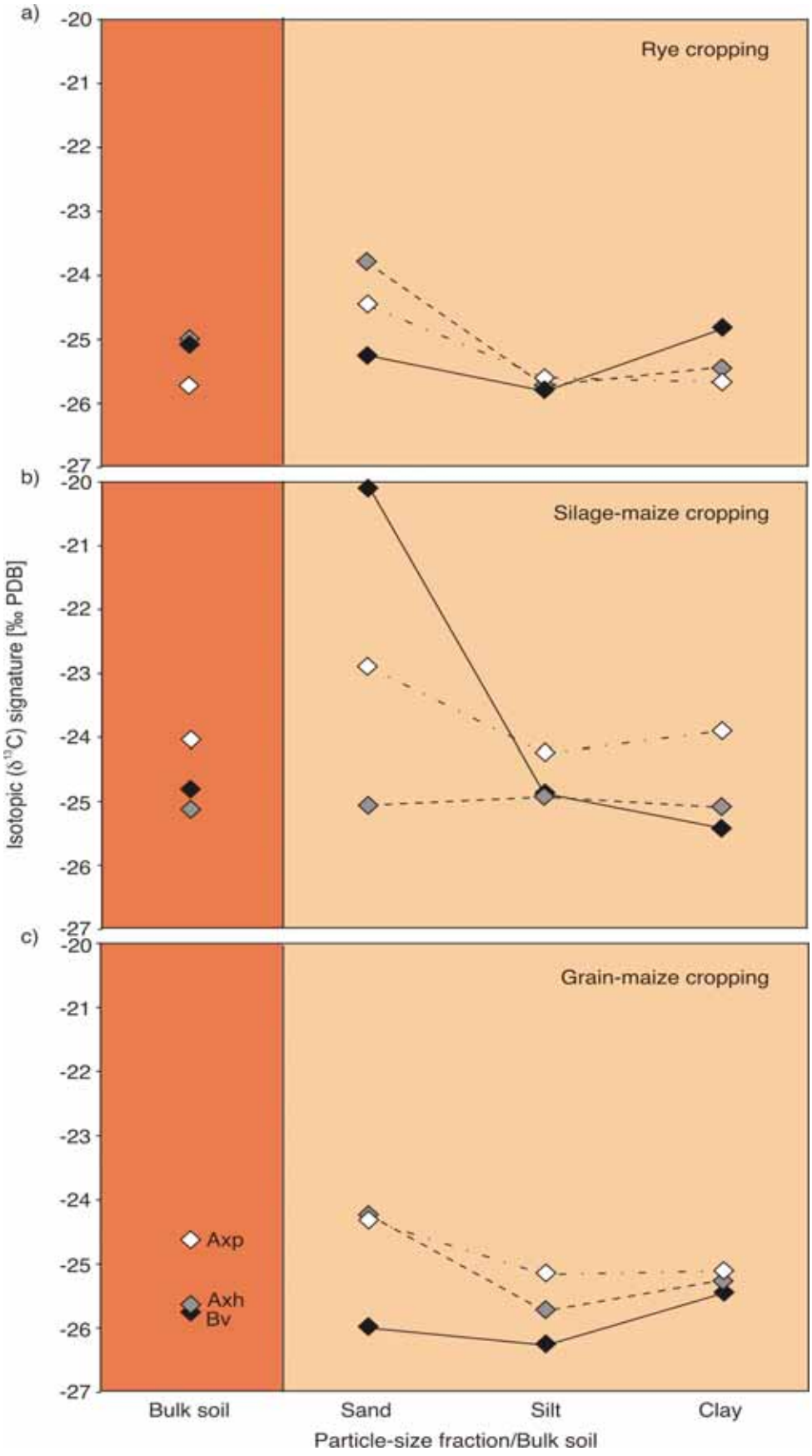


Figure 26. Carbon isotopic ($\delta^{13}C$) composition of particle-size separates and bulk soils of soil profiles for (a) rye cropped soil, (b) silage-maize cropped soil and (c) grain-maize cropped soil (data also shown in Table 7).

modern atmospheric carbon since the industrialization. The first process (preferential microbial degradation) would result in decreasing ^{13}C values with depth or decreasing particle-size, whereas the other proposed processes would yield the opposite trend. Thus, in the soils studied here, several competing processes could have produced the observed result. Additionally, a detailed explanation for the observed isotope contents got complicated by the fact, that the soils investigated here, are situated in a highly industrialized area and thus, contain lignite dust and charred fragments from mining and combustion (Wiesenberg *et al*. 2004b). In the ploughed horizons, lignite particles present in the sand-size separates were rich in organic carbon (480 g kg^{-1}) and ^{13}C contents (–25.4‰) were between rye (-30‰) and maize biomass (-13‰) (Wiesenberg *et al*., 2004b). Thus, the presence of fossil organic matter certainly contributed to the apparent elemental and isotopic ratios measured here, probably resulting in values different from corresponding soils without fossil carbon contamination.

5.4.1.3 Total lipid distribution

Lipids are a chemical class of compounds, operationally defined by their extractability in polar solvents. Lipids were extracted from bulk soils and particle-size separates of Halle soil profiles. Extract yields are shown in Table 7, first normalized to soil mass (mg kg^{-1}), and second normalized to total organic carbon (% of bulk). Where concentrations of extractable lipids were small, especially in coarse particle-size separates and subsoil horizons, weighing errors were relatively larger than for those separates producing larger lipid yields. Thus, calculated proportions of lipids from bulk soils and g kg^{-1} organic carbon (OC) contained large uncertainties.

Concentrations of extractable lipids followed the same trend that was already observed for organic carbon concentrations (Table 7). With increasing particle-size and soil depth, extractable lipid concentrations decreased systematically. The extract yields were similar to free lipid extract yields observed by Amblès *et al*. (1994b) and Lichtfouse *et al*. (1995b) for the ploughed horizons. Lipid yields increased linearly with increasing SOC concentrations, both in bulk soils and in particle-size separates (Table 7, Figure 27). Lipid yields for the rye cropped soil were larger than for the silage-maize cropped soil (Figure 27). These results were consistent with previous observations, that lipid extract yields of several rye plant parts were approximately twice as high as their corresponding parts of maize plants, although organic carbon concentrations were similar as demonstrated above. Consequently, yields from the grain-maize cropped soil (a mixture of pre-1961 rye biomass and one year of maize cultivation) were intermediately between rye and silage-maize cropped soils.

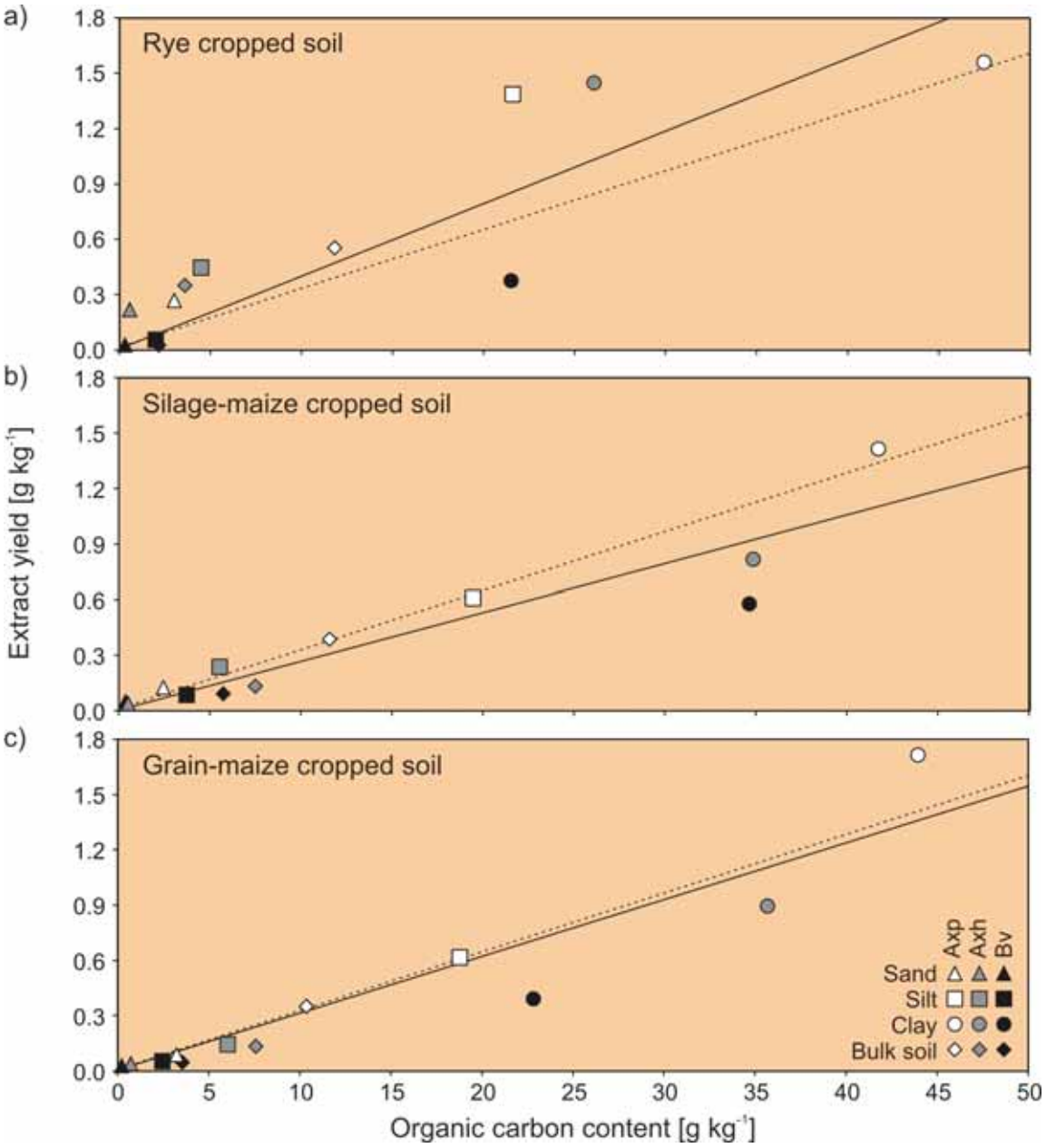


Figure 27. Extract yields of total lipids vs. total organic carbon concentrations for particle-size separates and bulk soils from (a) rye, (b) silage-maize, and (c) grain-maize cropped soils of Halle site (data also shown in Table 7). Correlations (black lines) for (a) rye cropped soil: y = 0.039 x (R^2 = 0.77), (b) silage-maize cropped soil: y = 0.026 x (R^2 = 0.87), and (c) grain-maize cropped soil: y = 0.031 x (R^2 = 0.90). The over-all correlation (dashed line): y = 0.032 x (R^2 = 0.78).

The distribution of the extractable lipids within the soil profile and between the particle-size separates from Halle site became clear when lipid yields were normalized to organic carbon concentrations. In the bulk soils, extractable lipids contributed 1 to 10% to the organic carbon present in the bulk sample (9-96 g lipids kg^{-1} organic carbon), consistently decreasing with depth for all investigated soil profiles (Axp>Axh>Bv). Also in the particle-size separates there seemed to be a general decrease of yields from large contributions of sand-size (3-31% of

the total organic carbon) to clay-size (2%) separates (sand>silt>clay), with few exceptions from that general trend (grain-maize Axp and Axh horizons). The number of investigated soils was limited to three profiles from one site, and spatial heterogeneity made it difficult to draw general conclusions. However, from these results could be concluded, that from sand- to clay-size separates the proportions of easily extractable biomass (e.g. relatively fresh plant material) decreased and the proportions of more altered biomass increased. Such a trend of increasing decomposition of soil organic matter with soil depth and decreasing particle-size was consistent with previous observations based on bulk chemical parameters, and individual compound classes, including lignin (reviewed by Christensen, 1996, Gleixner *et al*. 2001). A detailed identification of these lipids is the focus of a complementary publication (Wiesenberg *et al*. in preparation). When adding up all particle-size separates it could be observed, that higher lipid contents could be obtained from particle-size separates, than from bulk soils. This might result from three effects. First, lipid extract yields were determined gravimetrically and thus higher weighing errors, especially for samples with low lipid contents (preferentially of coarse-sized separates from deeper soil horizons) might have led to a sum up of weighing errors for individual size-separates in comparison to a lower error of the bulk soil extract. Second, preparatively separation of bulk soils into size separates made lipids available to extraction in separates, which were locked in aggregates of bulk soils. Third, when regarding lipid extract yields as a function of organic carbon content, high lipid contributions could be observed in samples with low organic carbon contents, most likely due to larger uncertainties in both parameters. Most likely, higher lipid contents of particle-size separates were caused preferentially by a combination of the first and the second effects.

5.4.1.4 Colour of particle-size separates and bulk soils

On visual inspection bulk soils and particle-size separates seemed to become lighter after lipid extraction but information about soil colours was not quantitative. Thus, a colour photo-spectrometer was used to infer reproducible information on the degree of lightness of all samples. For simplicity, lightness was expressed as value in the Munsell system, which is often used in soil science (Schulze *et al*., 1993).

Spectrometric measurements basically confirmed the visual observations. Lipid extraction made Halle samples (Figure 28) significantly lighter in soil colour, except for one sample (silt-size separate of Axh horizon of grain-maize cropped soil). Especially for bulk soils and clay-size separates Munsell values were very similar. After extraction Munsell values increased predominantly for bulk soils and clay-size separates. This suggested, that especially lipids extracted from clay-sized separates were important colouring soil constituents. Furthermore, we observed a uniform trend with particle-size for all soils. In ploughed (Axp) horizons,

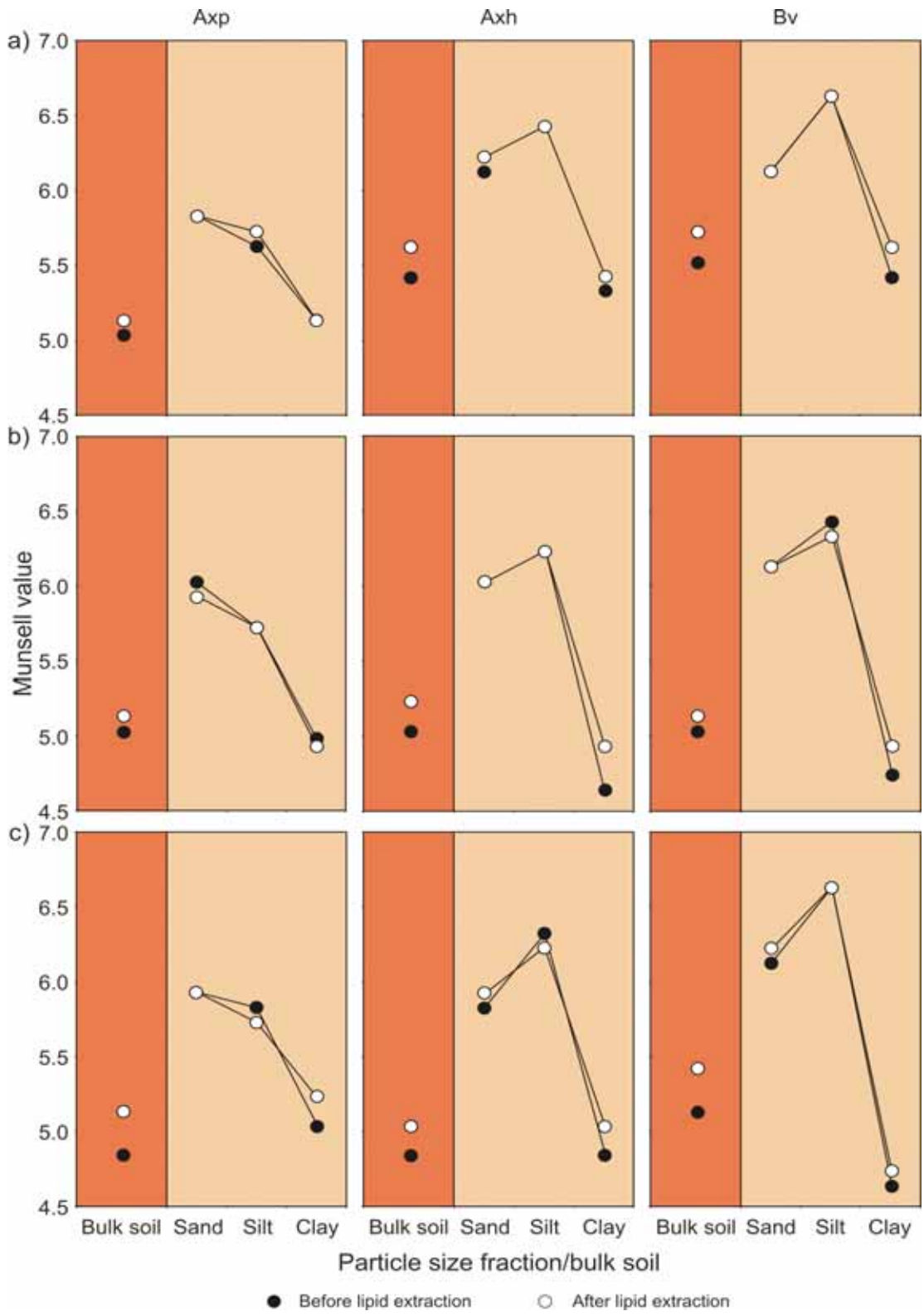


Figure 28. Munsell values of particle-size separates and bulk soils for the three soil horizons (Axp, Axh, Bv) before and after extraction of (a) rye cropped soil, (b) silage-maize cropped soil, and (c) grain-maize cropped soil. Silt-size separate from rye cropped Axh horizon before extraction was not measured, because not enough material was available for colour measurements. Standard deviations were always smaller than 0.05 for Munsell values and thus smaller than symbol size.

values decreased systematically with decreasing particle-size, whereas in subsoil horizons (Axh, Bv) values peaked in silt-size separates. Mineralogical informations on the samples were not available and it must be hypothesized, that probably the presence of quartz and mica particles contributed to the lighter colour of silt-size separates, and the presence of fresh plant material and fossil organic matter reduced the lightness in the sand-size separates.

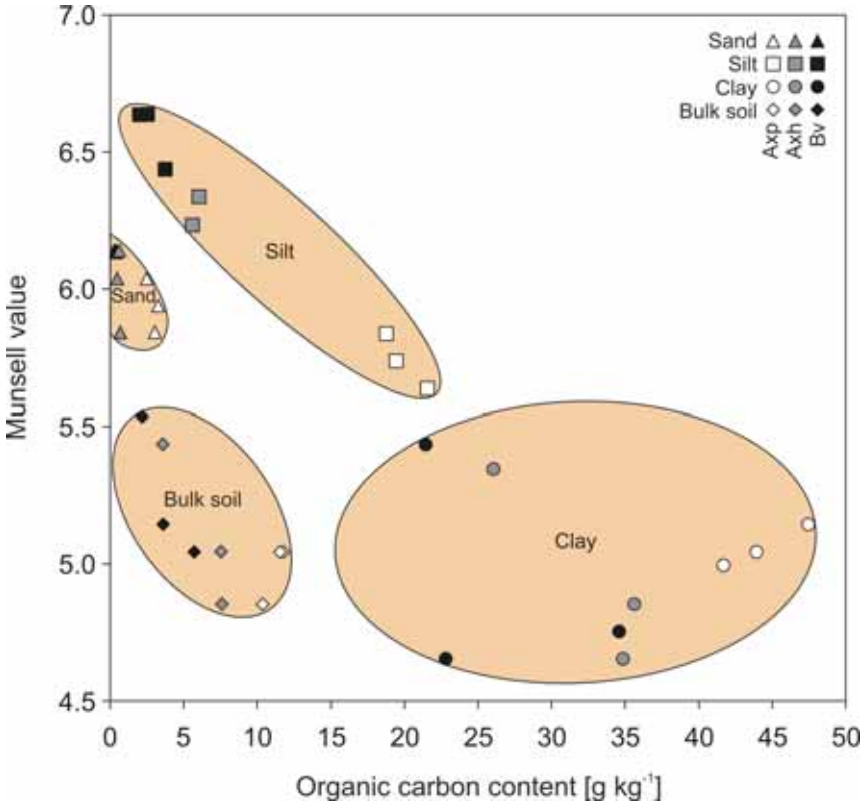


Figure 29. Munsell values vs. total organic carbon concentrations for particle-size separates and bulk soils of three soils with different croppings applied. Standard deviations were always smaller than 0.05 and thus smaller than symbol size.

To test, if the presence of organic matter can explain the different Munsell values, both parameters were combined in one graph (Figure 29). Surprisingly, no obvious relation was found between these two parameters for the analysed samples. Instead, bulk soils and the particle-size separates plotted in distinct, individual areas. Again, bulk soils and clay-size separates had the smallest Munsell values, but organic carbon concentrations differed between bulk soils and clay-size separates. Another interesting fact was, that sand-size separates for all soils, and bulk soils as well as particle-size separates for all ploughed horizons, plotted very close together, whereas subsoil horizons and other particle-size separates scattered more. One explanation for the uniform properties of the ploughed horizon could be the good homogenization due to ploughing. Probably, differences in microbial assemblages and different amounts of fossil carbon caused less uniform lightness and SOC relationship for clay-size separates in comparison to silt- and sand-size separates.

Summarizing, soil lipids follow similar decomposition trends like other compounds within particle-size separates and soil profiles. Clay-size separates show similar Munsell values like bulk soils and are thus predominantly colouring the coarse-size components of soils, which themselves reveal a larger lightness. Additionally, soil lipids are found as one soil colouring component, because after extraction of dark-coloured lipids lightness increases significantly.

5.4.2 Comparison of bulk soils from different sites

Bulk soil profiles of Halle site sampled in 2001 were described in detail above, but were shown additionally in Table 8 for a better comparison to the other bulk soils. Generally, topsoil samples taken in 2000 and 2001 for silage-maize and rye cropped soils revealed identical carbon, nitrogen, and isotopic compositions (Table 8). Lipid contents were different for rye cropped soils sampled in 2000 and 2001 most likely as a result of soil heterogeneity. Some minor variations could be observed when comparing grain-maize cropped soil samples of the years 2001 and 2002. Samples from Rotthalmünster and Boigneville sites were different from Halle soil samples, due to different soil types and thus different soil properties. Rotthalmünster soils are characterised by high proportions of silt (73%), and significantly lower proportions of clay (16%) and sand (11%) (John *et al.*, 2004). Contrastingly, Boigneville soils are characterised by nearly equal amounts of all particle-size separates (29% clay, 34% silt and 37% sand) for the topsoils. Particle-size distributions were not obtained for deeper soil horizons of Rotthalmünster and Boigneville sites. Slightly lower soil carbon concentrations for Rotthalmünster site significantly lower concentrations for Boigneville site could be obtained than for Halle soils. Contrastingly, nitrogen concentrations were higher in Rotthalmünster soils than for Halle soils. C:N ratios were the same for Rotthalmünster and Boigneville sites. These differences could be attributed to different soil types. Lipid extract yields were similar for all arable soils from sites shown here and in the literature (Amblès *et al.*, 1994b, Lichtfouse *et al.*, 1995b). While organic carbon contents and lipid extract yields were similar for the topsoils, they varied significantly for the underlying horizons between Rotthalmünster and Halle sites. Carbon and lipid contents decreased gradually with depth in Halle soil profiles. A large decrease could be observed between Axp1 and Axp2 horizons of Rotthalmünster sites and minor differences between the deeper soil horizons.

Stable carbon isotopic composition ($\delta^{13}C$) especially of topsoils strongly depended on monoculture croppings applied. Wheat and rye cropped soils showed identical compositions, while maize cropped soils revealed different isotopic compositions. After 23 years of grain-maize cropping Rotthalmünster and Boigneville soils had virtually identical isotopic compositions between -20‰ and -21‰, due to high maize biomass input with heavy isotopic signatures. Contrastingly, maize cropped soils from Halle site showed a minor isotopic

increase as a result of i) low biomass input over four decades for silage-maize cropped soil, ii) short-term managing of grain-maize cropping for grain-maize plot, iii) pollution with fossil carbon for all soils from Halle, and iv) ploughing modifications for silage-maize cropped soil as explained above. For Rotthalmünster wheat cropped soil, carbon isotopic signature increased with depth (Axp1 to Axp2 horizons), typical for most C3-cropped soils (Balesdent & Mariotti, 1996). Contrastingly, for the maize cropped soil, isotopy decreased with depth, typical for soils, where a C3-monoculture cropping was replaced by a C4-monoculture cropping (Balesdent & Mariotti, 1996).

Table 8. Soil properties of bulk soils from different sites.

Site and sampling year	Horizon	Organic C [g kg^{-1}][a]	$\delta^{13}C$ [‰][b]	N [g kg^{-1}][a]	C:N	Lipid extract yield [g kg^{-1}][a]	[g kg^{-1} OC][c]
Halle							
Rye cropped soil							
2000	Axp	11.9	-25.8	0.9	13	0.37	31
2001	Axp	11.8	-25.7	0.7	13	0.55	47
	Axh	3.6	-25.0	0.4	10	0.35	96
	Bv	2.2	-25.1	0.3	6	0.02	9
Silage-maize cropped soil							
2000	Axp	12.9	-24.1	1.0	13	0.39	31
2001	Axp	11.6	-24.0	0.8	14	0.38	33
	Axh	7.5	-25.1	0.8	10	0.13	17
	Bv	5.8	-24.8	0.7	9	0.09	15
Grain-maize cropped soil							
2001	Axp	12.3	-24.6	1.1	11	0.35	33
	Axh	7.7	-25.7	0.7	10	0.13	17
	Bv	3.6	-25.8	0.4	8	0.04	11
2002	Axp	11.5	-25.1	0.9	13	0.34	30
	Axh	8.0	-25.6	0.9	11	0.17	21
Rotthalmünster							
Wheat cropped soil							
2002	Axp1	11.3	-26.2	1.4	9	0.31	27
	Axp2	3.9	-25.8	0.6	7	0.05	13
	Sw-M	2.7	-25.6	0.5	6	0.04	14
	BtSd	1.6	-27.0	0.3	5	0.02	11
Grain-maize cropped soil							
2002	Axp1	12.5	-20.3	1.5	9	0.30	24
	Axh2	4.6	-23.5	0.7	7	0.08	18
	Sw-M	2.5	-23.4	0.4	6	0.03	13
	BtSd	1.6	-25.0	0.4	5	0.02	12
Boigneville							
Wheat cropped soil							
1993	Axp	10.3	-25.9	1.4	9	0.37	36
Grain-maize cropped soil							
1993	Axp	7.8	-20.8	1.1	9	0.32	40

[a] Expressed as g kg^{-1}.
[b] Carbon isotopy ($\delta^{13}C$) expressed as ‰ PDB.
[C] Expressed as g kg^{-1} organic carbon.

When regarding Munsell values of soils from different sampling sites (Figure 30), no obvious dependency of lightness (Munsell value) on organic carbon content could be observed for all soils. With increasing soil depth lightness increased slightly for all soils of Halle and Rotthalmünster sites. Despite of similar organic carbon distributions especially in all topsoils, Munsell values were significantly different for all sampling sites. These

differences could be related to differences in mineralogical, chemical and physical soil properties, reflected by distinct classifications for the analysed soils. Thus, soil lightness measured with a photo-spectrometer and expressed as Munsell value could be used as an additional parameter for the soil characterisation, as described by Schulze *et al*. (1993) in combination with Munsell chroma and hue.

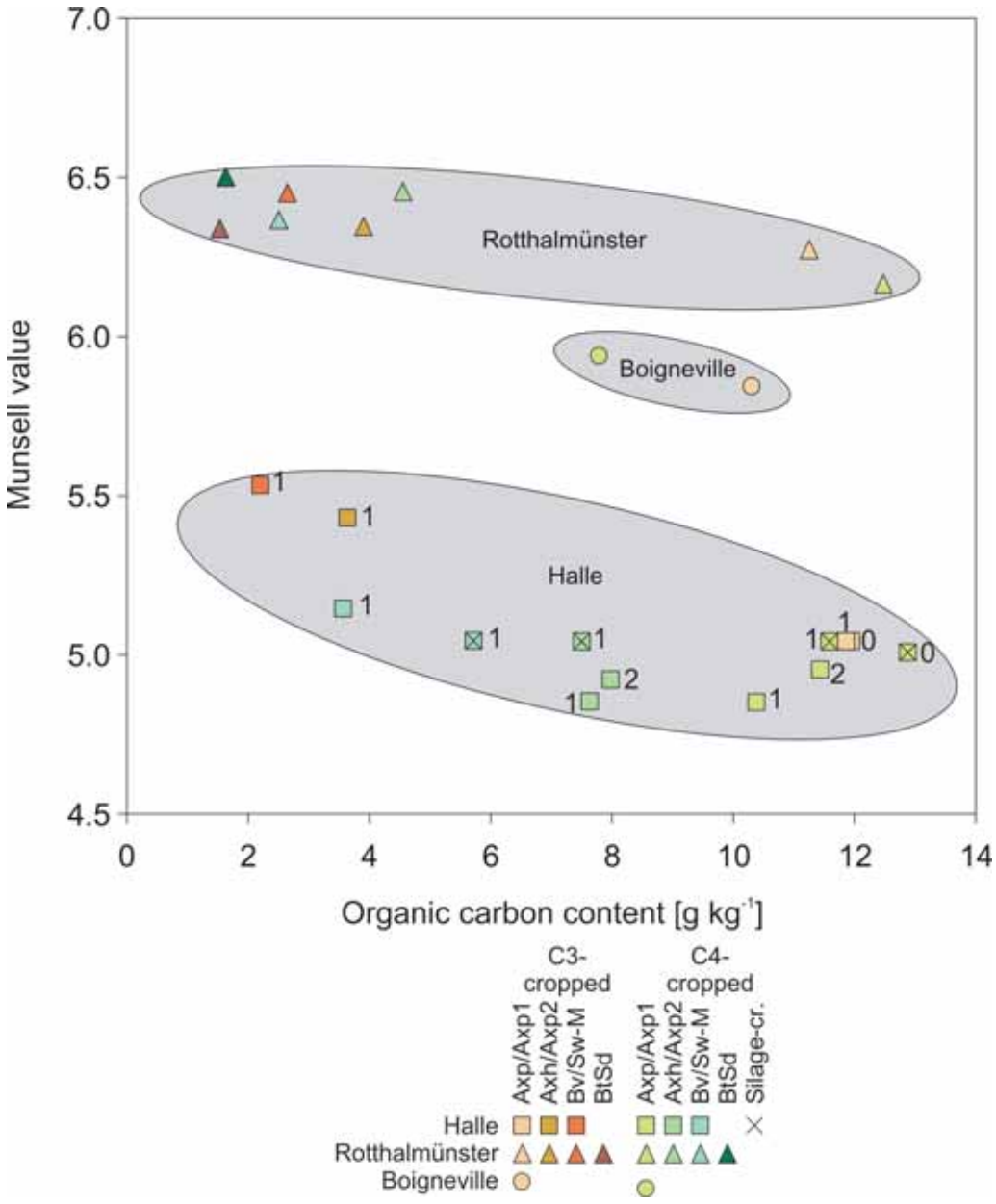


Figure 30. Munsell values vs. total organic carbon concentrations for bulk soils of several sampling sites with different monoculture croppings. Numbers beneath squares of Halle site indicate the year of sampling (2000 = 0, 2001 = 1, 2002 = 2). Bulk soils of several soil horizons were differentiated. For Halle site the soil profile was divided into Axp, Axh and Bv, while for Rotthalmünster site the following horizons could be differentiated: Axp1, Axp2, Sw-M and BtSd. Standard deviations were always smaller than 0.05 and thus smaller than symbol size.

5.4.3 Detailed lipid analyses of bulk topsoils

In the following the molecular composition of soil and plant *n*-alkanes and *n*-carboxylic acids of bulk soils from ploughed horizons of Halle, Rotthalmünster and Boigneville site are described. Thereafter, the compound-specific isotope signatures of the most abundant long-chain alkyl lipids are presented. In addition to soil samples, informations on plant samples of the individual plots, collected directly before harvest, are shown in the diagrams and discussed in relation to soil samples to obtain information, how the soils reflect the signature of the used crops. The results discussed in the following chapter were previously published by Wiesenberg *et al*. (2004b).

5.4.3.1 Aliphatic hydrocarbons

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

Generally, soils from all sites and different croppings showed similar alkane distribution patterns (Figure 31). All soils were dominated by long-chain, odd carbon-numbered *n*-alkanes with chain lengths between C_{25} and C_{35}, characteristic for biomass input from grasses (Lichtfouse & Budzinski, 1995, van Bergen *et al*. 1998, Marseille *et al*., 1999). In comparison to our analyses Lichtfouse (1998) determined identical free *n*-alkane distribution patterns in maize-cropped soil from Boigneville. The large amounts of *n*-C_{29} and *n*-C_{31} are related to a distinctive crop input (Lichtfouse *et al*., 1997a) with the maize cropped soils containing lower portions of *n*-C_{29} relative to *n*-C_{31}. The linear regression observed by Lichtfouse *et al*. (1994) for *n*-C_{27}/*n*-C_{29} vs. $\delta^{13}C_{27}$ - $\delta^{13}C_{29}$ proposed as molecular marker for differentiation of wheat vs. maize cropping could not be verified. Soils from Halle contained higher proportions of even-numbered long-chain *n*-alkanes (C_{26} to C_{34}). When compared with *n*-alkanes from brown coal (Figure 31e) the *n*-alkane distribution patterns of Halle soils and brown coal samples were virtually identical. Brown coal differed only marginally from the brown coal briquette. Thus, the higher proportions of even-numbered and in part of the odd-numbered *n*-alkanes in Halle soils must originate from brown coal pollution. Higher proportions of *n*-alkanes in Boigneville soils with chain length from C_{16} to C_{18} were previously related to anthropogenic pollution (Lichtfouse *et al*., 1997a), although a microbial origin could not be excluded. In Rotthalmünster soils *n*-alkanes with chain length around C_{20} were slightly enriched. This might be attributed to either a microbial origin (Dinel *et al*., 1990) or input of root biomass or biodegraded material.

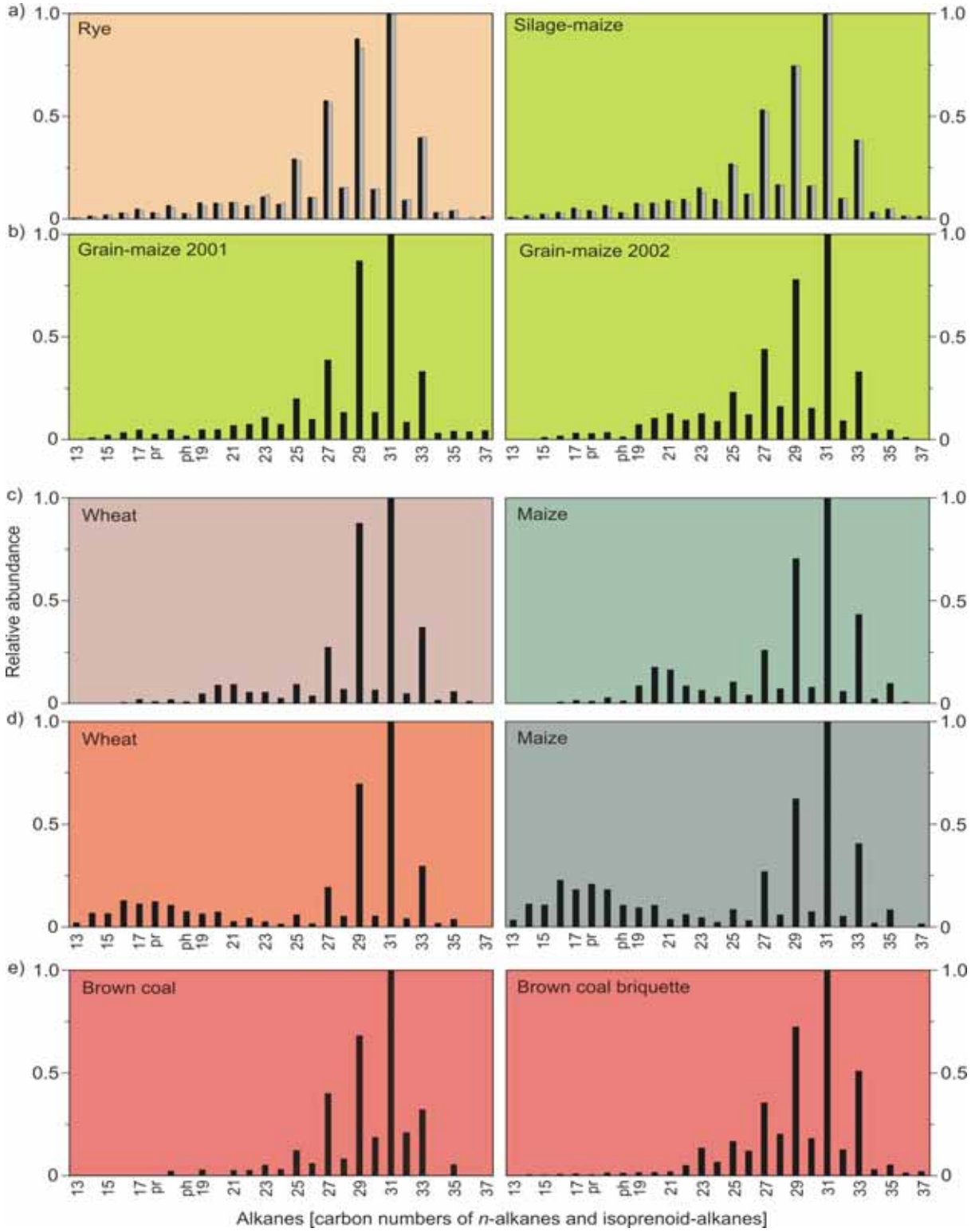


Figure 31. Relative distributions of *n*-alkanes and isoprenoid-alkanes extracted from soils and brown coals analysed, normalized to the most abundant compounds. Numbers beneath bars indicate carbon numbers of *n*-alkanes. Abbreviations for isoprenoid-alkanes: pr = pristane, ph = phytane. Soil samples from top to bottom originated from Halle (a, b), Rotthalmünster (c), and Boigneville sites (d). Halle rye and silage-maize cropped soils were analysed in duplicate (black and grey bars). Brown coal and brown coal briquette (e) potentially contributing to Halle soils were derived from the nearby Beuna mine.

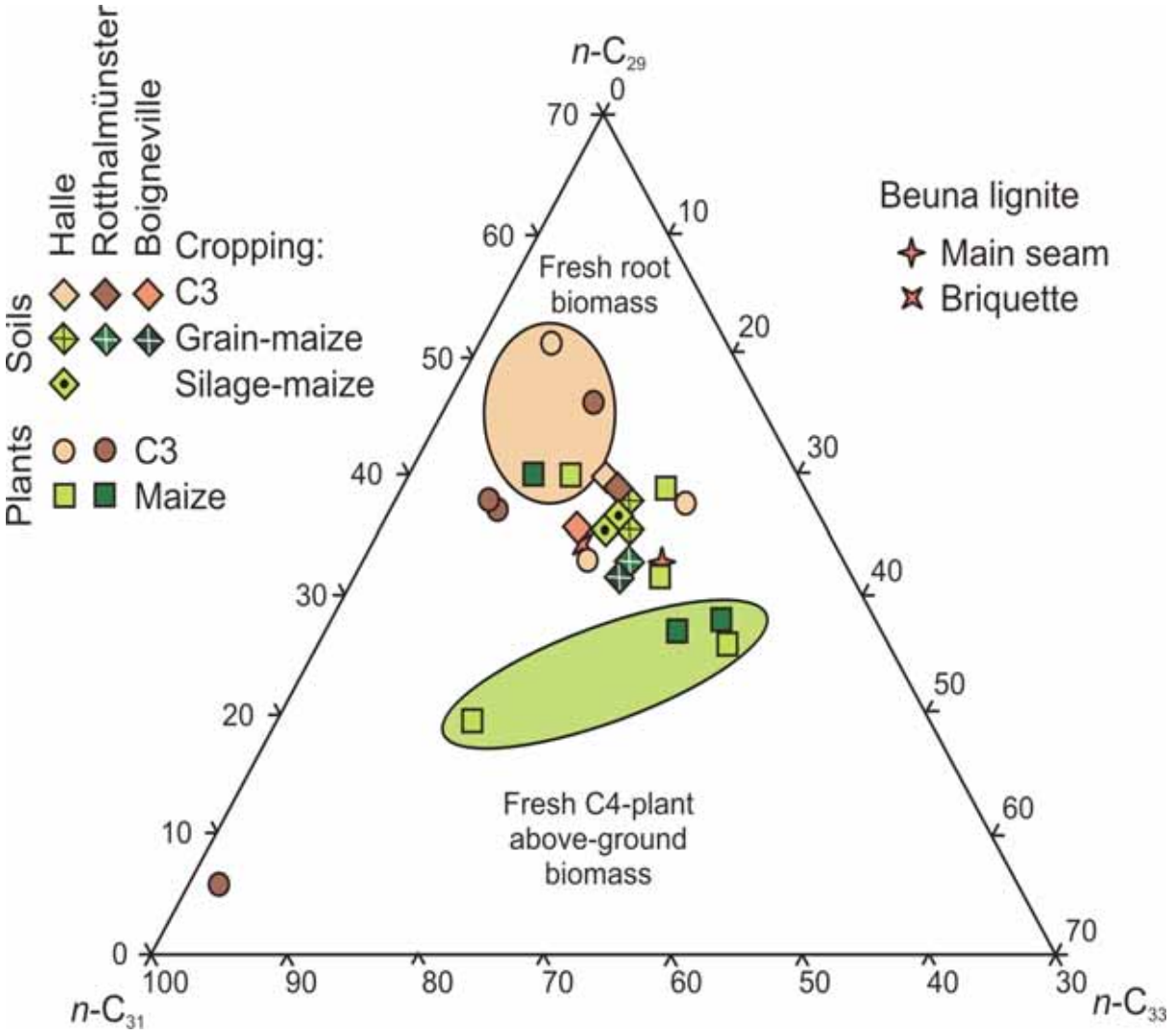


Figure 32. Ternary diagram showing the relative compositions of the three most abundant *n*-alkanes (C_{29}, C_{31}, C_{33}) in plants and soils.

Within most soils and plant parts odd carbon-numbered *n*-alkanes with a chain-length from C_{29} to C_{33} were most abundant and their relative distributions are shown in Figure 32 and Table 9. For wheat a trend from root biomass with nearly identical amounts of *n*-C_{29} and *n*-C_{31} to a dominance of more than 90% *n*-C_{31} in leaves was recognizable. For rye and maize plants different trends were observed. High amounts of *n*-C_{29} and *n*-C_{31} occurred in roots whereas higher contributions of *n*-C_{33} were characteristic of aboveground biomass. Within all soils similar trends from low (C3-cropped soils) to higher amounts of *n*-C_{33} could be observed after introduction of maize crops. This tendency was more pronounced in grain-maize cropped soil and for the Halle site already observed after one year, because of exceptional high incorporation of aboveground biomass. The Halle silage-maize cropped site revealed an intermediate increase in relative abundance of *n*-C_{33}. This was due to the presence of brown coal pollution and the low abundance of aboveground biomass left on the plot after silage harvesting.

Table 9. Relative abundance of long-chain *n*-alkanes and *n*-carboxylic acids as percent of each lipid fraction.

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

5.4.3.2 Carboxylic acids

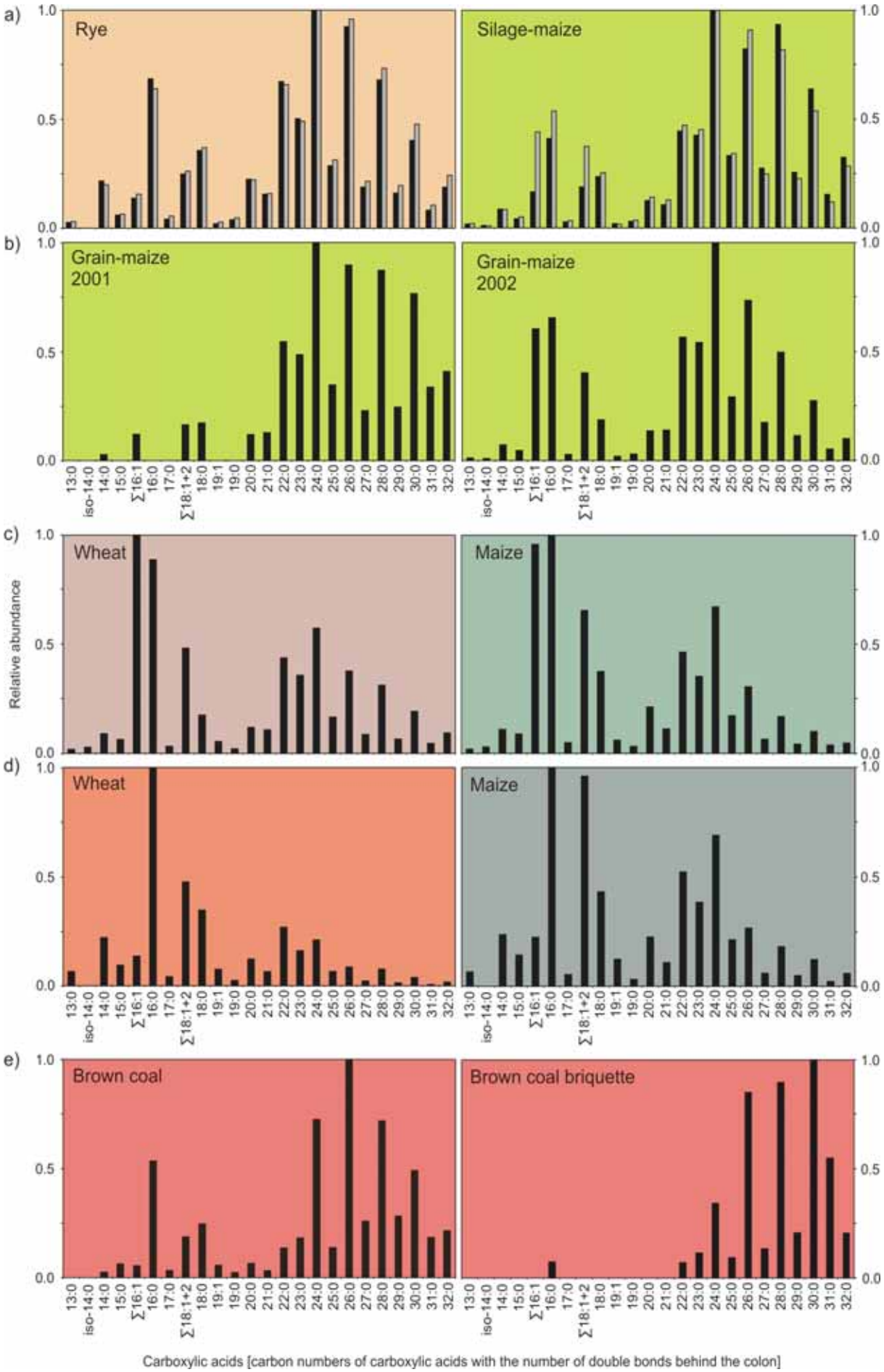
The carboxylic acid fraction consisted mainly of saturated and mono- as well as di-unsaturated straight-chain *n*-carboxylic acids. Polyunsaturated and branched acids occurred in trace amounts only and are not discussed further.

All soils (Figure 33) contained high proportions of even-numbered mid-chain carboxylic acids (between *n*-C_{16} and *n*-C_{18}) and their unsaturated counterparts as well as high values of even carbon-numbered long-chain carboxylic acids (*n*-C_{22+}). For the latter the *n*-C_{22}, *n*-C_{24}

and *n*-C_{26} were the most abundant homologues as previously observed for forest soils (Almendros *et al*., 1996) and for grassland soils (van Bergen *et al*., 1998). In Halle soils long-chain acids predominated over mid-chain carboxylic acids. Soils from Rotthalmünster and Boigneville were dominated by carboxylic acids with 16 and/or 18 carbon atoms. These mid-chain compounds could be derived from several sources including fungi, bacteria, algae and higher terrestrial plants (Dinel *et al*., 1990). Because they are ubiquitous in living organisms (Lichtfouse *et al*., 1995a, Bossio *et al*., 1998, Marseille *et al*., 1999, Boeschker & Middelburg, 2002) they are not suitable for plant biomass turnover determinations. Higher amounts of long-chain carboxylic acids with a predominance of even carbon numbers are preferentially due to carbon input from terrestrial biomass. Brown coal samples of Beuna (Figure 33e) had compositions similar to soils from the Halle site, characterised by lower amounts of mid-chain carboxylic acids and high amounts of long-chain acids (*n*-C_{22+}). This served as an additional indication for the high input of brown coal particles into soils at the urban Halle site.

Discrimination between C3- and C4-crop plants could be achieved by plotting long-chain *n*-carboxylic acid composition in a ternary diagram (Figure 34). In agreement with observations of Bianchi & Corbellini (1977) C3-plants were characterised by highest amounts of *n*-C_{22} (>40%), whereas *n*-C_{24} was most abundant (>40%) in C4-plants. Brown coal samples revealed lower amounts of *n*-C_{22} and *n*-C_{24} and increased *n*-C_{26} carboxylic acid concentrations (Table 9). C3- and C4-cropped soils from Rotthalmünster and Boigneville plotted on a mixing line between C3- and C4-plant biomass (Figure 34). For Halle soils a higher amount of *n*-C_{26} was observable, indicative for a significant brown coal contamination (Figure 34). Silage-maize cropped soils were only slightly enriched in *n*-C_{24}, compared to rye-cropped soils at the Halle site. These small differences between the rye and silage-maize cropping were due to the aboveground biomass removal upon silage harvesting. A shift of equal magnitude in the long-chain carboxylic acid composition was observed between silage- and grain-maize cropping after only one year for the Halle site. The grain harvesting technique led to a significant depletion in the *n*-C_{26} and a preferential increase in *n*-C_{24} carboxylic acid.

Figure 33. (On the following page.) Relative distributions of carboxylic acids extracted from soils and brown coals analysed, normalized to the most abundant compound. Numbers beneath bars indicate carbon numbers of carboxylic acids and number behind the colon depict the number of double bonds within the molecule. Soils originated from Halle (a, b), Rotthalmünster (c) and Boigneville (d) sites with C3-reference plots on the left and new introduced maize cropped plots on the right hand. Brown coal and brown coal briquette (e) were derived from Beuna near Halle. Halle rye and silage-maize cropped soils were analysed in duplicate.


a)
Rye
Silage-maize
b)
Grain-maize 2001
Grain-maize 2002
c)
Wheat
Maize
d)
Wheat
Maize
e)
Brown coal
Brown coal briquette
Relative abundance
1.0
0.5
0.0
13:0
iso-14:0
14:0
15:0
Σ16:1
16:0
17:0
Σ18:1+2
18:0
19:1
19:0
20:0
21:0
22:0
23:0
24:0
25:0
26:0
27:0
28:0
29:0
30:0
31:0
32:0
Carboxylic acids [carbon numbers of carboxylic acids with the number of double bonds behind the colon]

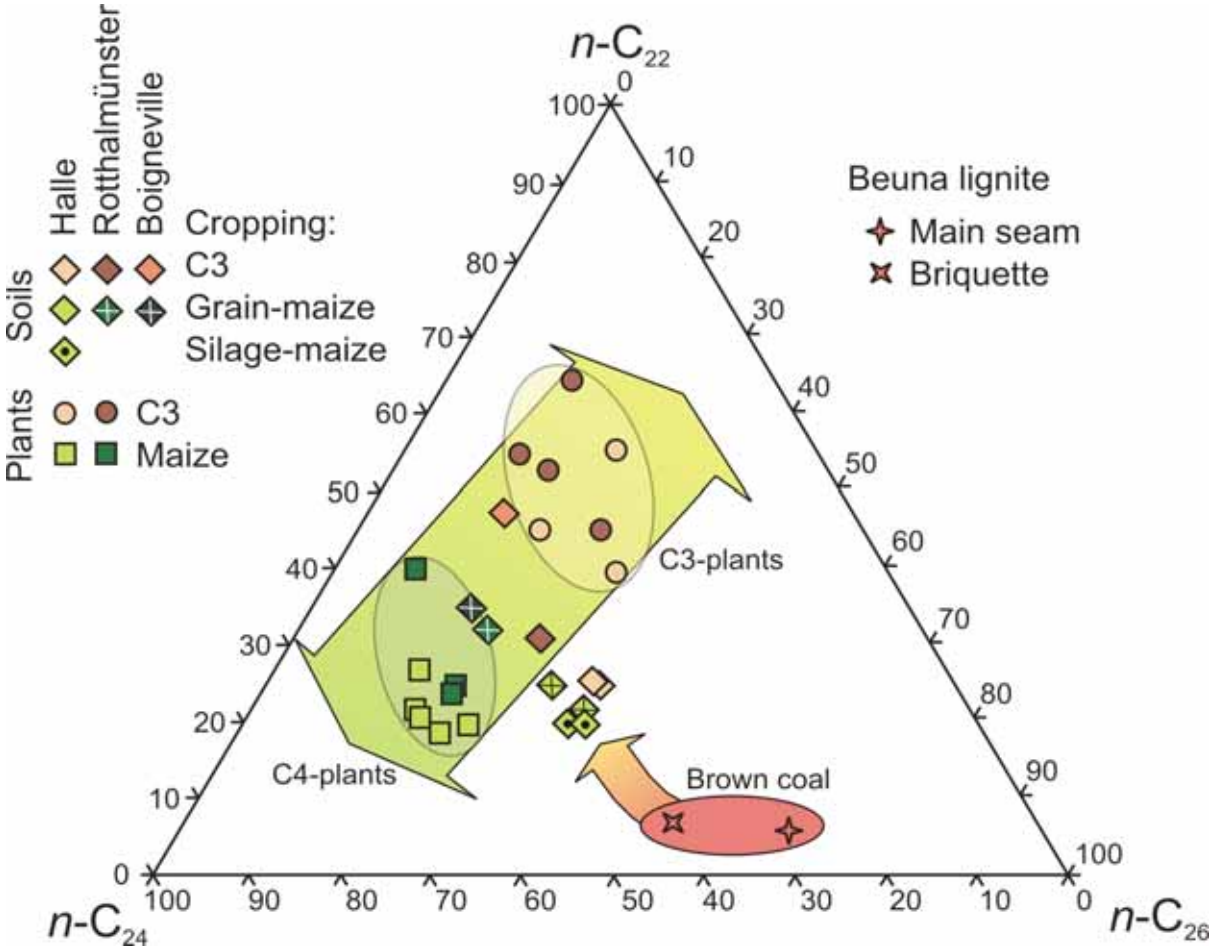


Figure 34. Ternary diagram showing the relative composition of the three most abundant long-chain *n*-carboxylic acids (C_{22}, C_{24}, C_{26}) as molecular indicators for plant biomass input.

When regarding the bulk isotopic ($\delta^{13}C$) composition of analysed bulk soils and plants vs. the CAR of those samples, it could be determined, that all C3-cropped soils showed similar isotopic compositions and CAR (Figure 35). Isotopic contents were usually a few permill higher for soils, than for corresponding crops as a result of isotopic fractionation processes during microbial decomposition of plant material. Similarly, CAR were slightly higher for soils than for C3-plants, probably as an effect of microbial degradation, too. Contrastingly, C4-cropped soils had heavier isotopic compositions and significantly higher CAR, especially when grain-maize cropping was applied for several decades as for Rotthalmünster and Boigneville sites. All C4-cropped soils plotted on a mixing line between the corresponding C3-plants and the C3-cropped soils on the one side and C4-plants of the individual plots on the other side. Probably, exclusively C4-cropped soils might show a similar isotopic enrichment and a slight enhancement of the CAR, similar to the relation between C3-cropped soils and plants. Thus, it could be estimated, that with increasing maize-derived carbon in the soils, both, the isotopy and the CAR would increase, maximising slightly above the respective means of the C4-plants. This would lead to isocons of C4-contents (lines of equal

C4-concentrations) for the soils. For the exact estimation of C3- or C4-plant-derived carbon content on an individual plot, the C3-cropped soil, as the reference is needed, giving the zero-point of C4-contents. Additionally, the means of C4-plant compositions are needed and those must be enhanced by at least 1‰ $\delta^{13}C$ and approximately 0.1 in the CAR. With these endpoints the isocons of identical C4-carbon compositions could be drawn. The lines drawn in Figure 35 led to less than 20% C4-derived carbon for Halle soils and nearly 40% C4-derived carbon for Rotthalmünster and Boigneville soils. For Halle soils, uncertainties due to fossil carbon pollution could not be excluded, leading to an underestimation of new C4-carbon contents. This method could be applied for a coarse approximation of C4-derived carbon contents of bulk carbon and/or carboxylic acids, if there was a reference C3-site under C3-grasses and a C4-site under C4-grasses. First results of ongoing studies showed, that several additional C3- and C4-grassed had similar CAR and isotopic compositions. Hence, this method probably can be applied for the source differentiation of C3- vs. C4-plant material for several other plants, soils, and eventually fossil matrices like sediments, respectively.

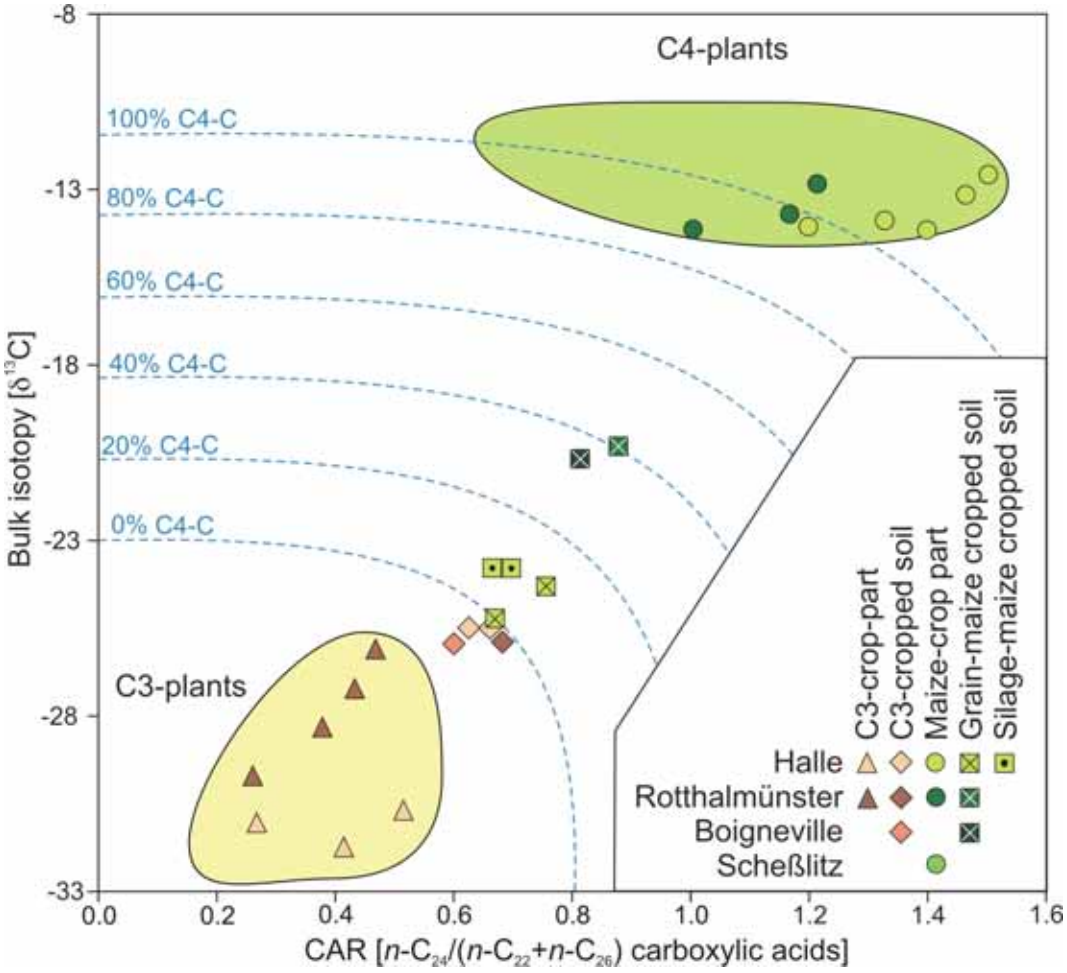


Figure 35. Bulk isotopy vs. CAR (n-C_{24}/(n-C_{22}+n-C_{26}) carboxylic acid ratio) for all analysed bulk soils of ploughed horizons and all plant samples. Interrupted blue lines are isocons of C4-plant-derived carbon contents in soils.

5.4.3.3 Compound-specific isotope analyses ($\delta^{13}C$)

To compare soils with plants, plant samples of Rotthalmünster and Halle sites directly sampled before harvest and additional degraded plant samples left on the soils after harvest are shown and discussed shortly in this chapter in addition to the extensive discussion in chapter 5.2.2. The results discussed in this chapter were previously published by Wiesenberg *et al*. (2004b).

The isotopic composition ($\delta^{13}C$) of the most abundant *n*-alkanes generally had an offset of approximately 9‰ against the bulk isotopic composition (Figure 36, Tables 6 and 9) as a result of biosynthetic fractionation (Hayes, 1993). C3-plant parts were depleted in ^{13}C as a result of the Hatch/Slack-photosynthesis metabolism, leading to bulk isotope values of -25‰ to -33‰ and corresponding *n*-alkane isotope values between -36‰ and -42‰ (Table 6).

For wheat plants the isotopic depletion of bulk samples and *n*-alkanes was larger for leaves and lower in roots, which could not be observed for plants sampled in May. Rye plants did not show the tendency of a larger isotopic depletion in root *n*-alkanes or bulk ^{13}C composition (Figure 36, Table 6).

Table 10. Isotopic ($\delta^{13}C$) signatures of bulk samples and predominant long-chain *n*-alkanes and *n*-carboxylic acids.

Sampling site / sample	Sampling time	Bulk sample [‰][a]	*n*-C_{27} alkane [‰]	*n*-C_{29} alkane [‰]	*n*-C_{31} alkane [‰]	*n*-C_{33} alkane [‰]	*n*-C_{22} carboxylic acid [‰]	*n*-C_{24} carboxylic acid [‰]	*n*-C_{26} carboxylic acid [‰]
Soils									
Halle									
Rye	2000a	-25.8±0.1	-31.2±0.0	-32.3±0.0	-33.1±0.1	-33.2±0.2	-33.3±0.4	-31.6±0.2	-30.7±0.2
	2000b	-25.8±0.1	-31.9±0.3	-32.7±0.3	-33.7±0.3	-33.8±0.3	-32.5±0.1	-31.8±0.5	-31.4±0.2
Silage-maize	2000a	-24.1±0.1	-29.1±0.2	-30.9±0.1	-30.7±0.1	-31.5±0.2	-27.0±0.4	-26.2±0.4	-28.3±0.6
	2000b	-24.1±0.1	-28.9±0.2	-30.8±0.3	-31.3±0.5	-31.7±0.2	-25.4±0.9	-26.6±0.2	-28.1±0.4
Grain-maize	2001	-25.1±0.1	-32.2±0.2	-35.1±0.1	-35.6±0.0	-34.3±0.4	-30.8±0.8	-33.3±0.5	-33.5±0.6
	2002	-24.6±0.1	-30.8±0.4	-34.0±0.2	-34.4±0.3	-32.1±0.6	-29.8±0.6	-30.4±0.0	-31.0±0.3
Rotthalmünster									
Wheat	2002	-26.0±0.1	-32.2±0.2	-34.1±0.3	-34.6±0.1	-39.4±0.3	-32.4±0.1	-32.5±0.1	-33.9±0.3
Grain-maize	2002	-20.6±0.1	-25.6±0.1	-29.4±0.1	-29.4±0.1	-33.1±0.6	-26.1±0.2	-27.5±0.6	-30.9±0.5
Boigneville									
Wheat	1993	-25.9±0.1	-33.6±0.3	-37.4±0.2	-38.6±0.4	-35.2±0.4	-32.5±0.0	-32.2±0.0	-32.5±0.4
Grain-maize	1993	-20.8±0.1	-30.2±0.5	-32.8±0.1	-32.7±0.2	-26.9±0.3	-25.8±0.5	-25.9±0.6	-28.4±0.8
Brown coal from Beuna									
Main seam[b]		-25.8±0.1	-30.8±0.5	-31.4±0.5	-32.2±0.5	-30.3±0.5	-29.6±0.5	-29.4±0.5	-32.7±0.5
Briquette[b]		-25.0±0.1	-29.6±0.5	-30.4±0.5	-31.3±0.5	-32.3±0.5	-[c]	-29.3±0.3	-29.6±0.7

[a] For single determinations of bulk isotopes a standard deviation of ±0.1‰ was assumed.
[b] Compound-specific isotope analysis based on a single analysis with an assumed standard deviation of ±0.5‰.
[c] Not detected.

C4 plants were relatively enriched in ^{13}C, leading to bulk isotopic values between -12.4‰ and -14.1‰ (Table 6). Relatively constant isotope values were observed for the bulk isotopy

of different maize plant parts but highly variable isotope values for *n*-alkanes (Table 6). While *n*-alkanes from fresh leaves were enriched in ^{13}C (average -24.2‰), those from fresh stems were slightly depleted (average -25.6‰). The *n*-alkanes of fresh and degraded maize roots as well as degraded stems gave the lightest isotopic ^{13}C-signatures (average -30.1‰), for details see Table 6. Probable reasons for these differences are discussed above. In contrast to fresh plant material, brown coal samples had intermediate bulk and *n*-alkane isotopic compositions (Table 10).

As a result of biomass input C3-cropped soils had the lowest bulk ^{13}C contents, but were still slightly enriched, when compared to fresh plant material (Figure 36 and Table 10). This effect was previously described for *in situ* preservation of resistant biopolymers and their stabilization through condensation reactions (Lichtfouse *et al*., 1995b). Lichtfouse *et al*. (1994, 1995b) reported an enrichment of about 3.0‰ in ^{13}C for wheat cropped soils when compared to aboveground biomass in Boigneville. This could also be observed for plants and wheat cropped soils from Rotthalmünster. Halle rye cropped soils showed nearly equivalent bulk ^{13}C signatures, when compared to C3-cropped soils from Rotthalmünster and Boigneville. The difference in bulk ^{13}C between C3-cropped soils from Halle and fresh aboveground plant biomass was 5.1‰ and thus significantly larger than for the other sites.

For all sites the bulk isotopic composition of SOC gave values intermediate between those of C3- and C4-plant biomass (Figure 36). In general isotopic differences between C3- and C4-cropped soils were larger, if the C4-cropped soil had been under grain-maize as compared to silage-maize harvesting. This was also observed in Halle where silage-maize cropping was practised for a longer time than grain-maize cropping on the other plots. In 2001 a new plot was established in Halle for grain-maize cropping on a previously rye cropped soil respective fallow land. Prior to introduction of maize this soil showed a slightly larger isotopic depletion in ^{13}C of *n*-alkanes in comparison to other C3-cropped soils in Halle, due to inhomogeneities in the soil properties of this trial. A significant enrichment in ^{13}C of *n*-alkanes and bulk SOC of the grain-maize plot in Halle could be observed after only one year.

Soil at the Halle site received input of lignite-derived lipids as shown by molecular compositions (Figure 34). Lignite bulk isotopic composition was identical to rye cropped soil whereas isotopy of lignite *n*-alkanes was slightly enriched in comparison to soils (Figure 36 and Table 10). Input of lignite-derived organic matter thus has diluted the incorporation of C4-plant lipids and total biomass into Halle soils for several decades.

In soils most abundant *n*-carboxylic acids generally gave results identical to those of *n*-alkanes, when compared with bulk isotopic composition (Figure 37). The mean biosynthetic fractionation was slightly lower (7‰) than for *n*-alkanes. Fractionation within carboxylic acids of C3-plants was similar to those observed for *n*-alkanes and yielded results typical for C3-

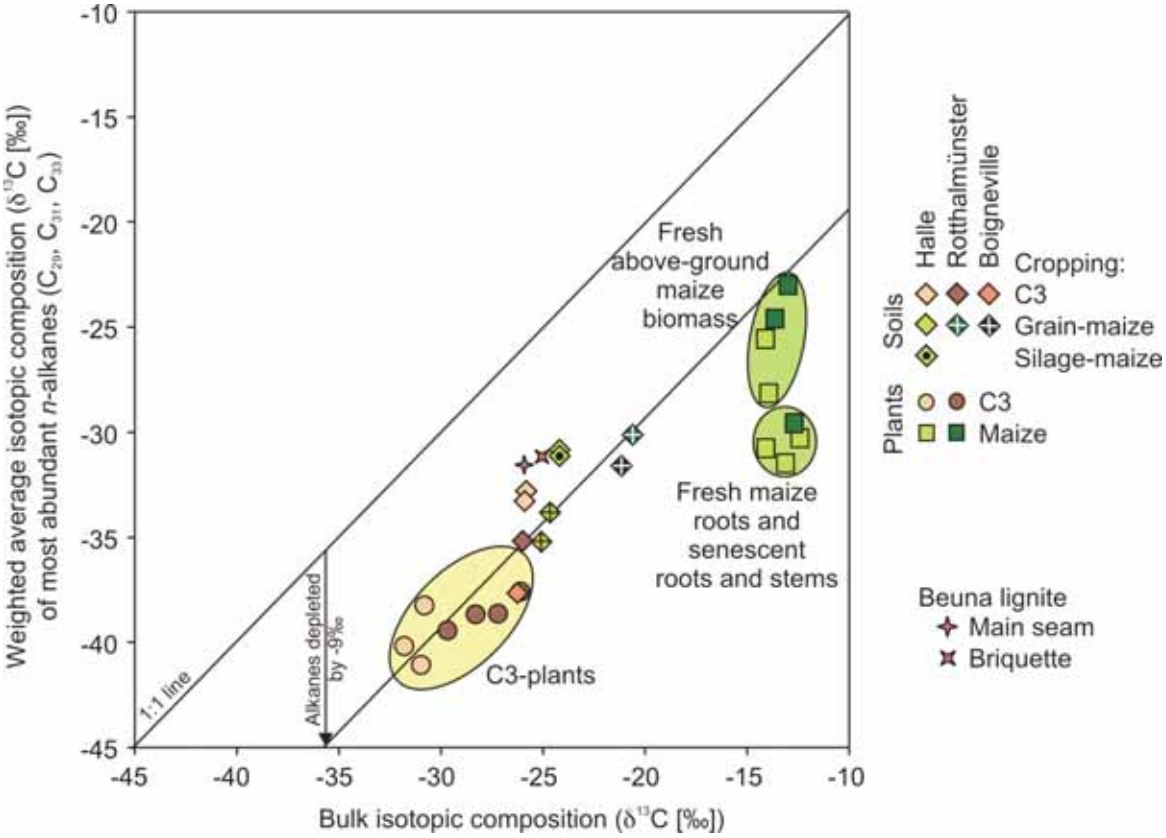


Figure 36. Weighted average isotopic composition of most abundant *n*-alkanes (C_{29}, C_{31}, C_{33}) compared to the bulk isotopic composition.

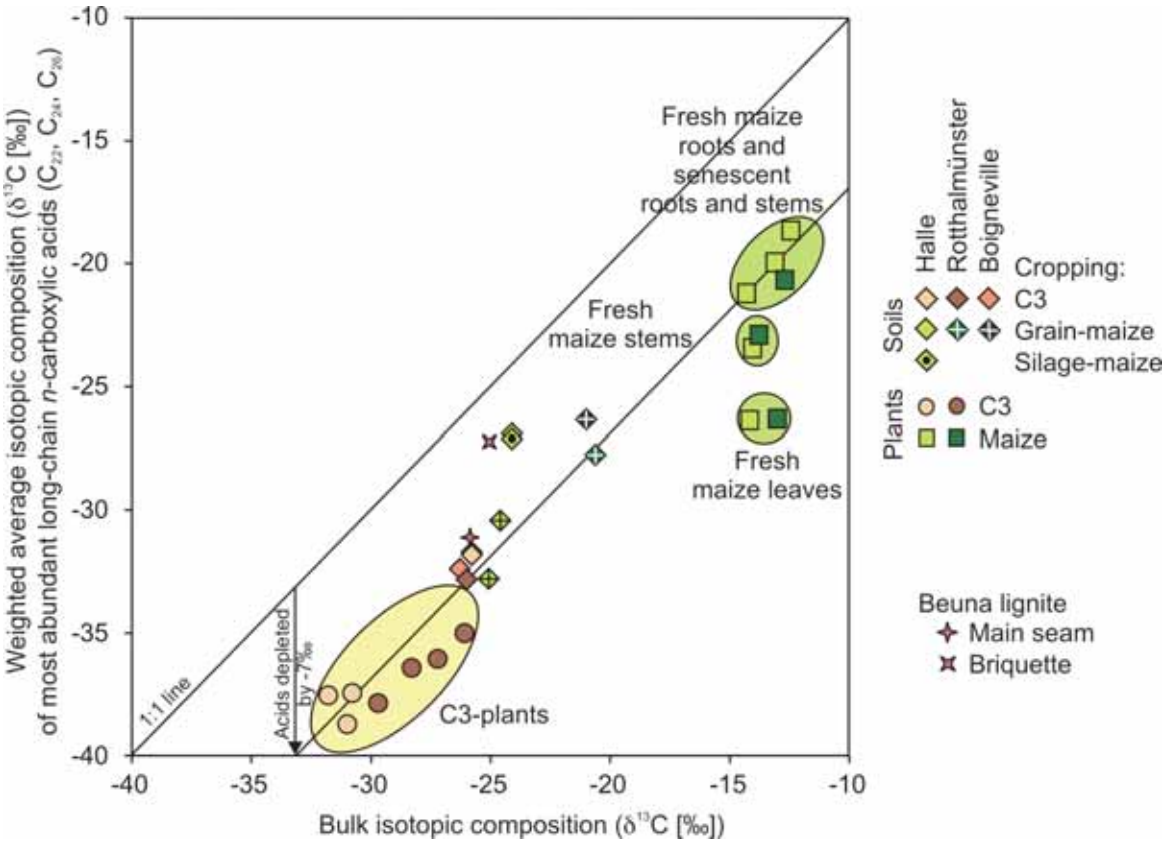


Figure 37. Weighted average isotopic composition of most abundant long-chain *n*-carboxylic acids (C_{22}, C_{24}, C_{26}) compared with bulk isotopic composition.

plants (Ballentine *et al*., 1998). Much larger isotopic differences occurred between various C4-plant parts. In contrast to *n*-alkanes the ^{13}C-content of *n*-carboxylic acids of maize plants was lowest for fresh leaves, slightly higher for fresh stems and highest for fresh and degraded roots as well as for degraded stems (Figure 37, Table 6). Results for maize leaves were similar to other C4-plants described by Ballentine *et al*. (1998). Reasons for this contrast in isotopic compositions between *n*-alkanes and *n*-carboxylic acids are still unknown but may be attributed to biosynthetic pathways (Kolattukudy *et al*., 1976, Lichtfouse, 1998).

The observations made on *n*-alkanes in soils and brown coal samples were also valid for *n*-carboxylic acids. Only Boigneville soils were enriched in ^{13}C, which might depend on deviating soil properties or microbial communities.

Comparison of isotopic compositions of most abundant *n*-carboxylic acids with those of most abundant *n*-alkanes (Figure 38) revealed only marginal differences between both lipid classes, except for maize plant biomass. The combination of results from both lipid classes improved discrimination between different fresh plant organs of maize and degraded biomass. As a result of the intra-plant isotopic variability of maize plants, the various C4-cropped soils showed different isotopic behaviour when compared with C3-cropped soils. This was due to the lack of significant intra-plant isotopic variability observed for C3-plants. The intra-plant isotopic variation was amplified by application of different cropping techniques, which selectively removed the aboveground biomass and its corresponding isotopic signatures from the plot (silage-maize) or preserved the isotopic signature of the aboveground biomass (grain-maize).

Grain-maize cropped soils were enriched in ^{13}C in both the *n*-alkane and *n*-carboxylic acid fractions as a result of high aboveground biomass input with ^{13}C -enriched stems and leaves. Only 15-17% of the total biomass input on these plots resulted from roots (Anderson, 1988), because nearly all biomass was left on the plot after harvesting. Contrastingly, on silage-maize cropped soils nearly all aboveground biomass was removed during harvest and mainly roots and the lowermost parts of the stems (up to 15cm) remained on the field. Thus, a ratio between shoot and root (S:R-ratio) of 1:1 must be assumed for plant biomass incorporation on this plot. This contrasting biomass input led to a preferential ^{13}C-enrichment in the *n*-carboxylic acids, whereas *n*-alkanes got only slightly enriched.

Summarizing, several new observations were made, concerning soil lipids of agricultural ecosystems. Lipids are an important part (1-10%) of the soil organic matter. Soil lipids follow similar decomposition trends with soil depth and decreasing particle-size like other compound classes like e.g. carbohydrates. Bulk soils show similar lipid compositions and distributions for all arable soils, but monoculture cropped soils can be differentiated by the new carboxylic acid ratio (CAR), which can also used for the crop differentiation. With this ratio and the bulk isotopy a first estimation of C3- and C4-derived carbon contents in soils can be practised. Compound-specific isotopy allows for the differentiation between cropped

soils and even cropping procedures like silage- and grain-cropping. Additionally, soil lipids can be used for source differentiation between e.g. plants, bacteria, fungi and fossil sources like brown coal.

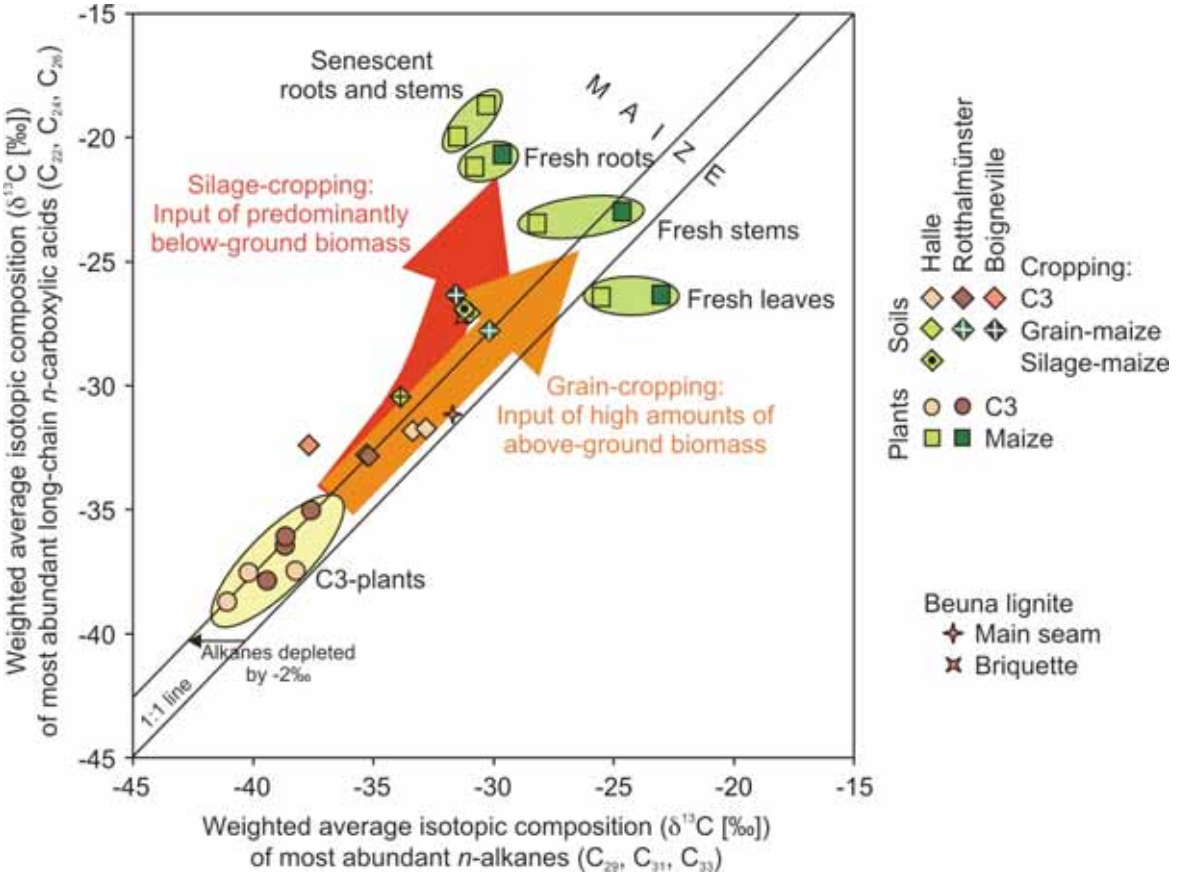


Figure 38. Weighted average isotopic composition of most abundant long-chain *n*-carboxylic acids plotted against weighted average isotopic composition of most abundant *n*-alkanes. Depending on harvesting techniques discrete incorporation pathways were observable: i) high aboveground biomass input of grain-maize cropping led to similar isotopic enrichment in soils for alkanes and carboxylic acids (orange arrow); ii) silage-maize cropping with preferential incorporation of belowground biomass led to an isotopic enrichment of carboxylic acids (red arrow).

5.5 Incorporation of new plant-derived carbon in top soils

The following chapter was previously published by Wiesenberg *et al*. (2004b). Only minor revisions were performed for this chapter.

New maize-C proportions were calculated for the long-term agricultural trials of Halle, Rotthalmünster and Boigneville sites. Maize-derived carbon proportions were determined for exclusive input of different plant parts and a combination of several plant parts like roots, stems and leaves, based on an assumed shoot to root ratios. First, determinations are discussed based on bulk isotopic determinations, followed by the most abundant lipids of two fractions: i) aliphatic hydrocarbons and ii) carboxylic acids.

5.5.1 New maize-derived carbon proportions

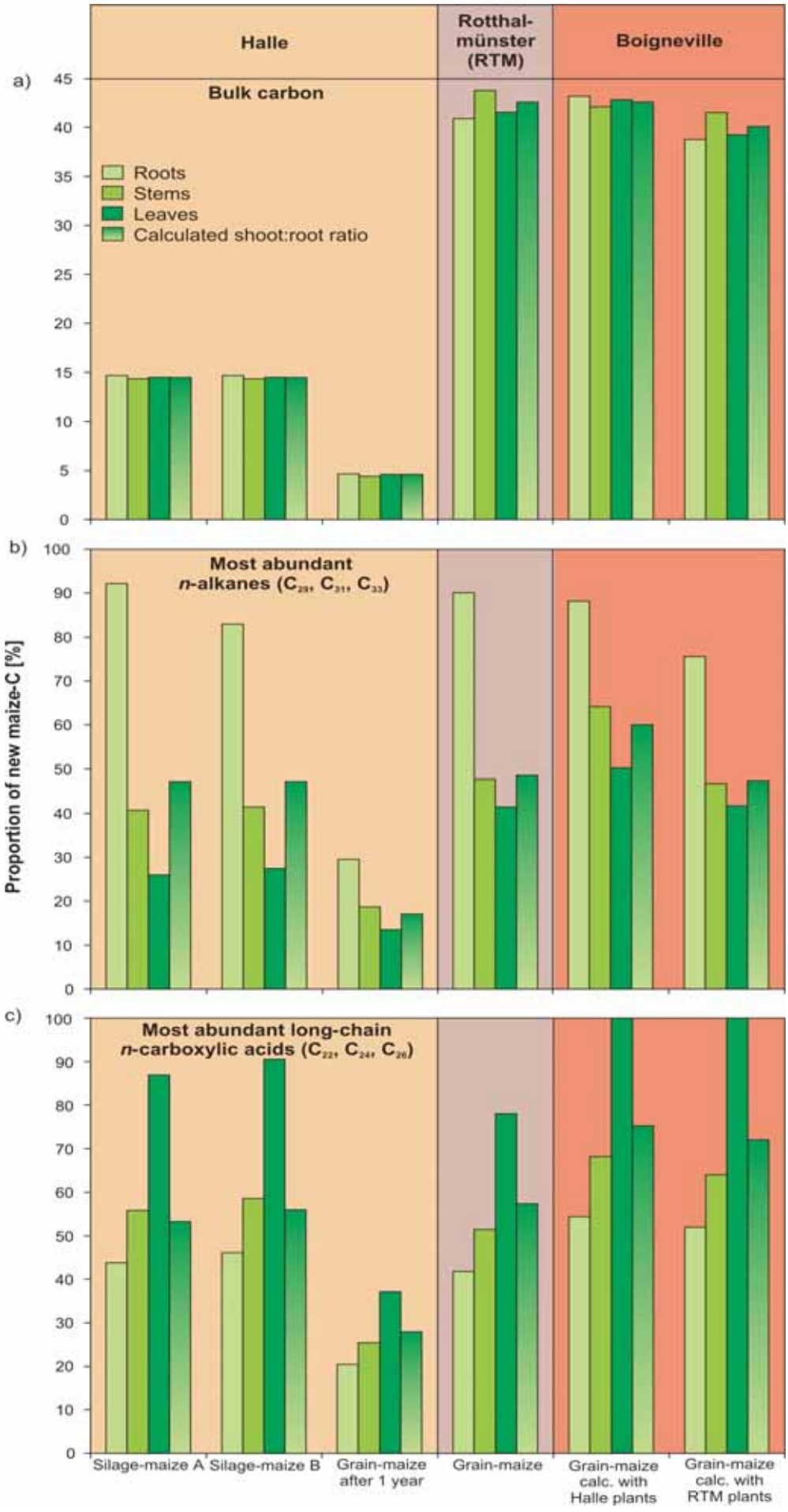
Previously in this study it was demonstrated for *n*-alkanes and *n*-carboxylic acids, that C3- and C4-plants had different molecular and isotopic compositions and that organic matter of maize plants differed significantly between plant parts. Consequently, the input of new maize-derived soil organic carbon must by calculated separately, assuming an exclusive input of several plant parts (roots, stems and leaves) and different shoot to root ratios (S:R-ratio). Adequate S:R-ratios were selected for each cropping technique. For grain-maize cropping a S:R-ratio of 5.7:1 or 15% root biomass (Anderson, 1988), and for silage-cropping a S:R-ratio of 1:1 was used. For all soils aboveground biomass was assumed to have a biomass proportion between leaf and stem of approximately 1:1. Proportions of new maize-derived carbon (Figure 39) in previously C3-cropped soils were calculated as:

$$M = 100 \times F_{C4} \quad (18)$$

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

For calculations based on bulk isotopic values (Figure 39a) only marginal differences (<5%) between various plant parts could be observed. Hence, input of separate plant parts was not significantly different for bulk isotopic signatures and consequently each plant part might be used for these calculations. Halle silage-maize cropped soils contained only 15% of new-maize C after 39 years of continuous cropping. In contrast after only one-year grain-maize cropping the soil from Halle contained 5% new biomass. This fast turnover was firstly caused by high biomass incorporation and secondly by the incorporation of labile carbon from e.g. sugars within the first year. In Rotthalmünster and Boigneville soils the proportions of new maize-C were around 41% (Figure 39a). Because fresh plant material for the Boigneville site was not available for analysis, calculation of biomass input was made using ^{13}C-signatures of plant material measured from the Halle and Rotthalmünster sites.

Figure 39. (On the following page.) Scenarios showing the calculated effects of incorporation of different plant parts on apparent proportions of new maize-derived carbon in previously C3-cropped soils. Scenarios were calculated for: only roots, only stems, only leaves, or a mixture of all. For the latter a shoot:root ratio of 1:1 for silage-maize in Halle and 5.7:1 for all other plots is used. A stem:leave ratio of 1:1 is assumed to represent shoot biomass. New maize-derived carbon was calculated for (a) bulk soil, (b) most abundant *n*-alkanes (C_{29}, C_{31}, C_{33}), and (c) most abundant long-chain *n*-carboxylic acids (C_{22}, C_{24}, C_{26}).


Halle
Rotthal-münster (RTM)
Boigneville
a)
Bulk carbon
Roots
Stems
Leaves
Calculated shoot:root ratio
b)
Most abundant n-alkanes (C29, C31, C33)
Proportion of new maize-C [%]
c)
Most abundant long-chain n-carboxylic acids (C22, C24, C26)
Silage-maize A
Silage-maize B
Grain-maize after 1 year
Grain-maize
Grain-maize calc. with Halle plants
Grain-maize calc. with RTM plants

Differences between calculations for biomass input into Boigneville soils based on Halle and Rotthalmünster plant isotopic values varied only marginally. For the Boigneville site Puget *et al*. (1995) and Cayet & Lichtfouse (2001) indicated proportions of new maize-derived carbon of 45% and 41%, respectively. The close match between three different analyses lends credibility to the analytical approach used.

Gregorich *et al*. (1996a) determined 20% and 30% new maize-C (maize-derived carbon) incorporation into agricultural soils after 35 years continuous maize cropping with less incorporation corresponding to non-fertilized plots. Slightly higher proportions of new maize-C were determined by Collins *et al*. (1999) ranging from 30% to 58% after 16 to 35 years of fertilized continuous maize cropping. Liang *et al*. (1998) observed lower proportions of new maize-C incorporation between 5% and 19% due to shorter duration of fertilized continuous maize cropping ranging between 3 to 11 years. Balesdent *et al*. (1987) reported new maize-C proportions of 30% after 13 years of corn cropping on previously C3-utilized soils. Similarly, Accoe *et al*. (2002) calculated 33% new maize-C after 19 years since the switch from C3-cropping occurred. Kristiansen *et al*. (2004) observed 10-20% new maize biomass for continuous silage-maize cropping after 14 years in Danish soils and 20-35% for additional application of aboveground biomass. The incorporation of maize-derived carbon into agricultural soils, managed by grain- and silage-maize cropping, as determined in this study, is in good agreement with data reported in the literature. The low proportions of maize-C in Halle silage-cropped soils are attributed to low biomass input due to the harvesting technique applied and the dilution of crop-derived carbon by lignite-derived carbon.

On a molecular level significant differences between various modes of plant biomass incorporation into soils (Figure 39b, c) were observed. For *n*-alkanes (Figure 39b) the maize-C proportions were highest (around 90%) for an incorporation of exclusively root biomass. This is related to the ^{13}C-depletion within maize roots. Contrastingly, biomass incorporation exclusively from stems showed lower maize-C proportions of 40-60% depending on sampling site and were lowest for the grain-maize plot in Halle (20%). The lowest maize-C proportions were calculated for an exclusive input of leaves. This led to an underestimation of new maize-C proportions calculated by Cayet & Lichtfouse (2001), who used exclusively leaf-derived *n*-alkane isotopic signatures in their calculations of new maize-C proportions. The largest differences between belowground and aboveground biomass were observed in Halle soils related to silage-maize cropping. For Boigneville soils differences up to a maximum of 20% were observed, when calculations were based on Halle or Rotthalmünster plant biomass values. Thus, it was required to use the plant biomass properties from each individual plot for the isotopic assessment of maize-C turnover based on *n*-alkanes. Maize-C calculations corrected for the S:R-ratio again showed results comparable to those based on

calculations using exclusively stem biomass. This implies that *n*-alkanes could be used as plot independent markers for SOC turnover. For silage-maize cropped soils from the Halle site similar results of maize-C incorporation could be observed compared to the other sites.

For *n*-carboxylic acids contrasting trends were observed (Figure 39c) in comparison to *n*-alkanes. Leaf-derived carboxylic acids seemed to be incorporated at a high degree, when compared with exclusively root or stem biomass. A proper correction had to be used for the *n*-carboxylic acid fraction, to obtain realistic proportions of new carbon incorporation and turnover rates. Calculations of S:R-ratio-corrected new carbon incorporation in soils led to similar maize-C yields in Halle silage-maize cropped soil and Rotthalmünster soil, which were almost in the range of exclusively stem-biomass input. The highest proportions of maize-C could be observed in Boigneville soils with identical results based on calculations using Halle and Rotthalmünster plant biomass properties. Grain-maize cropped soil in Halle yielded relatively high maize-derived carboxylic acid proportions of nearly 30% after only one year of C4-cropping. This is related to the preferential occurrence of *n*-carboxylic acids in shoots, in contrast to the dominance of *n*-alkanes in roots. For the Halle sites an enhanced proportion of new maize-derived carboxylic acids in the grain-maize cropped plot as compared to the silage-maize cropped plot were due to the grain-maize harvesting technique, which left more biomass on the field. This increase in biomass input is accompanied by a reduction in SOM dilution by lignite due to a cease of open pit mining in the Halle-Bitterfeld-Beuna region.

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

5.5.2 ^{13}C-based turnover time calculations

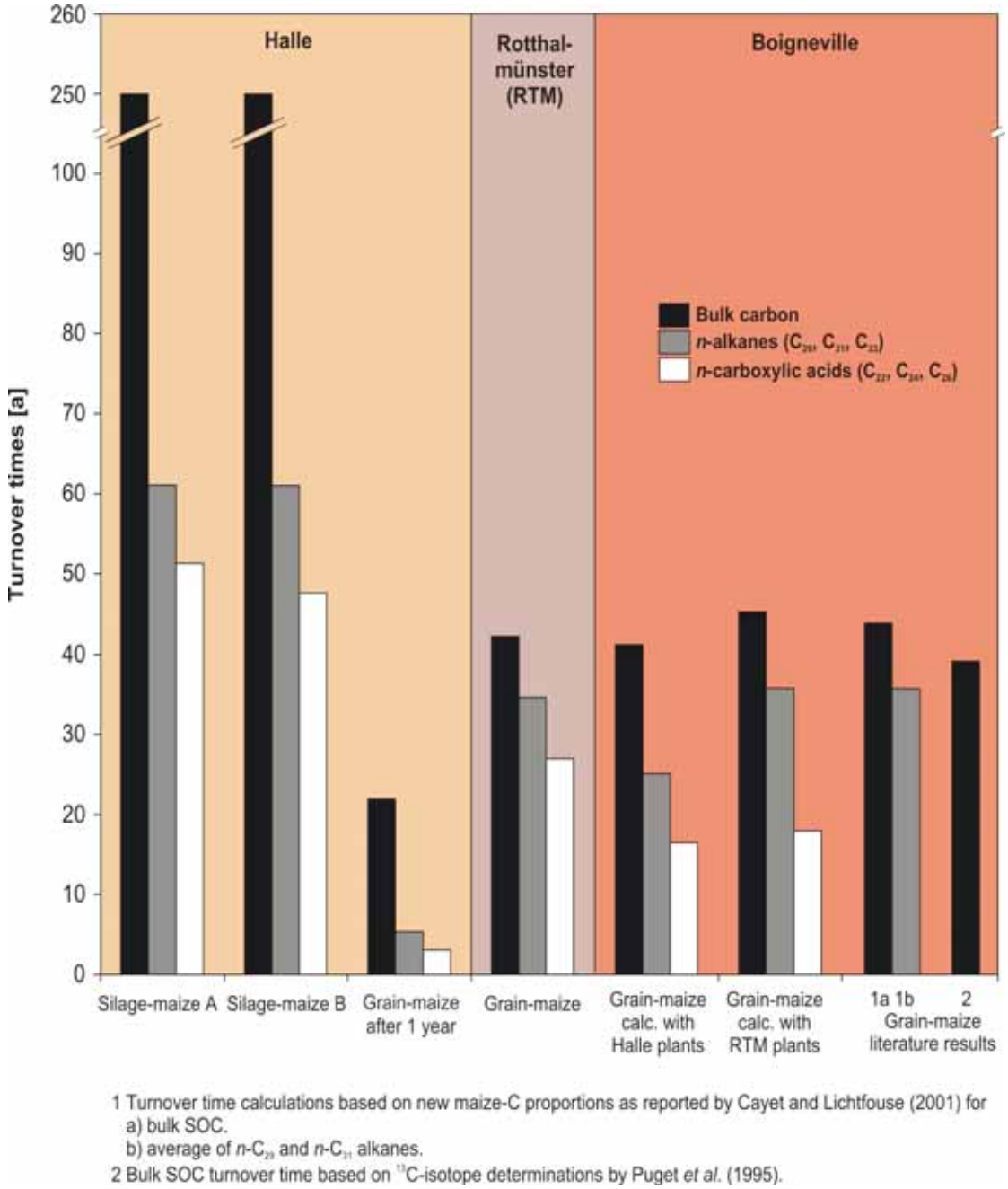
In order to account for the variable duration of long-term field experiments with respect to the proportion of new C4-incorporated carbon into SOM, turnover times or mean residence times are calculated based on formulas (6) and (7). As defined under methods, this approach requires steady state conditions in soil carbon balance and similar plant physiologies, e.g. vegetation periods (Balesdent & Mariotti, 1996).

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

For bulk SOC the obtained turnover times of 39 to 45 years are very similar (Figure 40). Turnover times for *n*-alkanes are virtually identical for the Rotthalmünster and Boigneville sites with 35 years, except when using isotopic composition of plants from Halle, which resulted in slightly shorter turnover times of 26 years (Figure 40). Turnover times of *n*-alkanes determined for Boigneville using data of averaged *n*-C_{29} and *n*-C_{31} as reported by Cayet & Lichtfouse (2001) or previous results for *n*-C_{31} derived from La Minère and Boigneville sites shown by Lichtfouse (1997) led to results identical with calculations based on data obtained in this study. This is taken as evidence for the high reliability of the methodological approach due to the complex work-up of lipid fractions required for compound-specific isotope analysis. Carboxylic acid fractions gave consistently shorter turnover time of 18 years for the Boigneville site than for the Rotthalmünster site with 28 years, irrespective of the isotopic composition used for the parent plant material. Soil lipid degradation can be affected by high soil acidity (Marseille *et al*., 1999, Moucawi *et al*., 1981, Bull *et al*., 2000) especially in forest or waterlogged soils. The near neutral conditions (pH 5.8 to 6.8) of the arable soils studied here exclude pH-influence on lipid turnover.

The grain-maize experiment in Halle has been running for only one year and thus could not have approached steady state conditions augmented by low input of plant biomass prior to maize cropping. Thus, the short turnover times of 21 years for bulk SOC, 5 years for *n*-alkanes and 3 years for *n*-carboxylic acids (Figure 40), are not comparable to the other grain-maize plots.

Silage-maize plots in Halle showed exceptionally long turnover times of 250 years for bulk carbon and 48 to 60 years for lipid fractions (Figure 40). The long turnover times are related to two processes: i) the lower direct plant biomass input as a consequence of the harvesting technique, which removes more than half of the standing biomass from the plot, and ii) the presence of exceptional high quantities of refractory fossil organic matter. The latter results from lignite input as described above (Figure 34) and further anthropogenic contamination (Rethemeyer *et al*., 2004a, Wiesenberg & Schwark, unpublished). Both of these two factors may have contributed to reducing microbial activity to a minimum in Halle soils (personal communication with A. Miltner and S. Scheu), which in turn would contribute to longer turnover times.



1 Turnover time calculations based on new maize-C proportions as reported by Cayet and Lichtfouse (2001) for
a) bulk SOC.
b) average of n-C_{29} and n-C_{31} alkanes.
2 Bulk SOC turnover time based on ^{13}C-isotope determinations by Puget *et al.* (1995).

Figure 40. Turnover times for bulk-C, most abundant *n*-alkanes and *n*-carboxylic acids, based on shoot:root-corrected maize-C proportions. Silage-maize cropping is only practised in Halle, all other sites employ grain-maize cropping.

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

Agricultural soils at all sites investigated show longer turnover times for total SOC than for lipid fractions. Within the lipid fractions *n*-alkanes had slightly longer turnover times than *n*-carboxylic acids. This corresponds to previous observations by Lichtfouse (1997) and

Lichtfouse *et al.* (1998), who concluded that *n*-alkanes are more stable against chemical and biological degradation because of the lack of functional groups that may serve as sites for microbial attack.

Consequently, application of grain-maize cropping on existing arable soils would lead to higher atmospheric CO_2 sequestration via incorporation and fixation of crop biomass in soils. In addition with introduction of non-disruptive ploughing procedures the CO_2-emission character of cropped soils as described by Janssens *et al*. (2003) might be changed towards a CO_2-sink character.

Summarizing, plant lipids turn over faster in arable soils than bulk carbon and than previously observed in peaty and acidic soils. For the first time it could be demonstrated, that especially for a realistic assessment of lipid turnover times exact lipid compositions and isotopic contents of incorporated plant parts are needed. Additionally, turnover times are influenced by fossil carbon contents in soils and harvesting techniques applied. Generally, lipids are part of the intermediate stable carbon pool in soils and thus important for soil carbon modelling.

5.6 Radiocarbon analyses

Parts of the following chapter were previously published by Rethemeyer *et al*. (2004a) and revised extensively in the context of this study.

Usually, radiocarbon ages should increase in soils with depth (Trumbore, 1996). Contrastingly, for soil profiles of Halle site radiocarbon ages decreased with soil depth (Rethemeyer *et al*., 2001). This is assumed to be derived from pollution in the topsoil horizons by brown coal or other fossil contaminations.

Radiocarbon analyses were practised for particle-size separates from Halle site to obtain informations, in which of those particle-size separates large amounts of recent respective fossil carbon were present. Thereafter, an industrialised and a rural site are compared with analyses of bulk soils and lipid fractions from both, Halle and Rotthalmünster sites. Exemplarily, source apportionment of some components from the alkane fraction was realised using pentacyclic triterpanes (hopanes) for the determination of recent and possible fossil carbon sources.

5.6.1 Particle-size separates

As described above and shown in Figure 41, ploughed horizon of silage-maize cropped soil from Halle site was dominated by sand (67%), followed by minor proportions of silt (22%) and clay (11%). Organic carbon content and extract yield increased with decreasing particle-

size. Paralleling this trend, ^{14}C-contents increased with decreasing particle-size from 23pMC in the sand-size separate over 43pMC in the silt-size separate to 70pMC in the clay-size separate. This means, that the highest proportions of recent carbon were present in the clay-size separate and thus, highest contents of fossil carbon were included in the sand-size separate. This observation was in contrast to previous observations within soils, where coarse recent plant biomass was supposed to be first incorporated into soils. With further degradation by microbial biomass, organic content was broken into smaller fragments and hence, in smaller particle-size separates smaller contents of recent carbon were determined. Thus, a contrasting trend was expected for analysed particle-size separates. As described above, pollution by brown coal fragments was observable in the ploughed horizon. The low percentage of modern carbon was caused by high proportions of fossil carbon, e.g. brown coal, in the sand-size fractions of soils from Halle site. The smaller the particle-size, the lower was the pollution by brown coal.

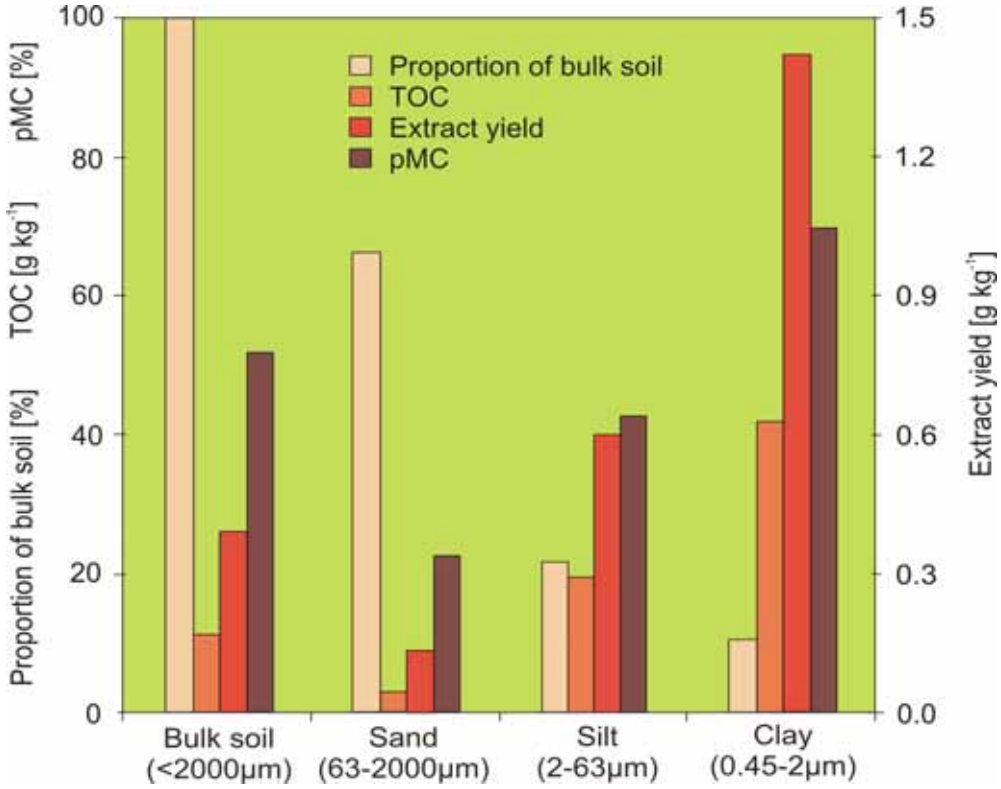


Figure 41. Distribution of texture, total organic carbon content, lipid extract yield and percent of modern carbon (pMC) for particle-size fractions and bulk soil of silage-maize cropped Axp-horizon from Halle site sampled in 2001.

5.6.2 Lipid fractions

The percentages of modern carbon of different lipid compound classes and distribution patterns of lipid fractions of soil samples of the Halle and Rotthalmünster field trials are shown in Figure 42.

Lipid compound classes of surface soil samples from Halle site revealed highly depleted ^{14}C values (Figure 42a). While soils before and residue after lipid extraction showed identical ^{14}C contents (51.7pMC), the lipid extract was depleted by 3.4pMC. Most lipid fractions revealed similar ^{14}C contents like the bulk soil and the lipid extract. Only the basic fraction (Q) showed a significant depletion, most likely due to higher contents of fossil carbon or uncertainties during the measurement. Uncertainties during measurement are a result of low carbon amounts available for ^{14}C-determinations. The largest depletion could be observed in the neutral fraction (N) with 16pMC. This fraction was separated into aliphatic (A) and aromatic (B) hydrocarbons and a low-polarity fraction (C). Lowest ^{14}C concentrations were measured in aromatic (5.7pMC),

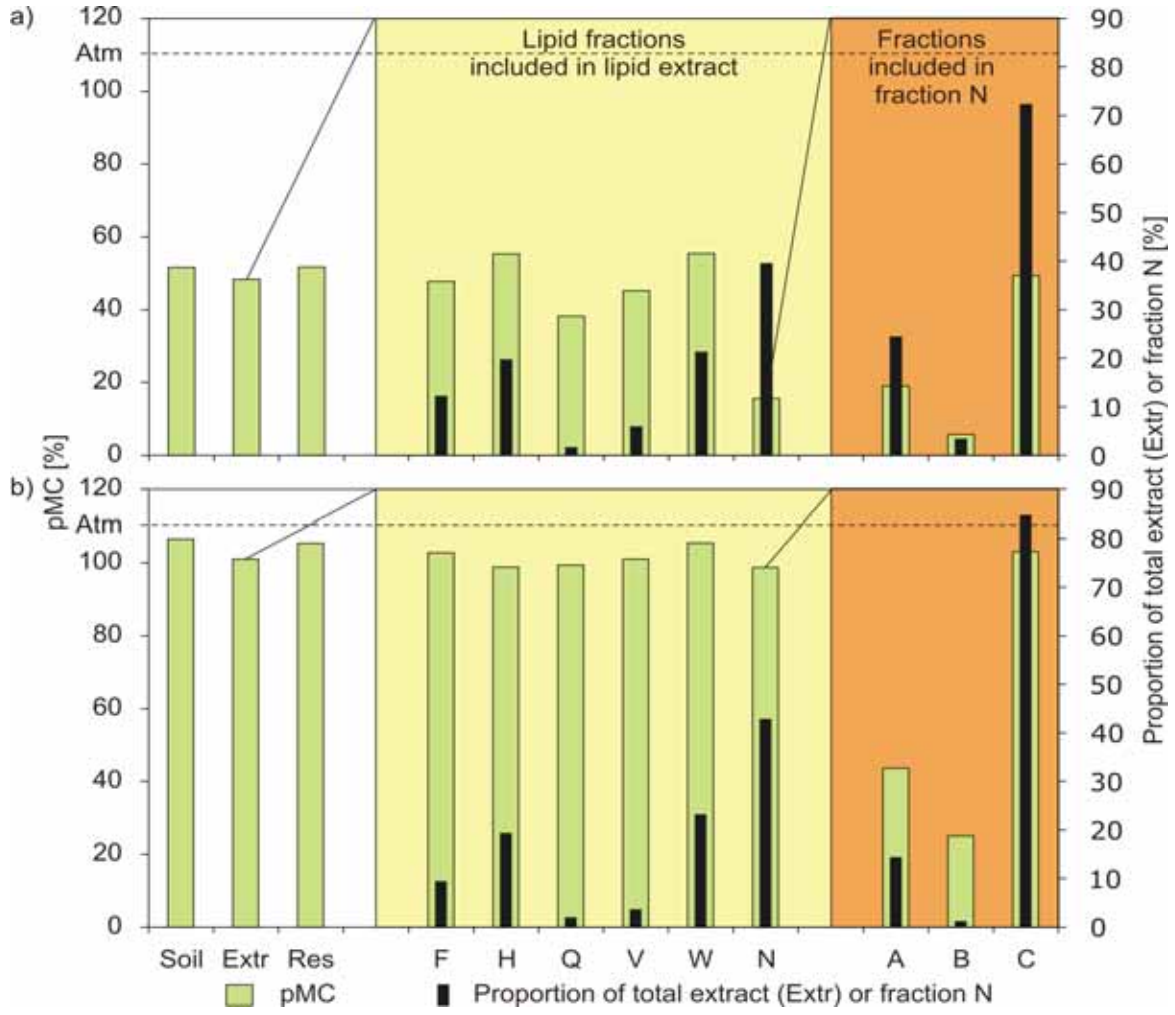


Figure 42. ^{14}C values of topsoils and lipid compound classes from topsoil samples (0-30cm) of a) the urban Halle site, and b) the rural Rotthalmünster site. The dashed lines represent the ^{14}C content of the modern atmosphere. Abbreviations: Atm = modern atmospheric ^{14}C-composition, Extr. = extract, Res. = extraction residue, F = fraction with mainly alcohols, H = fraction with mainly acids, Q = fraction with basic compounds, V = high polarity fraction, W = HMW fraction with mainly wax esters, N = neutral fraction, A = aliphatic fraction, B = aromatic fraction, C = low-polarity fraction with mainly ketones.

followed by aliphatic hydrocarbons (19pMC). This was attributed to a high contribution of old, ^{14}C-free carbon. Lichtfouse *et al*. (1997a) observed similar results for an aliphatic fraction, which was polluted by fossil fuel and thus contained 35pMC. As previously discussed lignite and coke particles in Halle soils were major sources of fossil carbon. Lignite was derived from nearby open pit mining, whereas coke particles were derived from steam trains running close to the experimental site until the 1990s. Lignite-derived fossil carbon compounds preferentially contributed to the aromatic and aliphatic hydrocarbon fractions. Relatively high contents of those fractions (1.2% for aromatic and 9.4% for aliphatic hydrocarbons of total lipids) were observable at Halle site. Most likely these elevated concentrations were due to the large contamination with fossil carbon.

Within most lipid fractions, individual compounds could not be related directly to recent and fossil sources. Exemplarily for source apportionment of compounds within one lipid fraction, selected compounds of the aliphatic hydrocarbons are discussed below. The aliphatic hydrocarbon fraction of soils consists of several different compound classes. While alkanes are most abundant in the aliphatic hydrocarbon fraction of soils, pentacyclic triterpanes (mainly hopanoids) and steranes are present only in low or trace amounts. As demonstrated previously in this study, alkanes of Halle soils showed the same distribution pattern as brown coal of a nearby lignite deposit and recent crop biomass. Thus, those compounds were not suitable to differ between several sources. Steranes were only abundant in trace amounts and thus a source apportionment could not be performed with this compound class. Alternatively, hopanoids (Figure 43) could be derived either from recent sources

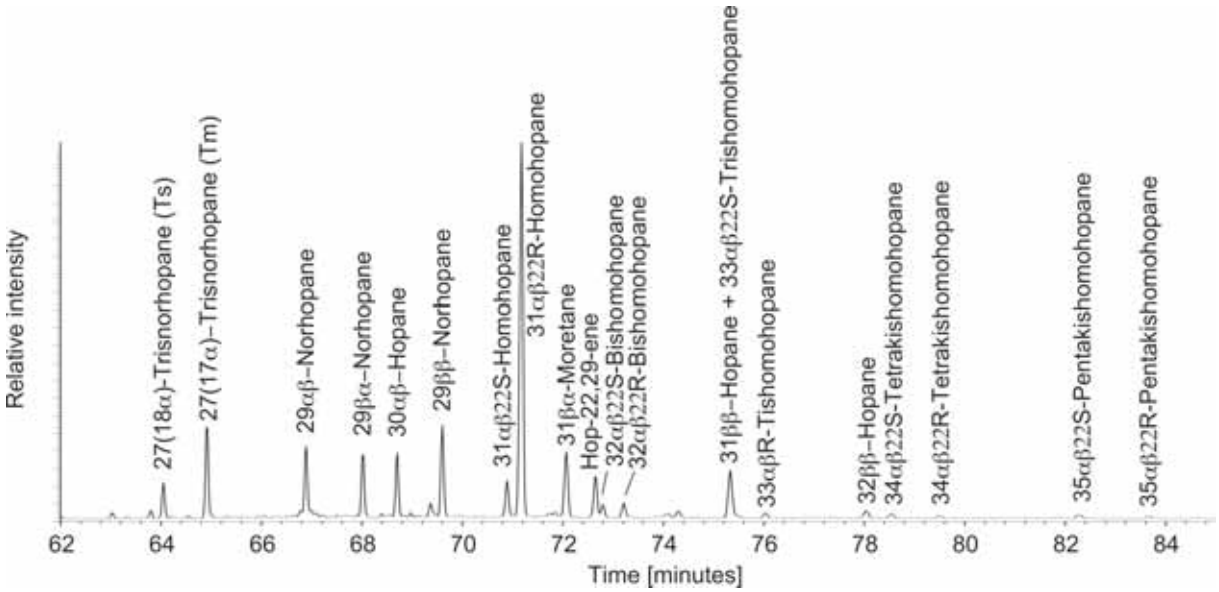


Figure 43. Mass fragment m/z 191 of GC/MS analysis of aliphatic hydrocarbons (fraction A) with pentacyclic triterpanes (hopanoids) of silage-maize cropped bulk soil from Axp horizon of Halle site.

(like bacteria, as observed by Ries-Kautt & Albrecht (1989)) as well as fossil sources (e.g. mineral oil as demonstrated by Peters & Moldowan (1993) and Lichtfouse *et al*. (1997b)). The ratio of Tm (27(17α)-Trisnorhopane) / Ts (27(18α)-Trisnorhopane) as shown by Peters & Moldowan (1993) for sediments and by Lichtfouse *et al.* (1997b) for soils was introduced as a maturity parameter, with Ts as the thermally more stable compound. Low amounts of Tm and thus low ratios (<1) indicate a high maturity, as observed for mineral oils or fossil fuel. High ratios (>1) are typical for immature material, e.g. unpolluted recent sediments or soils, because with increasing maturity Tm gets lost, while Ts is preserved. As observable in Figure 43, Tm was nearly three times as high as Ts and thus the soil of Halle showed low contributions by mature material like fossil fuel. Another parameter for maturity is the proportion of S-isomers within the homohopane series (Peters & Moldowan, 1993, Lichtfouse *et al.,* 1997b). With increasing maturity the S-isomer of the homohopanes is stabilized, while the R-isomer becomes successively degraded. For the homohopane series of different carbon numbers different ratios were determined within Halle soil (Figure 43). For 31αβ-Homohopanes a significant enrichment of the labile R-isomer could be obtained, related to immature, recent input. In comparison to 31αβ-Homohopanes, 32αβ-Bishomohopanes and 33αβ-Trishomohopanes showed decreasing amounts of the R-isomer with increasing carbon numbers. Thus, these series contained less amounts of recent compounds and higher contributions of fossil carbon. When comparing the contributions of the three shown homohopanes (Figure 44) to the entire homohopanes, the 31αβ-Homohopanes contributed approximately 90%, 32αβ-Bishomohopanes 6% and 33αβ-Trishomohopanes 4%. Thus, the analysed soil was dominated by recent material, while low amounts (approximately 10%) of fossil carbon were present as well. The low amounts of fossil, ^{14}C-free carbon led to the high ^{14}C-age for this fraction. Because of a large variability in hopane contents of fossil and recent samples, the discussed parameters only facilitated a first estimation of fossil and recent contributions. Additionally, the brown coal from Beuna (not shown here) had a similar hopane distribution pattern like the soil from Halle site, except for significant higher contents of the recent 31αβR-Homohopane and lower contents of 30αβ-Hopane in the soil. For detailed determinations of fossil respective recent sources of single lipids or compounds classes, additional analyses like ^{14}C-dating of single lipids as described by Rethemeyer *et al*. (2004a, 2004b) are needed.

Summarizing, ^{14}C-dating of lipid classes from Halle site, even of the less polluted fractions, which were assumed to be predominantly derived from recent plant biomass, showed ^{14}C concentrations of approximately 55pMC and reflected a significant contamination of approximately 50% fossil carbon. Thus, parts of the 'old', ^{14}C-free fossil carbon seemed to be recently remobilized and incorporated into the recent biomass, leading to high ^{14}C-ages.

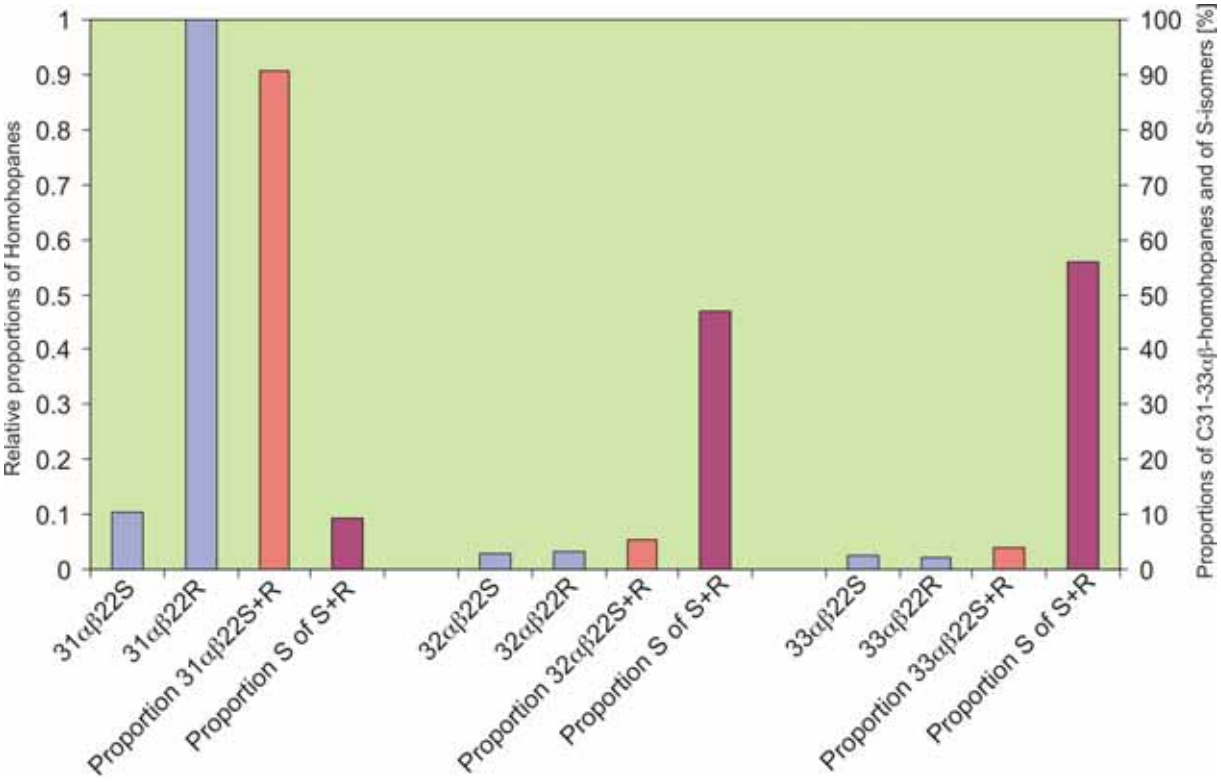


Figure 44. Relative proportions of 31αβ, 32αβ, and 33αβ homohopane 22S- and 22R-isomers derived from the m/z 191 mass fragment of silage-maize cropped bulk soil from Axp horizon of Halle site. Blue bars represent relative proportions of homohopanes in comparison to the most abundant compound. Orange bars show relative proportion of 31, 32, or 33 homohopanes in relation to all displayed homohopanes. Red bars represent the proportion of the S isomer within each homohopane isomer pair (S+R).

^{14}C-values of compound classes of Rotthalmünster sites were close to the modern atmospheric ^{14}C-level (Figure 42b). Similar to Halle site, the total lipid extract was depleted by 3.3pMC compared to the bulk soil (106.5pMC). The residue after lipid extraction showed similar ^{14}C-contents (105.4pMC). Radiocarbon contents of the isolated compound classes ranged from 99pMC of the neutral lipids (N), with similar values for the acid (H) and basic fractions (Q), to 105pMC of the high molecular fraction (W). Aliphatic (A) and aromatic (B) hydrocarbons showed low ^{14}C-contents of 44pMC (A), respective 25pMC (B). Similar to Halle site, these low ^{14}C-contents were mainly caused by a contribution of fossil carbon to these fractions, especially for the aromatic hydrocarbon fraction. In contrast to Halle site, this fraction mainly consisted of residues of incomplete fossil fuel burning or house burning, while brown coal derived compounds were not abundant. The aliphatic hydrocarbon fraction consisted mainly of plant-derived compounds, but low amounts of fossil carbon, derived from fossil fuel or fossil fuel burning, were abundant as well. Within the aliphatic fraction only trace amounts of hopanoids could be observed showing a strong domination of the recent 31αβR-Homohopane. Thus, the hopanoids of Rotthalmünster site were not shown here. These old

fractions represented only small proportions (approximately 0.5% (B) to 6.0% (A)) of the total extract in comparison to Halle site, which revealed low contamination by fossil carbon of the Rotthalmünster site. Main constituents (approximately 85%) of neutral lipids were low polar hetero-compounds (C) with a relatively high ^{14}C-content of 103.4pMC. Since N was subdivided into fractions A, B, and C, the weighted mean ^{14}C-concentration of the three fractions had to be equal to the ^{14}C-content of N. Actually, the resulting ^{14}C-value, calculated by mass balance of fractions A, B, and C, suggested a small loss of ca. 4.4% modern carbon compared to the higher radiocarbon content of the directly measured fraction N. This might result from volatilization of substances during solvent evaporation and/or incomplete recovery in the preparation of fractions A, B, and C.

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

5.6.3 ^{14}C-based mean residence time calculations

After Trumbore (1996), mean residence times (MRT ^{14}C), based on ^{14}C-data, can be calculated for soil organic carbon as follows:

$$\text{MRT } ^{14}C = - (1 / \lambda) \times \ln (pMC / 100) \quad (19)$$

with λ as the rate constant for the radioactive decay of ^{14}C (λ=0,000121 per year).

Mean residence time of Halle bulk soil (Table 11, 2372 years) was significantly longer than that of Rotthalmünster soil with -227 years. The negative result was caused by larger ^{14}C contents than 100pMC. This indicated a larger radiocarbon content, than obtained in the atmosphere of the year 1950, when the atmospheric bomb-^{14}C peaked. Thus, this soil and the lipid fractions with negative mean residence times have to be called recent, due to low residence times. Residence times of lipid fractions varied between 1283 and 5333 years for particle-size separates of Halle soil and between -191 and 4976 years for Rotthalmünster site. As previously described, the fossil carbon content inhibited turnover of carbon in Halle soil, leading to large mean residence times. Residence times of most lipid fractions from Rotthalmünster site were less than 50 years and mostly seemed to be larger, than the residence time of the bulk soil. Hence, samples with high pMC (>100%) indicate an absolutely radiocarbon age of less than 54 years (since bomb-^{14}C peak 1950), resulting in improbable mean residence times, based on the calculation by Trumbore (1996). Thus, for those samples reliable calculations of mean residence times are still needed.

Table 11. ^{14}C-based mean residence times (years) of soils and lipid fractions of ploughed horizons.

	Halle	Rotthalmünster
Bulk soil	2372 ± 15	-227 ± 9
Sand-size separate	5333 ± 33	n.d.[a]
Silt-size separate	3052 ± 15	n.d.[a]
Clay-size separate	1283 ± 11	n.d.[a]
Total lipid extract	2596 ± 24	-106 ± 8
Extract residue	2367 ± 12	-188 ± 8
Lipid fractions		
F (alcohols, sterols)	2652 ± 26	-98 ± 8
H (carboxylic acids)	2026 ± 23	42 ± 9
Q (basic compounds)	3452 ± 38	23 ± 14
V (high-polarity compounds)	2853 ± 30	-37 ± 11
W (HMW-fraction)	2091 ± 27	-191 ± 8
N (neutral fraction)	6664 ± 89	47 ± 10
Fractions included in lipid fraction N		
A (aliphatic hydrocarbons)	5959 ± 41	2980 ± 22
B (aromatic hydrocarbons)	10295 ± 203	4976 ± 237
C (e.g. ketones)	2542 ± 18	-106 ± 10

[a] Not detected.

Summarizing, ^{14}C-based mean residence times were significantly larger than turnover times based on stable carbon isotope measurements (^{13}C). The carboxylic acid fraction of the unpolluted Rotthalmünster site showed a ^{14}C-mean residence time of 42 years, which is only twice as high as the ^{13}C-turnover time. Bulk soil and acid fraction of Halle site as well as alkane fractions of Halle and Rotthalmünster sites showed ^{14}C-mean residence times, which were 20 to 100 times higher than the turnover times based on ^{13}C calculations. Hence, contaminations by fossil carbon could not be excluded even in Rotthalmünster alkane fraction. Most reliable results of turnover times of 'natural', predominantly plant-derived lipids and especially those for carboxylic acids were derived from Rotthalmünster and Boigneville sites, because their ^{13}C-turnover times were very close to those of the ^{14}C-based data. Probably, there are higher uncertainties in ^{14}C-based calculations, because low contributions of fossil carbon without ^{14}C could cause large depletions of the whole sample.

6. Synthesis

The main aims of this study were to i) analyse lipids of different crop plants in order to find specific crop biomarkers, ii) obtain new insights into long-term carbon changes of agro-ecosystems, iii) determine turnover of bulk soil organic matter and individual plant-derived lipids, iv) check residence times of soil organic matter and lipids with ^{13}C and verify those against ^{14}C results, and finally, as a result of all analyses v) determine the importance of lipids for total soil organic matter turnover.

All planned analyses could be carried out, and in addition to the initial hypotheses numerous innovations and new findings were obtained. Lipids are a major part (1-10%) of the total soil organic carbon of arable soils. Hence, they form an important part of the terrestrial carbon cycle. Soil lipids can be derived from several sources including plants, fungi, and bacteria as well as fossil sources like brown coal or mineral oil. As a result of distinctive lipid distribution patterns of the different sources, lipids are a suitable compound class for source apportionment in soils. In contrast to previous determinations on peaty, acidic soils, plant-derived lipids are less stable in arable soils, i.e. turnover times involve only a few decades. Lipids are less stable in soils than bulk carbon and provide a part of the intermediate stable carbon pool.

The main results of this study are as follows:

i. Long-chain carboxylic acids are suitable for the differentiation of C3- and C4-grasses. Grasses following different photosynthesis pathways and correspondingly their input to cropped soils can be differentiated using the carboxylic acid ratio (CAR = (n-C_{24} / (n-C_{22} + n-C_{26}) carboxylic acids)). Large CAR-values were found in C4-cropped soils and low CAR-values in C3-cropped soils. In combination with bulk carbon isotopy, the CAR can be used as a first approximation of C3- and C4-plant-derived carbon contents in soils. The newly established CAR still has to be verified for other C3- and C4-plants, which is subject of ongoing studies.

ii. On a long-term scale, i.e. over several decades or centuries, changes in atmospheric carbon, such as the recent increase in CO_2 and the corresponding carbon isotopic (^{13}C) depletion, as a result of fossil fuel burning (Suess effect), cause changes in plant biosynthates (e.g. Zhao *et al*., 2001) and thus in soils. As a consequence of the slow response of soil on atmospheric and plant modifications, changes during the next decades must be expected. Exact predictions of soil organic matter and carbon isotopic

developments, especially for agro-ecosystems, are very difficult, because e.g. deeper ploughing during several decades and low biomass input influence the soil carbon isotopic values more than the atmospheric-derived Suess effect. Nevertheless, modifications can be observed for Halle soils and expected for all soils as a result of atmospheric and plant changes. These may include variations in molecular composition or the local favorisation of plants following either the C3- or the C4-type photosynthesis metabolism. Analyses of time-series of archived arable soils from long-term field experiments with a well-documented cropping history, without crop management modifications would provide information, how soils reflect the atmospheric Suess effect.

iii. In arable soils, plant-derived lipids (alkanes and carboxylic acids) turn over faster than the bulk organic carbon, as derived from stable carbon isotope analyses ($\delta^{13}C$). This is most likely due to the fact that the bulk soil organic carbon consists of a mixture of more and less stable compounds of recent (e.g. plant-derived) and fossil (e.g. brown coal) origin, while only recent plant-derived lipids were chosen for turnover time determinations. Lipids are a part of the intermediate stable carbon pool in arable soils and turn over within several decades. As a new finding, lipid distribution patterns and isotopic compositions ($\delta^{13}C$) of different plant parts (roots, stems, leaves) are significantly different. Hence, the assessment of realistic biomass incorporation into soils, e.g. proportions and the compositions of roots, stems and leaves, is a prerequisite for a realistic turnover time determination of plant-derived lipids in soils.

iv. Even small contents of fossil carbon, e.g. from fossil fuel burning, cause very large apparent turnover times, based on radiocarbon analyses. Generally, alkanes were found to have an intermedeiate turnover time between fossil carbon, derived from fossil fuel burning (estimated from PAH data), and recent lipids like carboxylic acids, even in soils of rural regions. Hence, ^{13}C-turnover time determinated for alkanes must be regarded with care in all soils, even of rural sites, because contamination with fossil carbon often cannot be excluded. Contrastingly, carboxylic acids show shorter ^{14}C turnover times in the range of those of the bulk soils. Hence, carboxylic acids are best suitable for both, ^{13}C and ^{14}C turnover rate determinations of recent biomass. Lipids are part of the intermediate stable carbon pool with a turnover of several decades to centuries in arable soils. Previous observations made for lipids in peaty soils, with a turnover of several centuries to millennia, cannot be confirmed for lipids from arable soils of temperate climates.

v. Free extractable lipids represent 1-10%, averaging around 4% of the total soil organic carbon and thus comprise an important group of soil organic matter. Plant-derived lipids

are characterized by lowest turnover times followed by total lipids with intermediate turnover time and bulk soil organic carbon with the largest turnover times.

Summarizing, in agreement with Nieder & Richter (2000) sustainable management of arable soils over large areas can lead to an enrichment of soil carbon. Thus, a stabilization or even a reduction of atmospheric CO_2 over long-term periods as recommended by the Kyoto-Protocol (IGBP, 1998) can be realized, leading to an enhanced fixation of carbon in soils. Alternatively, a further depletion in carbon can be postulated for arable soils, as shown by Janssens *et al*. (2003).

Results and new findings obtained in this study offer an important basis for future studies on soil organic matter (SOM) dynamics, and may provide valuable tools for several other disciplines. Especially the newly introduced CAR can be useful for archaeological and paleoenvironmental analyses of sediments and soils, to reconstruct the cropping history or the general input of C3- and C4-crop biomass on a molecular level. Previous lipid turnover determinations were based exclusively on alkanes. The new results derived from ^{13}C and ^{14}C analyses of lipid fractions showed that carboxylic acids are suitable for turnover rate determinations of recent biomass, while alkanes are less suitable due to potential contamination by fossil carbon.

Numerous new questions arose from the results obtained in this study. First of all, it is unclear if the introduced CAR can be applied to other C3- and C4-crops, or even other grasses and if it offers any potential to generally differentiate between C3- and C4-plants. Long-term effects of the atmospheric Suess effect like carbon isotopic and budget changes in arable, forest and grassland soils need to be analysed from archived soils. In this study, analysed archived soils from the Halle site were not suitable due to fossil carbon contamination. Thus, changes in soil carbon isotope ratios induced by the atmospheric Suess effect seem to be possible, but were in the case of the Halle site not observed at a magnitude as expected. The effects of tillage and fertilization on lipid turnover in arable soils were not determined in this study. Thus, till and no-till soils as well as soils will different fertilizers still need to be compared. In comparison to tilled soils, no-till might lead to a long-term stabilization or even enrichment in soil lipid contents similar to carbon contents as observed by Balesdent *et al*. (2000). Finally, lipids were not found to remain stable in arable soils over periods of several hundred or thousand years, as previously determined for peaty, acidic soils (Bol *et al*., 1996). Thus, further research is necessary to identify those recalcitrant components in soils, which contribute to the stable soil organic carbon pool.

7. References

Accoe, F., Boeckx, P., van Cleemput, O., Hofman, G., Xu, H., Huang, B., Chen, G., 2002. Characterization of soil organic matter fraction from grassland and cultivated soils via C-content and $\delta^{13}C$ signature. *Rapid Communications in Mass Spectrometry* **16**, 2157-2164.

Almendros, G., Sanz, J., Velasco, F., 1996. Signatures of lipid assemblages in soils under continental Mediterranean forest. *European Journal of Soil Science* **47**, 183-196.

Amblès, A., Jambu, P., Jacquesy, J.-C., Parlanti, E., Secouet, B., 1993. Changes in the ketone portion of lipidic components during the decomposition of plant debris in a hydromorphic forest podsol. *Soil Science* **156**, 49-56.

Amblès, A., Jambu, P., Parlanti, E., Joffre, J., Riffe, C., 1994a. Incorporation of natural monoacids from plant residues into a hydromorphic forest podsol. *European Journal of Soil Science* **45**, 175-182.

Amblès, A., Parlanti, E., Jambu, P., Mayoungou, P., Jacquesy, J.-C., 1994b. *n*-Alkane oxidation in soil. Formation of internal monoalkenes. *Geoderma* **64**, 111-124.

Amelung, W., Flach, K.W., Zech, W., 1997. Climatic effects on soil organic matter composition in the Great Plains. *Soil Science Society of America Journal* **61**, 115-123.

Amthor, J.S., 1995. Terrestrial higher-plant response to increasing atmospheric CO_2 in relation to the gobal carbon cycle. *Global Change Biology* **1**, 243-274.

Anderson, E.L., 1988. Tillage and N fertilization effects on maize root growth and root:shoot ratio. *Plant and Soil* **108**, 245-251.

Arens, N.C., Jahre, A.H., Amundson, R., 2000. Can C_3 plants faithfully record the carbon isotopic composition of atmospheric carbon dioxide? *Paleobiology* **26**, 137-164.

Bakker, M.I., Casado, B., Koerselman, J.W., Tolls, J., Kollöffel, C., 2000. Polycyclic aromatic hydrocarbons in soil and plant samples from the vicinity of an oil refinery. *The Science of the Total Environment* **263**, 91-100.

Balabane, M., Balesdent J., 1992. Input of fertilizer-derived labelled N to soil organic matter during a growing season of maize in the field. *Soil Biology and Biochemistry* **24**, 89-96.

Balesdent, J., 1987. The turnover of soil organic fractions estimated by radiocarbon dating. *The Science of the Total Environment* **62**, 405-408.

Balesdent, J., Mariotti, A., 1996. Measurement of soil organic matter turnover using ^{13}C-natural abundance. In: *Mass Spectrometry of Soils* (eds. Boutton, T.W., Yamasaki, S.), pp. 83-111. Marcel Dekker, New York.

Balesdent, J., Mariotti, A., Guillet, B., 1987. Natural ^{13}C abundance as a tracer for studies of soil organic matter dynamics. *Soil Biology and Biochemistry* **19**, 25-30.

Balesdent, J., Wagner, G.H., Mariotti, A., 1988. Soil organic matter turnover in long-term field experiments as revealed by carbon-13 natural abundance. *Soil Science Society of America Journal* **52**, 118-124.

Balesdent, J., Chenu, C., Balabane, M., 2000. Relationship of soil organic matter dynamics to physical protection and tillage. *Soil and Tillage Research* **53**, 215-230.

Ballentine, D.C., Macko, S.A., Turekian, V.C., 1998. Variability of stable carbon isotopic compositions in individual fatty acids from combustion of C4 and C3 plants: implications for biomass burning. *Chemical Geology* **152**, 151-161.

Berset, J.D., Ejem, M., Holzer, R., Lischer, P., 1999. Comparison of different drying, extraction and detection techniques for the determination of priority polycyclic aromatic hydrocarbons in background contaminated soil samples. *Analytica Chimica Acta* **383**, 263-275.

Batjes, N.H., 1996. Total carbon and nitrogen in the soils of the world. *European Journal of Soil Science* **47**, 151-163.

Bianchi, G., 1994. Plant Waxes. In: *Waxes: Chemistry, Molecular Biology and Functions* (ed. Hamilton, R.J.), pp. 175-222. The Oily Press, Dundee.

Bianchi, A., Bianchi, G., 1990. Surface lipid composition of C3 and C4 plants. *Biochemical Systematics and Ecology* **18**, 533-537.

Bianchi, G., Corbellini, M., 1977. Epicuticular wax of *Triticum aestivum* Demar 4. *Phytochemistry* **16**, 943-945.

Bianchi, G., Avato, P., Salamini, F., 1982. Epicuticular waxes of albino maize. *Phytochemistry* **21**, 129-131.

Bird, M.I., Pousai, P., 1997. Variations of $\delta^{13}C$ in the surface soil organic carbon pool. *Global Biogeochemical Cycles* **11**, 313-322.

Bird, M., Kracht, O., Derrien, D., Zhou, Y., 2003. The effect of soil texture and roots on the stable carbon isotope composition of soil organic carbon. *Australian Journal of Soil Research* **41**, 77-94.

Boeschker, H.T.S., Middelburg, J.J., 2002. Stable isotopes and biomarkers in microbial ecology. *FEMS Microbiology Ecology* **40**, 85-95.

Bol, R., Huang, Y., Meridith, J.A., Eglinton, G., Harkness, D.D., Ineson, P., 1996. The ^{14}C age and residence time of organic matter and its lipid constituents in a stagnohumic gley soil. *European Journal of Soil Science* **47**, 215-222.

Bolinder, M.A., Angers, D.A., Dubuc, J.P., 1997. Estimating shoot to root ratios and annual carbon inputs in soils for cereal crops. *Agriculture, Ecosystems and Environment* **63**, 61-66.

Bonkowski, M., 2004. Protozoa and plant growth: the microbial loop and soil revisited. *New Phytologist* **162**, 617-631.

Boom, A., Marchant, R., Hooghiemstra, H., Sinninghe Damsté, J.S., 2002. CO_2- and temperature-controlled altitudinal shifts of C4- and C3-dominated grasslands allow reconstruction of paleoatmospheric pCO_2. *Palaeogeography, Palaeoclimatology, Palaeoecology* **177**, 151-168.

Bossio, D.A., Scow, K.M., Gunapala, N., Graham, K.J., 1998. Determinants of soil microbial communities: Effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. *Microbial Ecology* **36**, 1-12.

Boutton, T.H., Archer, S.R., Midwood, A.J., Zitzer, S.F., Bol, R., 1998. $\delta^{13}C$ values of soil organic carbon and their use in documenting vegetation change in a subtropical savanna ecosystem. *Geoderma* **82**, 5-41.

Bull, I.D., van Bergen, P.F., Poulton, P.R., Evershed, R.P., 1998. Organic geochemical studies of soils from Rothamsted Classical Experiments – II. Soils from the Hoosfield, Spring Barley Experiment treated with different quantities of manure. *Organic Geochemistry* **28**, 11-26.

Bull, I.D., van Bergen, P.F., Nott, C.J., Poulton, P.R., Evershed, R.P., 2000. Organic geochemical studies of soils from Rothamsted Classical Experiments – V. The fate of lipids in different long-term experiments. *Organic Geochemistry* **31**, 389-408.

Cayet, C., Lichtfouse, E., 2001. $\delta^{13}C$ of plant-derived *n*-alkanes in soil particle-size fractions. *Organic Geochemistry* **32**, 253-258.

Chikaraishi, Y., Naraoka, H., 2003. Compound-specific δD-$\delta^{13}C$ analyses of *n*-alkanes extracted from terrestrial and aquatic plants. *Phytochemistry* **63**, 361-371.

Chikaraishi, Y., Naraoka, H., Poulson, S.R., 2004. Hydrogen and carbon isotopic fractionations of lipid biosynthesis among terrestrial (C3, C4 and CAM) and aquatic plants. *Phytochemistry* **65**, 1369-1381.

Christensen, B.T., 1996. Carbon in primary and secondary organomineral complexes. In: *Advances in soil science: Structure and organic matter in agricultural soils* (eds. Carter, M.R., Stewart, B.A.), pp. 97-165. CRC, Boca Raton.

Collins, H.P., Blevins, R.L., Bundy, R.G., Christenson, D.R, Dick, W.A., Huggins, D.R., Paul, E.A., 1999. Soil carbon dynamics in corn-based agroecosystems : Results from carbon-13 natural abundance. *Soil Science Society of America Journal* **63**, 584-591.

Collister, J.M., Rieley, G., Stern, B., Eglinton, G., Fry, B., 1994. Compound-specific $\delta^{13}C$ analyses of leaf lipids from plants with differing carbon dioxide mechanisms. *Organic Geochemistry* **21**, 619-627.

Conte, M.H., Weber, J.C., Carlson, P.J., Flanagan, L.B., 2003. Molecular and carbon isotopic composition of leaf wax in vegetation and aerosols in a northern prairie ecosystem. *Oecologia* **135**, 67-77.

Dean, J.R., Xiong, G., 2000. Extraction of organic pollutants from environmental matrices: selection of extraction technique. *Trends in Analytical Chemistry* **19**, 553-564.

Desjardins, T., Andreux, F., Volkoff, B., Cerri, C.C., 1994. Organic carbon and ^{13}C contents in soils and soil size-fractions, and their changes due to deforestation and pasture installation in eastern Amazonia. *Geoderma* **61**, 103-118.

Dinel, H., Schnitzer, M, Mehuys, G.R., 1990. Soil lipids: origin, nature, contents, decomposition and effect on soil physical properties. In: *Soil Biochemistry, Vol.6* (eds. Bollag, J.M., Stotzky, G.), pp. 397-427. Marcel Dekker, New York.

Eglinton, G., Gonzalez, A.G., Hamilton, R.J., Raphael, R.A., 1962. Hydrocarbon constituents of the wax coatings of plant leaves: a taxonomic survey. *Phytochemistry* **1**, 89-102.

Eglinton, T.I., Aluwihare, L.I., Bauer, J.E., Druffel, E.R.M., McNichol, A.P., 1996. Gas chromatographic isolation of individual compounds from complex matrices for radiocarbon dating. *Analytical Chemistry* **68**, 904-912.

Eglinton, T.I., Benitez-Nelson, B.C., Pearson, A., McNichol, A.P., Bauer, J.E., Druffel, E.R.M., 1997. Variability in radiocarbon ages of individual organic compounds from marine sediments. *Science* **277**, 796-799.

Ehleringer, J.R., Cerling, T.E., 1995. Atmospheric CO_2 and the ratio of intercellular to ambient CO_2 concentrations in plants. *Tree Physiology* **15**, 105-111.

FAO-UNESCO, 1994. *Soil Map of the World, Revised Legend*. FAO, Rome.

Feng, X., 1998. Long-term c_i/c_a response of trees in western North America to atmospheric CO_2 concentration derived from carbon isotope chronologies. *Oecologia* **117**, 19-25.

Fernandez, I., Mahieu, N., Cadisch, G., 2003. Carbon isotopic fractionation during decomposition of plant materials of different quality. *Global Biogeochemical Cycles* **17**, 1075.

Flessa, H., Ludwig, B., Heil, B., Merbach, W., 2000. The origin of soil organic C, dissolved organic C and respiration in a long-term maize experiment in Halle, Germany, determined by ^{13}C natural abundance. *Journal of Plant Nutrition and Soil Science* **163**, 157-163.

Fortuna, A., Harwood, R.R., Paul, E.A., 2003. The effects of compost and crop rotations on carbon turnover and the particulate organic matter fraction. *Soil Science* **168**, 434-444.

Freeman, K.H., 2001. Isotope biogeochemistry of marine organic carbon. In: *Stable isotope geochemistry, Reviews in Mineralogy & Geochemistry, Vol. 43* (eds. Valley, J.M., Cole, D.R.), pp. 579-605. Mineralogical Society of America and Geochemical Society of America.

Friedli, H., Lötscher, H., Oeschger, H., Siegenthaler U., Stauffer, B., 1986. Ice core record of the $^{13}C/^{12}C$ ratio of atmospheric CO_2 in the past two centuries. *Nature* **324**, 237-238.

Gleixner, G., Czimczik, C.J., Kramer, C., Lühker, B., Schmidt, M.W.I., 2001. Plant compounds and their turnover and stabilization as soil organic matter. In: *Global Biogeochemical Cycles in the Climate System* (eds. Schulze, E.-D., Heimann, M., Harrison, S., Holland, E., Lloyd, J., Prentice, I.C., Schimel, D.), pp. 201-215. Academic Press, San Diego.

Gobé, V., Lemée, L., Amblès, A., 2000. Structure elucidation of soil macromolecular lipids by preparative pyrolysis and thermochemolysis. *Organic Geochemistry* **31**, 409-419.

Gregorich, E.G., Ellert, B.H., Drury, C.F., Liang, B.C., 1996a. Fertilization effects on soil organic matter turnover and corn residue C storage. *Soil Science Society of America Journal* **60**, 472-476.

Gregorich, E.G., Monreal, C.M., Schnitzer, M., Schulten, H.-R., 1996b. Transformation of plant residues into soil organic matter: Chemical characterization of plant tissue, isolated soil fractions, and whole soils. *Soil Science* **161**, 680-693.

Guggenberger, G., Zech, W., Thomas, R.J., 1995. Lignin and carbohydrate alteration in particle-size separates of an oxisol under tropical pastures following native savanna. *Soil Biology and Biochemistry* **27**, 1629-1638.

Guil-Guerrero, J.L., Rodríguez-García, I., 1999. Lipids classes, fatty acids and carotenes of the leaves of six edible wild plants. *European Food Research Technology* **209**, 313-316.

Harwood, J.L., Russell, N.J., 1984. *Lipids in plants and microbes*. George Allen & Unwin, London.

Hayes, J.M., 1993. Factors controlling ^{13}C contents of sedimentary organic compounds: Principles and evidence. *Marine Geology* **113**, 111-125.

Hayes, J.M., 2001. Fractionation of carbon and hydrogen isotopes in biosynthetic processes. In: *Stable isotope geochemistry, Reviews in Mineralogy & Geochemistry, Vol. 43* (eds. Valley, J.M., Cole, D.R.), pp. 225-278. Mineralogical Society of America, Geochemical Society of America.

Hedges, J.I., 1991. Lignin, cutin, amino acid and carbohydrate analysis of marine particulate organic matter. In: *Marine Particles: Analysis and Characterization, Geophysical Monographs, Vol. 63* (eds. Hurd, D.C., Spencer, D.W.), pp. 129-137. American Geophysical Union, Washington.

Henderson, S.A., von Caemmerer, S., Farquhar, G.D., 1992. Short-term measurements of carbon isotope discrimination in several C_4 species. *Australian Journal of Plant Physiology* **19**, 263-285.

Hobbie, E.A., Werner, R.A., 2004. Intramolecular compound-specific, and bulk carbon isotope patterns in C3 and C4 plants: a review and synthesis. *New Phytologist* **161**, 371-385.

Hubert, A., Wenzel, K.-D., Manz, M., Weissflog, L., Engewald, W., Schüürmann, G., 2000. High extraction efficiency for POPs in real contaminated soil samples using accelerated solvent extraction. *Analytical Chemistry* **72**, 1294-1300.

Huggins, D.R., Clapp, C.E., Allmaras, R.R., Lamb, J.A., Layese, M.F., 1998. Carbon dynamics in corn-soybean sequences as estimated from natural carbon-13 abundance. *Soil Science Society of America Journal* **62**, 195-203.

Idso, C.D., Idso, S.B., Balling Jr., R.C., 2001. An intensive two-week study of an urban CO_2 dome in Phoenix, Arizona, USA. *Atmospheric Environment* **35**, 995-1000.

Idso, S.B., Idso, C.D., Balling Jr., R.C., 2002. Seasonal and diurnal variations of near-surface atmospheric CO_2 concentration within a residential sector of the urban CO_2 dome of Phoenix, AZ, USA. *Atmospheric Environment* **36**, 1655-1660.

IGBP, 1998. The terrestrial carbon cycle: Implications for the Kyoto Protocol. *Science* **280**, 1393-1394.

Jambu, P., Amblès, A., Dinel, H., Secouet, B., 1991. Incorporation of natural hydrocarbons from plant residues into a hydromorphic humic podsol following afforestation and fertilization. *Journal of Soil Science* **42**, 629-636.

Jambu, P., Amblès, A., Jaquesy, J-C., Secouet, B., Parlanti, E., 1993. Incorporation of natural alcohols from plant residues into a hydromorphic forest-podsol. *Journal of Soil Science* **44**, 135-146.

Janssens, I.A., Freibauer, A., Ciais, P., Smith, P., Nabuurs, G.-J., Folberth, G., Schlamadinger, B., Hutjes, R.W.A., Ceulemans, R., Schulze, E.-D., Valentini, R., Dolman, A.J., 2003. Europe´s terrestrial biosphere absobs 7 to 12% of European anthropogenic CO_2 emissions. *Science* **300**, 1538-1542.

Jenkinson, D.S., Rayner, J.H., 1977. The turnover of soil organic matter in some of the Rothamsted classical experiments. *Soil Science* **123**, 298-305.

John, B., Ludwig, B., Flessa, H., 2001. Stabilisierung von maisbürtigem Kohlenstoff in Abhängigkeit der Korngrößenfraktionen im Dauerversuch “Ewiger Roggenbau”. *Mitteilungen der Deutschen Bodenkundlichen Gesellschaft* **96**, 209-210.

John, B., Yamashita, T., Ludwig, B., Flessa, H., 2004. Storage of organic carbon in aggregate and density fractions of silty soils under different types of land use. *Geoderma*, submitted.

Kögel-Knabner, I., 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. *Soil Biology and Biochemistry* **34**, 139-162.

Körschens, M., Pfefferkorn, A., 1998. *Bad Lauchstätt: The static fertilization experiment and other long-term field experiments.* Umweltforschungszentrum Leipzig-Halle, Leipzig.

Kolattukudy, P.E., Croteau, R., Buckner, J.S., 1976. Biochemistry of plant waxes. In: *Chemistry and biochemistry of natural waxes* (ed. Kolattukudy P.E.), pp. 290-347. Elsevier, Amsterdam.

Krauss, M., Wilcke, W., Zech, W., 2000. Polycyclic aromatic hydrocarbons and polychlorinated biphenyls in forest soils: depth distribution as indicator of different fate. *Environmental Pollution* **110**, 79-88.

Kristiansen, S.M., Hansen, E.M., Jensen, L.S., Christensen, B.T., 2004. Natural ^{13}C abundance and carbon storage in Danish soils under continuous silage maize. *European Journal of Agronomy* **22**, 107-117.

Krull, E.S., Skjemstad, J.O., 2003. $\delta^{13}C$ and $\delta^{15}N$ in ^{14}C-dated Oxisols and Vertisols as a function of soil chemistry and mineralogy. *Geoderma* **112**, 1-29.

Kuzyakov, Y.V., 2001. Tracer studies of carbon translocation by plants from the atmosphere into the soil. *Eurasian Soil Science* **34**, 36-51.

Liang, B.C., Gregorich, E.G., MacKenzie, A.F., Schnitzer, M., Voroney, R.P., Monreal, C.M., Beyaert, R.P., 1998. Retention and turnover of corn residue carbon in some eastern Canadian soils. *Soil Science Society of America Journal* **62**, 1361-1366.

Lichtfouse, E., 1997. Heterogeneous turnover of molecular organic substances from crop soils as revealed by ^{13}C labelling at natural abundance with *Zea mays*. *Naturwissenschaften* **84**, 23-25.

Lichtfouse, E., 1998. Isotope and biosynthetic evidence for the origin of long-chain aliphatic lipids in soils. *Naturwissenschaften* **85**, 76-77.

Lichtfouse, E., Budzinski, H., 1995. ^{13}C analysis of molecular organic substances, a novel breakthrough in analytical sciences. *Analusis* **23**, 364-369.

Lichtfouse, E., Elbisser, B., Balesdent, J., Mariotti, A., Bardoux, G., 1994. Isotope and molecular evidence for direct input of maize leaf wax *n*-alkanes into crop soils. *Organic Geochemistry* **22**, 349-351.

Lichtfouse, E., Berthier, G., Houot, S., Barriuso, E., Bergheaud, V., Vallaeys, T., 1995a. Stable carbon isotope evidence for the microbial origin of C_{14}-C_{18} *n*-alkanoic acids in soils. *Organic Geochemistry* **23**, 849-852.

Lichtfouse, E., Dou, S., Girardin, C., Grably, M., Balesdent, J., Behar, F., Vandenbroucke, M., 1995b. Unexpected ^{13}C-enrichment of organic components from wheat crop soils: evidence for the in situ origin of soil organic matter. *Organic Geochemistry* **23**, 865-868.

Lichtfouse, E., Bardoux, G., Mariotti, A., Balesdent, J., Ballentine, D.C., Macko, S.A., 1997a. Molecular, ^{13}C and ^{14}C evidence for the allochthonous and ancient origin of C_{16}-C_{18} *n*-alkanes in modern soils. *Geochimica et Comsochimica Acta* **61**, 1891-1898.

Lichtfouse, E., Budzinski, H., Garrigues, P., Eglinton, T.I., 1997b. Ancient polycyclic aromatic hydrocarbons in modern soils: ^{13}C, ^{14}C and biomarker evidence. *Organic Geochemistry* **26**, 353-359.

Lichtfouse, E., Wehrung, P., Albrecht, P., 1998. Plant wax *n*-alkanes trapped in soil humin by noncovalent bonds. *Naturwissenschaften* **85**, 449-452.

Lockheart, M.J., van Bergen, P.F., Evershed, R.P., 1997. Variations in the stable carbon isotope compositions of individual lipids from the leaves of modern angiosperms: implications for the study of higher land plant-derived sedimentary organic matter. *Organic Geochemistry* **26**, 137-153.

Ludwig, B., John, B., Ellerbrock, R., Kaiser, M., Flessa, H., 2003. Stabilization of carbon from maize in a sandy soil in a long-term experiment. *European Journal of Soil Science* **54**, 117-126.

Lüniger, G., 2002. Chemofazies der oligozänen Schwarzpelite von Enspel (Westerwald, Rheinland-Pfalz). Universität zu Köln, Köln.

Marino, B.D., McElroy, M.B., 1991. Isotopic composition of atmospheric CO_2 inferred from carbon in C4 plant cellulose. *Nature* **349**, 127-131.

Marseille, F., Disnar, J.R., Guillet, B., Noack, Y., 1999. *N*-alkanes and free fatty acids in humus and A1 horizons of soils under beech, spruce and grass in the Massif-Central (Mont-Lozère), France. *European Journal of Soil Science* **50**, 433-441.

Merbach, W., Schmidt, L., Wittenmayer, L., 1999. *Die Dauerdüngungsversuche in Halle (Saale).* B.G. Teubner, Stuttgart.

Merbach, W., Garz, J., Schliephake, W., Stumpe, H., Schmidt, L., 2000. The long-term fertilization experiments in Halle (Saale), Germany – Introduction and survey. *Journal of Plant Nutrition and Soil Science* **163**, 629-638.

Moucawi, J., Fustec, E., Jambu, P., Jacquesy, R., 1981. Decomposition of lipids in soils: Free and esterified fatty acids, alcohols and ketones. *Soil Biology and Biochemistry* **13**, 461-468.

Nadeau, M.-J., Schleicher, M., Grootes, P.M., Erlenkeuser, H., Gottdang, A., Mous, D.J.W., Sarnthein, J.M., Willkomm, H., 1997. The Leibniz-Labor AMS facility at the Christian-Albrechts-University, Kiel, Germany. *Nuclear Instruments and Methods in Physics Research* **B123**, 22-30.

Nadeau, M.-J., Grootes, P.M., Schleicher, M., Hasselberg, P., Rieck, A., Bitterling, M., 1998. Sample throughput and data quality at the Leibniz-Labor AMS facility. *Radiocarbon* **40**, 239-245.

Nguyen Tu, T.T., Derenne, S., Largeau, C., Bardoux, G., Mariotti, A., 2004. Diagenesis effects on specific carbon isotope composition of plant *n*-alkanes. *Organic Geochemistry* **35**, 317-329.

Nieder, R., Richter, J., 2000. C and N accumulation in arable soils of West Germany and its influence on the environment – Developments 1970-1998. *Journal of Plant Nutrition and Soil Science* **163**, 65-72.

Nierop, K.G.J., 1998. Origin of aliphatic compounds in a forest soil. *Organic Geochemistry* **29**, 1009-1016.

Nierop, K.G.J., Pulleman, M.M., Marinissen, J.C.Y., 2001. Management induced organic matter differentiation in grassland and arable soil: a study using pyrolysis techniques. *Soil Biology and Biochemistry* **33**, 2001.

O´Brien, B.J., 1986. The use of natural and anthropogenic ^{14}C to investigate the dynamics of soil organic carbon. *Radiocarbon* **28**, 358-362.

O´Leary, M.H., 1981. Carbon isotope fractionation in plants. *Phytochemistry* **20**, 553-567.

Paul, E.A., Morris, J.S., Boehm, S., 2001. The determination of soil C pool sizes and turnover rates: biophysical fractionation and tracers. In: *Assessment method for soil C pools* (eds. Lal, R., Kimble, J.M., Follett, R.F., Stewart, B.A.), pp. 193-206. CRC Press, Boca Raton.

Peters, K.E., Moldowan, J.M., 1993. *The Biomarker Guide*. Prentice Hall, Englewood Cliffs.

Pörschmann, J., Plugge, J., Toth, R., 2001. In situ derivatization using pressurised liquid extraction to determine phenols, sterols and carboxylic acids in environmental samples and microbial biomasses. *Journal of Chromatography A* **909**, 95-109.

Prasad, R.B.N., Gülz, P.-G., 1990. Development and seasonal variations in the epicuticular waxes of beech leaves (*Fagus sylvatica* L.). *Zeitschrift für Naturforschung* **45c**, 805-812.

Prentice, I.C., Farquhar, G.D., Fasham, M.J.R., Goulden, M.L., Heimann, M., Jaramillo, V.J., Kheshgi, K.H., Le Quéré, C., Scholes, R.J., Wallace, D.W.R., 2001. The carbon cycle and atmospheric carbon dioxide. In: *Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel of Climate Change* (eds. Houghton, J.T., Ding, Y., Griggs, D.J., Noguer, M., van der Linden, P. J., Dai, X., Maskell, K., Johnson, C.A.), pp. 183-238. Cambridge University Press, Cambridge.

Puget, P., Chenu, C., Balesdent, J., 1995. Total and young organic matter distributions in aggregates of silty cultivated soils. *European Journal of Soil Science* **46**, 449-459.

Radke, M., Willsch, H., Welte, D.H., 1980. Preparative hydrocarbon group type determination by automated medium pressure liquid chromatography. *Analytical Chemistry* **52**, 406-411.

Read, D.B., Bengough, A.G., Gregory, P.J., Crawford, J.W., Robinson, D., Scrimgeour, C.M., Young, I.M., Zhang, K., Zhang, X., 2003. Plant roots release phospholipid surfactants that modify the physical and chemical properties of soil. *New Phytologist* **157**, 315-326.

Rethemeyer, J., Bruhn, F., P.M. Grootes, 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**, 267-268.

Rethemeyer, J., Kramer, C., Gleixner, G., Wiesenberg, G.L.B., Schwark, L., Andersen, N., Nadeau, M.-J., Grootes, P.M., 2004a. Complexity of soil organic matter: AMS ^{14}C analysis of soil lipid fractions and individual compounds. *Radiocarbon* **46**, 465-473.

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

Ries-Kautt, M., Albrecht, P., 1989. Hopane-derived triterpenoids in soils. *Chemical Geology* **76**, 143-151.

Rumpel, C., Balesdent, J., Grootes, P., Weber, E., Kögel-Knabner, I., 2003. Quantification of lignite- and vegetation-derived soil carbon using ^{14}C activity measurements in a forested chronosequence. *Geoderma* **112**, 155-166.

Scharpenseel, H.W., Becker-Heidmann, P., 1992. Twenty-five years of radiocarbon dating soils: Paradigm of erring and learning. *Radiocarbon* **34**, 541-549.

Schmidt, M.W.I., 1998. *Organic matter in natural soils and in soils contaminated by atmospheric organic particles from coal processing industries.* Shaker Verlag, Aachen.

Schmidt, L., Warnstorff, K., Dörfel, H., Leinweber, P., Lange, H., Merbach, W., 2000. The influence of fertilization and rotation on soil organic matter and plant yields in the long-term *Eternal Rye* trial in Halle (Saale), Germany. *Journal of Plant Nutrition Soil Science* **163**, 639-648.

Schulze, D.G., Nagel, J.L., Van Scoyoc, G.E., Henderson, T.L., Baumgardner, M.F., Stott, D.E., 1993. Significance of organic matter in determining soil colors. In: *Soil Color* (eds. Bigham, J.M., Ciolkosz, E.J.), pp. 71-90. Soil Science Society of America, Madison.

Stevenson, F.J., 1994. *Humus Chemistry: Genesis, Composition, Reactions, 2nd edition*. John Wiley, New York.

Stuiver, M., Polach, H.A., 1977. Discussion reporting of ^{14}C data. *Radiocarbon* **22**, 1-24.

Stumpe, H., Garz, J., Hagedorn, E., 1990. Die Dauerdüngungsversuche auf dem Versuchsfeld in Halle. In: *Dauerfeldversuche* (ed. Körschens, M.), pp. 25-71. Akademie der Landwirtschaftswissenschaften, Berlin.

Torn, M.S., Lapenis, A.G., Timofeev, A., Fischer, M.L., Babikov, B.V., Harden, J.W., 2002. Organic carbon and carbon isotopes in modern and 100-year-old-soil archives of the Russian steppe. *Global Change Biology* **8**, 941-953.

Trumbore, S.E., 1993. Comparison of carbon dynamics in tropical and temperate soils using radiocarbon measurement. *Global Biogeochemical Cycles* **7**, 275-290.

Trumbore, S.E., 1996. Applications of accelerator mass spectrometry to soil science. In: *Mass spectrometry of soils* (eds. Boutton, T.W., Yamasaki, S.), pp. 311-340. Marcel Dekker, New York.

Trumbore, S.E., Zheng, S., 1996. Comparison of fractionation methods for soil organic matter ^{14}C analysis. *Radiocarbon* **38**, 219-229.

Trumbore, S.E., Bonani, G., Wölfli, W., 1990. The rates of carbon cycling in several soils from AMS ^{14}C measurements of fractionated soil organic matter. In: *Soils and the Greenhouse Effect* (ed. Bouwman, A.F.), pp. 407-414. John Wiley, New York.

Tulloch, A.P., Hoffman, L.L., 1971. Leaf wax of durum wheat. *Phytochemistry* **10**, 871-876.

Tulloch, A.P., Hoffman, L.L., 1973. Leaf wax of *Triticum aestivum. Phytochemistry* **12**, 2217-2223.

Uchida, M., Shibata, Y., Kawamura, K., Yoneda, M., Mukai, H., Tanaka, A., Uehiro, T., Morita, M., 2000. Isolation of individual fatty acids in sediments using preparative capillary gas chromatography (PCGC) for radiocarbon analysis at NIES-TERRA. *Nuclear Instruments and Methods* **B172**, 583-588.

van Bergen, P.F., Bull, I.D., Poulton, P.R., Evershed, R.P., 1997. Organic geochemical studies of soil from the Rothamsted Classical Experiments – I. Total lipid extracts,

solvent insoluble residues and humic acids from Broadbalk Wilderness. *Organic Geochemistry* **26**, 117-135.

van Bergen, P.F., Nott, C.J., Bull, I.D., Poulton, P.R., Evershed, R.P., 1998. Organic geochemical studies of soils from Rothamsted Classical Experiments – IV. Preliminary results from a study of the effect of soil pH on organic matter decay. *Organic Geochemistry* **29**, 1779-1795.

Wedin, D.A., Tieszen, L.L., Dewey, B., Pastor, J., 1995. Carbon isotope dynamics during grass decomposition and soil organic matter formation. *Ecology* **76**, 1383-1392.

Whelan, T., Sackett, W.M., Benedict, C.R., 1973. Enzymatic fractionation of carbon isotopes by phosphoenolpyruvate carboxylase from C_4 plants. Plant Physiology **51**, 1051-1054.

Wiesenberg, G.L.B., Schwark, L., Schmidt, M.W.I., 2004a. Improved automated extraction and separation procedure for soil lipid analyses. *European Journal of Soil Science* **55**, 349-356.

Wiesenberg, G.L.B, Schwarzbauer, J., Schmidt, M.W.I., Schwark, L., 2004b. Sources and turnover of organic matter in agricultural soils derived from *n*-alkane/*n*-carboxylic acid compositions and C-isotope signatures. *Organic Geochemistry* **35***,* 1371-1393.

Wiesenberg, G.L.B., Schwark, L., Schmidt, M.W.I., 2004c. Extractable lipid contents and colour in particle-size separates and bulk arable soils. *European Journal of Soil Science*, submitted.

Wiesenberg, G.L.B., Schmidt, M.W.I., Schwark, L., in preparation. Distribution patterns of alkanes and carboxylic acids in particle-size separates and bulk arable soils.

Willsch, H., Clegg, H., Horsfield, B., Radke, M. Wilkes, H., 1997. Liquid chromatographic separation of sediment, rock, and coal extracts and crude oil into compound classes. *Analytical Chemistry* **69**, 4203-4209.

Winkler, F.J., Wirth, E., Latzko, E., Schmidt, H.-L., Hoppe, W., Wimmer, P., 1978. Influence of growth conditions and development on $\delta^{13}C$ values in different organs and constituents of wheat, oat and maize. *Zeitschrift für Pflanzenphysiologie* **87**, 255-263.

Yoneyama, T., Handley, L.L., Scrimgeour, C.M., Fisher, D.B., Raven, J.A., 1997. Variations of the natural abundances of nitrogen and carbon isotopes in *Triticum aestivum*, with special reference to phloem and xylem exudates. *New Phytologist* **137**, 205-213.

Zhao, F.-J., Spiro, B., McGrath, S.P., 2001. Trends in $^{13}C/^{12}C$ ratios and C isotope discrimination of wheat since 1845. *Oecologia* **128**, 336-342.

8. Appendix

A: Index of figures
B: Index of tables
C: List of abbreviations
D: Acknowledgements
E: Erklärung
F: Lebenslauf

A: Index of figures

B: Index of tables

C: List of abbreviations

AMS	Accelerator mass-spectrometry
AR	Alkane ratio
ASE	Accelerated solvent extraction
BSTFA	N,O-bis(trimethylsilyl)trifluoracetamide
C	Carbon
C3-plants	Plants following the Hatch/Slack-photosynthesis metabolism (e.g. rye, wheat)
C4-plants	Plants following the C4-photosynthesis metabolism (e.g. maize)
CAR	Carboxylic acid ratio
CSIA	Compound-specific isotope analyses
DCM	Dichloromethane
GC-FID	Gas-chromatography
GCirmMS	Gas-chromatography isotope ratio monitoring mass-spectrometry
GC/MS	Gas-chromatography/mass-spectrometry
HMW	High molecular weight
H-MPLC	Hetero-compound medium-pressure liquid chromatography
k	Decomposition rate
Maize-C	Maize-derived carbon
MeOH	Methanol
MPLC	Medium-pressure liquid chromatography
MRT	Mean residence time
MS	Mass-spectrometer
N	Nitrogen
NPK	Nitrogen, phosphorous, potassium fertilizer
PAH	Polycyclic aromatic hydrocarbons
PBD	PeeDee Belemnite
Ph	Phytane
pMC	Percent modern carbon
Pr	Pristane
RTM	Rotthalmünster
SOC	Soil organic carbon
SOM	Soil organic matter
S:R-ratio	Shoot to root ratio
TC	Total carbon content
TN	Total nitrogen content
TOC	Total organic carbon content

D: Acknowledgements

Many people provided excellent encouragement in supporting my studies on several ways. Huge support was performed e.g. in solving any kinds of problems, giving thoughtful comments, preparing and carrying out measurements as well as supporting financially and mentally. Among these I am especially grateful to

HD. Dr. Lorenz Schwark (University of Cologne) for the suggestion of the interesting topic, joint field work in Halle, thoughtful discussions and continuous help while preparing papers and this thesis.

Prof. Dr. Michael W.I. Schmidt (University of Zurich) for the suggestion of the interesting topic, introduction into soil science, didactical instructions, establishing contacts with interesting discussants, helpful comments, providing samples and continuous support.

Prof. Dr. Ulrich Radtke (University of Cologne) for reviewing this thesis and very helpful comments.

Prof. Dr. Wolfgang Merbach, Dr. Lothar Schmidt (University of Halle/Wittenberg) for the possibility to sample 'Eternal Rye' trial, for preparing and sending numerous soil as well as plant samples and for the establishment of a grain-maize trial after our stimulus.

Mr. Schnellhammer (Staatliche Höhere Lanbauschule Rotthalmünster) for the possibility to sample the long-term agricultural plots, for preparing and sending of numerous plant samples

Dr. Jan Schwarzbauer (RWTH Aachen) for the possibility to use the GCirmMS and for helpful comments in interpreting the generated data.

Janet Rethemeyer and Dr. Peter Grootes (University Kiel) for carrying out and supporting ^{14}C measurements and constructive discussions on the '^{14}C in soils' problem.

Members of the DFG-SPP 1090 for helpful, interesting discussions as well as excellent loyalty during numerous meetings and sampling at Rotthalmünster site.

Kay Scheffler (University Bonn), Silvia Strecker (University of Cologne), and Dr. Stefano Bernasconi for the bulk ^{13}C-analyses of numerous samples.

Udo Keldenich (GEW Rheinenergy Cologne) for derivatising numerous carboxylic acid samples.

Prof. Dr. Heiner Flessa (University Göttingen) for intensive discussions, extensive comparison of data and the possibility to present results at the University Göttingen.

Deutsche Forschungsgemeinschaft (DFG) for funding the project within the priority program (SPP) 1090 "Soils as sources and sinks for CO_2".

The staff of the Geological and Mineralogical Institute of the University of Cologne for continuous help and support.

Hanna Cieszynski (University of Cologne) for excellent laboratory assistance and keeping the laboratory running.

Daniela Garding, Yassin Hardi, Bianca Stapper (University of Cologne) for caring for the GC and GC/MS instruments.

Thorsten Bauersachs, Eva Lehndorff, Birgit Nabbefeld, Oliver Paech and Alexandra Richter (University of Cologne) for laboratory assistance as preparing and measuring samples with e.g. H-MPLC, MPLC, elemental analyser.

Olaf Butenschön (University Darmstadt), Ute Hamer (University Bochum), Bettina John (University Göttingen), Michael Kaiser (University Müncheberg), Christiane Kramer (Max-Planck-Institute Jena), Sven Marhan (University Darmstadt), Janet Rethemeyer (University Kiel), Ariane Serafin (University Hamburg) and many other Ph.D. students of the DFG-SPP 1090 for cooperations within the SPP and unforgettable, constructive late-night discussions during several meetings.

Nicole Juraschek and Eva Lehndorff (University of Cologne) for a lot of intensive discussions during entertaining coffee and tea breaks, intensive reviewing of the manuscript of this thesis, numerous helpful comments and encouraging promises (e.g. ’männlicher Schwan’, ’gedrungenes Pferd’... – to be continued).

Ingrid and Jochen Wiesenberg for numerous and especially mental as well as financial support during the last three decades and for helpful instructions to find and keep the right way.

Finally, I want to thank my wife Simone Wiesenberg for continuous support in many ways, loving me and giving me new power, day by day.

E: Erklärung

Ich versichere, daß ich die von mir vorgelegte Dissertation selbständig angefertigt, die benutzten Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit - einschließlich Tabellen, Karten und Abbildungen -, die anderen Werken im Wortlaut oder dem Sinn nach entnommen sind, in jedem Einzelfall als Entlehnung kenntlich gemacht habe; daß diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegen hat; daß sie - abgesehen von den angegebenen Teilpublikationen - noch nicht veröffentlicht worden ist, sowie daß ich eine solche Veröffentlichung vor Abschluß des Promotionsverfahrens nicht vornehmen werde. Die Bestimmungen der Promotionsordnung der Mathematisch-Naturwissenschaftlichen Fakultät der Universität zu Köln sind mir bekannt. Die von mir vorgelegte Dissertation ist von HD Dr. Lorenz Schwark betreut worden.

(Guido Lars Bruno Wiesenberg)

Publications in peer-reviewed journals:

Wiesenberg, G.L.B., Schwark, L., Schmidt, M.W.I., 2004a. Improved automated extraction and separation procedure for soil lipid analyses. *European Journal of Soil Science* **55**, 349-356.

Wiesenberg, G.L.B, Schwarzbauer, J., Schmidt, M.W.I., Schwark, L., 2004b. Sources and turnover of organic matter in agricultural soils derived from *n*-alkane/*n*-carboxylic acid compositions and C-isotope signature. *Organic Geochemistry* **35***,* 1371-1393.

Wiesenberg, G.L.B., Schwark, L., Schmidt, M.W.I., 2004c. Extractable lipids and colour in particle-size fractions and bulk arable soils. *European Journal of Soil Science*, submitted.

Wiesenberg, G.L.B., Schmidt, M.W.I., Schwark, L., in preparation. Distribution patterns of alkanes and carboxylic acids in particle-size separates and bulk arable soils.

Rethemeyer, J., Kramer, C., Gleixner, G., Wiesenberg, G.L.B., Schwark, L., Andersen, N., Nadeau, M.-J., Grootes, P.M., 2004a. Complexity of soil organic matter: AMS ^{14}C analysis of soil lipid fractions and individual compounds. *Radiocarbon* **46**, 465-473.

F: Lebenslauf

Guido Lars Bruno Wiesenberg

GEBURTSDATUM:	23.05.1974
GEBURTSORT:	Köln
STAATSANGEHÖRIGKEIT:	deutsch
FAMILIENSTAND:	verheiratet

SCHULBILDUNG:

1980 – 1984	Gemeinschaftsgrundschule Garthestraße in Köln
1984 – 1993	Erich-Kästner-Gymnasium in Köln, Abitur: Juni 1993

STUDIUM:

10/1993 – 10/2000	Geologie/Paläontologie an der Universität zu Köln, Vordiplom 1996, Diplomkartierung 1998: „Erläuterungen zur geologischen Karte (1:10.000) des mittleren Teils der Blankenheimer Mulde – Die Geologie der Umgebung von Blankenheim“, Diplomarbeit 2000: „Der rezente Schadstoffeintrag in den Harkortsee (Nordrhein-Westfalen) anhand der Oberflächensedimente seines Einzugsgebietes“
11/2000 – 11/2004	Promotion (Geologie/Paläontologie) an der Universität zu Köln

PRAKTISCHE TÄTIGKEITEN:

06/1993 – 09/1993	Vermessungsgehilfe im Ingenieur-, Vermessungs- und Planungsbüro Diel in Köln
02/1995 – 04/1995	Werksstudent bei der Maschinenfabrik Reifenhäuser in Troisdorf
07/1995 – 09/1995	Werksstudent bei der Maschinenfabrik Reifenhäuser in Troisdorf
01/1996 – 12/1999	studentische Hilfskraft am Geologischen Institut der Universität zu Köln
01/2000 – 10/2000	wissenschaftliche Hilfskraft am Geologischen Institut der Universität zu Köln
11/2000 – 10/2002	wissenschaftlicher Mitarbeiter am Geographischen Institut der Universität zu Köln
seit 11/2002	wissenschaftlicher Mitarbeiter am Geologischen Institut der Universität zu Köln