Composition and stability of organic matter fractions in soils under different land use regimes

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List of Abbreviations

A.D.	Anno Domini
AMS	accelerator mass spectrometry
BC	black carbon
Years BP	years before present (1950)
C	carbon
C_3-plant	plant with C_3 pathway of photosynthesis (Calvin-Cycle)
C_4-plant	plant with C_4 pathway of photosynthesis (Hatch-Slack-Cycle)
CFE	chloroform fumigation extraction
CO_2	carbon dioxide
CPMAS	cross-polarization magic angle spinning
Delta (δ)^{13}C	$^{13}C/^{12}C$ ratio expressed relative to the PDB standard
DON	dissolved organic nitrogen
$fPOM_{<1.6}$	free particulate organic matter, density < 1.6 g cm^{-3}
g	gravity acceleration
IAEA	International Atomic Energy Agency
IOM	inert organic matter
IR-MS	isotope ratio-mass spectrometry
$Mineral_{>2.0}$	mineral-associated fraction, density < 1.6 g cm^{-3}
N	nitrogen
NIST	National Institute of Standards and Technology
NMR	nuclear magnetic resonance spectroscopy
N_t	total soil nitrogen
$oPOM_{<1.6}$	occluded particulate organic matter, density < 1.6 g cm^{-3}
$oPOM_{1.6-2.0}$	occluded particulate organic matter, density 1.6–2.0 g cm^{-3}
PDB	Pee Dee Belemnite, carbonate standard for ^{13}C analysis
Pg	petagramm (1 Pg = 10^{15} g)
PLFA	phospholipid fatty acids
pMC	percent modern carbon
POM	particulate organic matter
ppb	parts per billion
ppm	parts per million
rpm	rotations per minute
SD	standard deviation
SE	standard error
SOC	soil organic carbon
SOM	soil organic matter
TOC	total organic carbon
VCT	variable contact time

Summary

Soil organic matter (SOM) vitally impacts all soil functions and plays a key role in the global carbon (C) cycle. More than 70% of the terrestric C stocks that participate in the active C cycle are stored in the soil. Therefore, quantitative knowledge of the rates of C incorporation into SOM fractions of different residence time is crucial to understand and predict the sequestration and stabilization of soil organic carbon (SOC). Consequently, there is a need of fractionation procedures that are capable of isolating functionally SOM fractions, i.e. fractions that are defined by their stability. The literature generally refers to three main mechanisms of SOM stabilization: protection of SOM from decomposition by (i) its structural composition, i.e. recalcitrance, (ii) spatial inaccessibility and/or (iii) interaction with soil minerals and metal ions. One of the difficulties in developing fractionation procedures for the isolation of functional SOM fractions is the marked heterogeneity of the soil environment with its various stabilization mechanisms – often several mechanisms operating simultaneously – in soils and soil horizons of different texture and mineralogy. The overall objective of the present thesis was to evaluate present fractionation techniques and to get a better understanding of the factors of SOM sequestration and stabilization.

The first part of this study is attended to the structural composition of SOM. Using ^{13}C cross-polarization magic-angle spinning (CPMAS) nuclear magnetic resonance (NMR) spectroscopy, (i) the effect of land use on SOM composition was investigated and (ii) examined whether SOM composition contributes to the different stability of SOM in density and aggregate fractions. The second part of the present work deals with the mineral-associated SOM fraction. The aim was (iii) to evaluate the suitability of chemical fractionation procedures used in the literature for the isolation of stable SOM pools (stepwise hydrolysis, treatments using oxidizing agents like $Na_2S_2O_8$, H_2O_2, and NaOCl as well as demineralization of the residue obtained by the NaOCl treatment using HF (NaOCl+HF)) by pool sizes, ^{13}C and ^{14}C data. Further, (iv) the isolated SOM fractions were compared to the inert organic matter (IOM) pool obtained for the investigated soils using the Rothamsted Carbon Model and isotope data in order to see whether the tested chemical fractionation methods produce SOM fractions

capable to represent this pool. Besides chemical fractionation, (v) the suitability of thermal oxidation at different temperatures for obtaining stable SOC pools was evaluated. Finally, (vi) the short-term aggregate dynamics and the factors that impact macroaggregate formation and C stabilization were investigated by means of an incubation study using treatments with and without application of ^{15}N labeled maize straw of different degradability (leaves and coarse roots). All treatments were conducted with and without the addition of fungicide.

Two study sites with different soil properties and land managements were chosen for these investigations. The first one, located at Rotthalmünster, is a Stagnic Luvisol (silty loam) under different land use regimes. The Ah horizons of a spruce forest and continuous grassland and the Ap and E horizons of two plots with arable crops (continuous maize and wheat cropping) were examined. The soil of the second study site, located at Halle, is a Haplic Phaeozem (loamy sand) where the Ap horizons of two plots with arable crops (continuous maize and rye cropping) were investigated. Both study sites had a C_3-/C_4-vegetational change on the maize plot for the purpose of tracing the incorporation of the younger, maize-derived C into different SOM fractions and the calculation of apparent C turnover times of these. The Halle site is located near a train station and industrial areas, which caused a contamination with high amounts of fossil C.

The investigation of aggregate and density fractions by ^{13}C CPMAS NMR spectroscopy revealed that density fractionation isolated SOM fractions of different composition. The consumption of a considerable part (10–20%) of the easily available O-alkyl-C and the selective preservation of the more recalcitrant alkyl-C when passing from litter to the different particulate organic matter (POM) fractions suggest that density fractionation was able to isolate SOM fractions with different degrees of decomposition. The spectra of the aggregate fractions resembled those of the mineral-associated SOM fraction obtained by density fractionation and no considerable differences were observed between aggregate size classes. Comparison of plant litter, density and aggregate size fractions from soil under different land use showed that the type of land use markedly influenced the composition of SOM. While SOM of the acid forest soil was characterized by a large content (> 50%) of POM, which contained high amounts of spruce-litter derived alkyl-C, the organic matter in the biologically

more active grassland and arable soils was dominated by mineral-associated SOM (> 95%). This SOM fraction comprised greater proportions of aryl- and carbonyl-C and is considered to contain a higher amount of microbially-derived organic substances. Land use can alter both, structure and stability of SOM fractions.

All applied chemical treatments induced considerable SOC losses (> 70–95% of mineral-associated SOM) in the investigated soils. The proportion of residual C after chemical fractionation was largest in the arable Ap and E horizons and increased with decreasing C content in the initial SOC after stepwise hydrolysis as well as after the oxidative treatments with H_2O_2 and $Na_2S_2O_8$. This can be expected for a functional stable pool of SOM, because it is assumed that the more easily available part of SOC is consumed first if C inputs decrease. All chemical treatments led to a preferential loss of the younger, maize-derived SOC, but this was most pronounced after the treatments with $Na_2S_2O_8$ and H_2O_2. After all chemical fractionations, the mean ^{14}C ages of SOC were higher than in the mineral-associated SOM fraction for both study sites and increased in the order: NaOCl < NaOCl+HF ≤ stepwise hydrolysis << H_2O_2 ≈ $Na_2S_2O_8$. The results suggest that all treatments were capable of isolating a more stable SOM fraction, but the treatments with H_2O_2 and $Na_2S_2O_8$ were the most efficient ones. However, none of the chemical fractionation methods was able to fit the IOM pool calculated using the Rothamsted Carbon Model and isotope data.

In the evaluation of thermal oxidation for obtaining stable C fractions, SOC losses increased with temperature from 24–48% (200°C) to 100% (500°C). In the Halle maize Ap horizon, losses of the young, maize-derived C were considerably higher than losses of the older C_3-derived C, leading to an increase in the apparent C turnover time from 220 years in mineral-associated SOC to 1158 years after thermal oxidation at 300°C. Most likely, the preferential loss of maize-derived C in the Halle soil was caused by the presence of the high amounts of fossil C mentioned above, which make up a relatively large thermally stable C_3-C pool in this soil. This agrees with lower overall SOC losses for the Halle Ap horizon compared to the Rotthalmünster Ap horizon. In the Rotthalmünster soil only slightly more maize-derived than C_3-derived SOC was removed by thermal oxidation. Apparent C turnover times increased slightly

from 58 years in mineral-associated SOC to 77 years after thermal oxidation at 300°C in the Rotthalmünster Ap and from 151 to 247 years in the Rotthalmünster E horizon. This led to the conclusion that thermal oxidation of SOM was not capable of isolating SOM fractions of considerably higher stability.

The incubation experiment showed that macroaggregates develop rapidly after the addition of easily available plant residues. Within the first four weeks of incubation, the maximum aggregation was reached in all treatments without addition of fungicide. The formation of water-stable macroaggregates was related to the size of the microbial biomass pool and its activity. Furthermore, fungi were found to be crucial for the development of soil macroaggregates as the formation of water-stable macroaggregates was significantly delayed in the fungicide treated soils. The C concentration in the obtained aggregate fractions decreased with decreasing aggregate size class, which is in line with the aggregate hierarchy postulated by several authors for soils with SOM as the major binding agent. Macroaggregation involved incorporation of large amounts maize-derived organic matter, but macroaggregates did not play the most important role in the stabilization of maize-derived SOM, because of their relatively low amount (less than 10% of the soil mass). Furthermore, the maize-derived organic matter was quickly incorporated into all aggregate size classes. The microaggregate fraction stored the largest quantities of maize-derived C and N – up to 70% of the residual maize-C and -N were stored in this fraction.

Zusammenfassung

Die organische Bodensubstanz hat einen entscheidenden Einfluss auf alle Bodenfunktionen und spielt eine wichtige Rolle im globalen Kohlenstoffkreislauf. Mehr als 70% der terrestrischen Kohlenstoffvorräte, die am aktiven Kohlenstoffkreislauf teilnehmen, sind im Boden gespeichert. Deshalb ist es wichtig, ein quantitatives Verständnis bezüglich der Größenordnung von Kohlenstoffeinträgen in Fraktionen der organischen Bodensubstanz unterschiedlicher Verweilzeit zu erlangen, um die Sequestrierung und Stabilisierung organischen Kohlenstoffes im Boden verstehen und vorhersagen zu können. Dementsprechend werden spezielle Fraktionierungsverfahren benötigt, welche es ermöglichen, funktionelle Fraktionen der organischen Bodensubstanz zu isolieren, d.h. Fraktionen, die über ihre Stabilität definiert sind. Im Allgemeinen werden in der Literatur drei Haupt-Stabilisierungsmechanismen beschrieben: Schutz der organischen Bodensubstanz vor Abbau durch (i) ihre strukturelle Zusammensetzung, d.h. Rekalzitranz, (ii) räumliche Unzugänglichkeit und/oder (iii) Wechselwirkung mit der Mineralphase oder Metall-Ionen. Ein Problem, welches bei der Entwicklung von Fraktionierungsverfahren zur Isolierung funktioneller Fraktionen der organischen Bodensubstanz auftritt, ist die Heterogenität des Bodens mit einer Vielzahl an Stabilisierungsmechanismen, welche in Böden und Bodenhorizonten unterschiedlicher Textur und Mineralogie vorkommen – oft sind es mehrere zur gleichen Zeit. Das übergreifende Ziel dieser Arbeit war es, vorhandene Fraktionierungsverfahren zu testen und ein besseres Verständnis der Faktoren die die Verteilung und Stabilisierung organischer Bodensubstanz beeinflussen, zu erarbeiten.

Der erste Teil der vorliegenden Studie beschäftigt sich mit der strukturellen Zusammensetzung der organischen Bodensubstanz. Mittels ^{13}C CPMAS NMR Spektroskopie wurde (i) der Einfluss der Landnutzung auf die Zusammensetzung der organischen Bodensubstanz untersucht und (ii) geprüft, ob diese zu den unterschiedlichen Stabilitäten der organischen Bodensubstanz in Dichte- und Aggregatfraktionen beiträgt. Der zweite Teil der Arbeit beschäftigt sich mit der mineral-assoziierten Fraktion der organischen Bodensubstanz. Das Ziel war es (iii), die Eignung von in der Literatur verwendeten chemischen Fraktionierungsverfahren zur Isolierung stabiler Pools der organischen Bodensubstanz anhand

von Pool-Größen sowie ^{13}C und ^{14}C Daten zu testen (schrittweise Hydrolyse, Verfahren mit oxidierenden Mitteln wie $Na_2S_2O_8$, H_2O_2 und NaOCl, sowie Demineralisierung des Residuums der NaOCl-Behandlung mittels Fluss-Säure (NaOCl+HF)). Die isolierten Fraktionen der organischen Bodensubstanz wurden (iv) mit dem Pool an inerter organischer Substanz verglichen, welcher mit dem Rothamsted Carbon Modell und Isotopendaten für die untersuchten Böden ermittelt wurde. Ziel des Vergleiches war, herauszufinden, ob die mittels der getesteten Verfahren erhaltenen Fraktionen sich dazu eignen, diesen Pool zu repräsentieren. Neben der chemischen Fraktionierung wurde die Eignung (v) der thermischen Oxidation zum Erhalt stabiler Pools der organischen Bodensubstanz getestet. Im letzten Teil der vorliegenden Arbeit wurden mittels einer Inkubationsstudie mit und ohne Zugabe von ^{15}N-markierter Maisstreu unterschiedlich guter Zersetzbarkeit (Blätter und Grobwurzeln) mit und ohne Zugabe eines Fungizides (vi) die kurzfristige Aggregatdynamik und die Faktoren, welche die Makroaggregatbildung und Kohlenstoff-Stabilisierung beeinflussen, untersucht.

Für die Analysen wurden zwei Untersuchungsgebiete mit unterschiedlichen Bodeneigenschaften und Landnutzungen für die Analysen ausgewählt. Bei dem ersten, in Rotthalmünster, handelt es sich um eine pseudovergleyte Parabraunerde (schluffiger Lehm) mit unterschiedlichen Landnutzungsarten. Für die Analysen wurden die Ah Horizonte eines Fichtenforstes und eines Dauergrünlandes, sowie der Ap und E Horizont von zwei Standorten mit Ackerkulturen (Mais- und Weizenanbau) herangezogen. Das zweite Untersuchungsgebiet befindet sich in Halle („Ewiger Roggen“). Es handelt sich hier um eine degradierte Schwarzerde (lehmiger Sand), von dem die Ap Horizonte zweier Plots mit Ackerkulturen (Mais- und Roggenanbau) beprobt wurden. Bei beiden Untersuchungsgebieten hat ein C_3-/C_4-Vegetationswechsel auf der Maisfläche stattgefunden. Dieser Wechsel ermöglicht es, den Einbau jungen, maisbürtigen Kohlenstoffes in unterschiedliche Fraktionen der organischen Bodensubstanz zu verfolgen und deren Umsatzraten zu errechnen. Nahe dem Untersuchungsgebiet in Halle befinden sich ein Bahnhof und Industriegebiete, wodurch dieser Boden mit hohen Gehalten an fossilem Kohlenstoff belastet ist.

Die Erforschung von Aggregat- und Dichtefraktionen mittels ^{13}C CPMAS NMR Spektroskopie zeigte, dass die Dichtefraktionierung es ermöglichte, Fraktionen der organischen Bodensubstanz unterschiedlicher Zusammensetzung zu isolieren. Die Abnahme des leicht verfügbaren O-Alkyl-Kohlenstoffes um einen beachtlichen Teil (10–20%) und die selektive Anreicherung des abbau-resistenteren Alkyl-Kohlenstoffes beim Übergang von Streu zu den einzelnen Fraktionen partikulärer organischer Substanz, weisen darauf hin, dass die Dichtefraktionierung Fraktionen unterschiedlicher Zersetzungsstadien isoliert hat. Die Spektren der Aggregatfraktionen ähnelten in ihrer Zusammensetzung denen der mittels Dichtefraktionierung gewonnenen mineral-assoziierten Fraktion und es gab keine deutlichen Unterschiede zwischen den einzelnen Aggregat-Größenklassen. Der Vergleich von Pflanzenstreu, Dichte- und Aggregatfraktionen aus Böden unterschiedlicher Landnutzung zeigte, dass der Landnutzungstyp einen starken Einfluss auf die Zusammensetzung der organischen Bodensubstanz hat. Während die organische Bodensubstanz im sauren Waldboden einen hohen Anteil (> 50%) an partikulärem organischen Material aufwies, welches große Mengen an fichtenstreubürtigem Alkyl-C enthielt, wurde die organische Bodensubstanz der biologisch aktiveren Grünland- und Ackerböden von mineral-assoziierter organischer Bodensubstanz dominiert (> 95%), welche höhere Anteile an aromatischem und Carbonyl-C barg und vermutlich mehr mikrobiell entstandene organische Substanzen beinhaltete. Folglich kann durch die Landnutzung beides, die Struktur und die Stabilität von Fraktionen der organischen Bodensubstanz beeinflusst werden.

Die chemische Fraktionierung mineral-assoziierter organischer Bodensubstanz führte zu beachtlichen Verlusten organischen Kohlenstoffes (> 70–95%) bei allen angewendeten Verfahren und untersuchten Böden. Der Anteil an residualem Kohlenstoff war im Falle der schrittweisen Hydrolyse, sowie der oxidativen Behandlung mit H_2O_2 und $Na_2S_2O_8$ am höchsten in den Ober- und Unterböden der Ackerstandorte und stieg mit sinkendem Ausgangskohlenstoffgehalt der Böden. Dies spricht für den Erhalt eines funktionellen stabilen Pools der organischen Bodensubstanz, da angenommen wird, dass die leichter verfügbaren Kohlenstoffanteile bei reduzierten Kohlenstoffeinträgen zuerst konsumiert werden. Alle chemischen Fraktionierungsverfahren entfernten präferentiell den

jungen, maisbürtigen Kohlenstoff, aber am ausgeprägtesten war dies bei den $Na_2S_2O_8$- und H_2O_2-Behandlungen zu beobachten. Das mittlere ^{14}C Alter des Bodenkohlenstoffes war in beiden untersuchten Standorten nach allen angewandten chemischen Fraktionierungsverfahren höher als zuvor und stieg in folgender Reihenfolge an: NaOCl < NaOCl+HF < schrittweise Hydrolyse << H_2O_2 ≈ $Na_2S_2O_8$. Die Ergebnisse zeigen, dass alle Verfahren eine stabilere Fraktion der organischen Bodensubstanz isolieren konnten. Am besten geeignet schienen jedoch die oxidativen Verfahren mit H_2O_2 und $Na_2S_2O_8$. Allerdings stimmte keine der residualen Kohlenstofffraktionen mit dem mittels Rothamsted Carbon Modell und Isotopendaten für die Flächen errechneten inerten Pool der organischen Substanz überein.

Bei der Evaluierung der thermischen Oxidation als Verfahren zum Erhalt stabiler Kohlenstofffraktionen stiegen die Kohlenstoffverluste mit der Temperatur von 24–48% bei 200°C auf 100% bei 500°C an. Im Ap Horizont des Maisbodens in Halle gab es einen deutlich stärkeren Verlust von jungem Maiskohlenstoff im Vergleich zum älteren, C_3-bürtigen Kohlenstoff, was zu einem Anstieg der C Umsatzzeit von 220 Jahren in der mineral-assoziierten Kohlenstofffraktion auf 1158 Jahre nach thermischer Oxidation bei 300°C führte. Höchstwahrscheinlich wurde dieser präferentielle Verlust an maisbürtigem Kohlenstoff jedoch durch die hohen Mengen fossilen Kohlenstoffes verursacht, welcher einen relativ großen, thermisch stabilen C_3-Kohlenstoff-Pool in diesem Boden stellt. Dies stimmt auch mit den insgesamt geringeren Verlusten an Kohlenstoff im Halle Boden gegenüber dem Rotthalmünster Ap Horizont überein. Im Rotthalmünster Boden wurde durch die thermische Oxidation nur geringfügig mehr maisbürtiger Kohlenstoff im Vergleich zum C_3-bürtigem Kohlenstoff entfernt. Im Ap Horizont stieg die C Umsatzzeit lediglich von 58 Jahren in der mineral-assoziierten Bodenkohlenstofffraktion auf 77 Jahre nach thermischer Oxidation bei 300°C an und im E Horizont von 151 auf 247 Jahre. Das führte zu der Schlussfolgerung, dass es nicht möglich war, mittels thermischer Oxidation Fraktionen der organischen Bodensubstanz von erheblich größerer Stabilität zu isolieren.

Das Inkubationsexperiment zeigte auf, dass die Entwicklung von Makro-aggregaten nach Zugabe leicht verfügbarer Pflanzenreste sehr schnell erfolgt.

Binnen der ersten vier Wochen der Inkubation wurde die maximale Aggregierung in allen Varianten ohne Fungizid-Zugabe erreicht. Die Bildung wasserstabiler Makroaggregate war eng mit der Größe des Pools der mikrobiellen Biomasse und deren Aktivität verbunden. Auch der starke Einfluss der Pilze auf die Makroaggregierung wurde deutlich, da sich die Bildung wasserstabiler Makroaggregate in den Varianten mit Fungizid-Zugabe signifikant verzögerte. Die Kohlenstoffkonzentration in den erhaltenen Aggregatfraktionen sank mit abnehmender Aggregatgröße, was mit der von mehreren Autoren postulierten Theorie der Aggregathierarchie für Böden, in denen die Aggregierung hauptsächlich biotisch gesteuert ist, übereinstimmt. Die Makroaggregierung ging mit der Einbindung großer Mengen maisbürtiger organischer Substanz einher, aber aufgrund der geringen Menge an Makroaggregaten (weniger als 10% des Bodens) spielten diese nicht die wichtigste Rolle in der Stabilisierung maisbürtiger organischer Bodensubstanz. Außerdem wurde die maisbürtige organische Substanz schnell in alle Aggregatgrößenklassen eingebunden. Die Mikroaggregatfraktion barg die größten Mengen an maisbürtigem C und N – bis zu 70% des noch vorhandenen maisbürtigen C und -N waren in dieser Fraktion gespeichert.

1 General introduction

1.1 Soils as source and sink for CO_2

Global climate change is a major environmental issue of current times. Evidence for global climate change is growing and there is rising consent that anthropogenic interference into the natural cycle of greenhouse gases is the main reason for this (IPCC, 2001). Greenhouse gases are named after their ability to entrap the thermal radiation of the sun in the atmosphere – the so-called greenhouse effect. Carbon dioxide (CO_2) is known to be the most important among the greenhouse gases.

In the pre-industrial era, the atmospheric CO_2 concentration remained constant at about 280 ppm. Ice core measurements and (since 1957) direct measurement of CO_2 concentrations in the atmosphere revealed a rising CO_2 level with beginning industrialization up to about 370 ppm in 1999 (IPCC, 2001). The major source of CO_2 emissions into the atmosphere was found to be fossil fuel combustion (6.3 ± 0.4 Pg C $year^{-1}$, Figure 1.1). Evidence for this derives from (i) a decrease in atmospheric O_2 levels of the same magnitude as CO_2 levels increase (combustion consumes O_2), (ii) a characteristic isotopic signature of fossil fuel (a lack of ^{14}C and a depletion in the ^{13}C content) that labels the atmosphere and (iii) a faster increase in CO_2 concentration in the northern hemisphere where most fossil fuel burning occurs (IPCC, 2001).

Besides fossil fuel burning, significant anthropogenic CO_2 emissions are caused by changes in land use. In the 1980s they ranged from 0.6–2.5 Pg C $year^{-1}$ with a central estimate of 1.7 Pg C $year^{-1}$ (IPCC, 2001). Ninety percent of these emissions derived from deforestation. Assumptions based on data for the early 1990s led to the estimation that emissions due to land use change remained at the same level in the 1990s (1.6 ± 0.8 Pg C $year^{-1}$) (Schimel *et al.*, 2001).

The overall increase in the atmospheric CO_2 level was found to amount 3.2 ± 0.1 Pg C $year^{-1}$ (IPCC, 2001), which is lower than suggested by the amounts of CO_2 from anthropogenic emissions. The reason for this is an uptake of atmospheric CO_2 by the ocean (1.7 ± 0.5 Pg C $year^{-1}$) and by the terrestrial biosphere (1.4 ± 0.7 Pg C $year^{-1}$) (Figure 1.1). When calculating the difference between the above

mentioned CO_2 sources and sinks, an annual deficit of about 1.6 Pg C remains in the global C budget. Estimates – deriving from several sources such as land inventory data (Brown & Schroeder, 1999), atmospheric CO_2 (Tans *et al.*, 1990) and oceanic O_2 data (Bender *et al.*, 1996; Keeling *et al.*, 1996), isotopic analyses (Ciais *et al.*, 1995) and ecosystem models (Schimel *et al.*, 2000; White *et al.*, 2000; McGuire *et al.*, 2001) – suggest that this "missing" CO_2 is taken up by terrestrial ecosystems in the northern hemisphere. This leads to an overall terrestrial CO_2 uptake of 3.2 Pg C $year^{-1}$, which points out the vital importance of terrestrial ecosystems as a C sink.

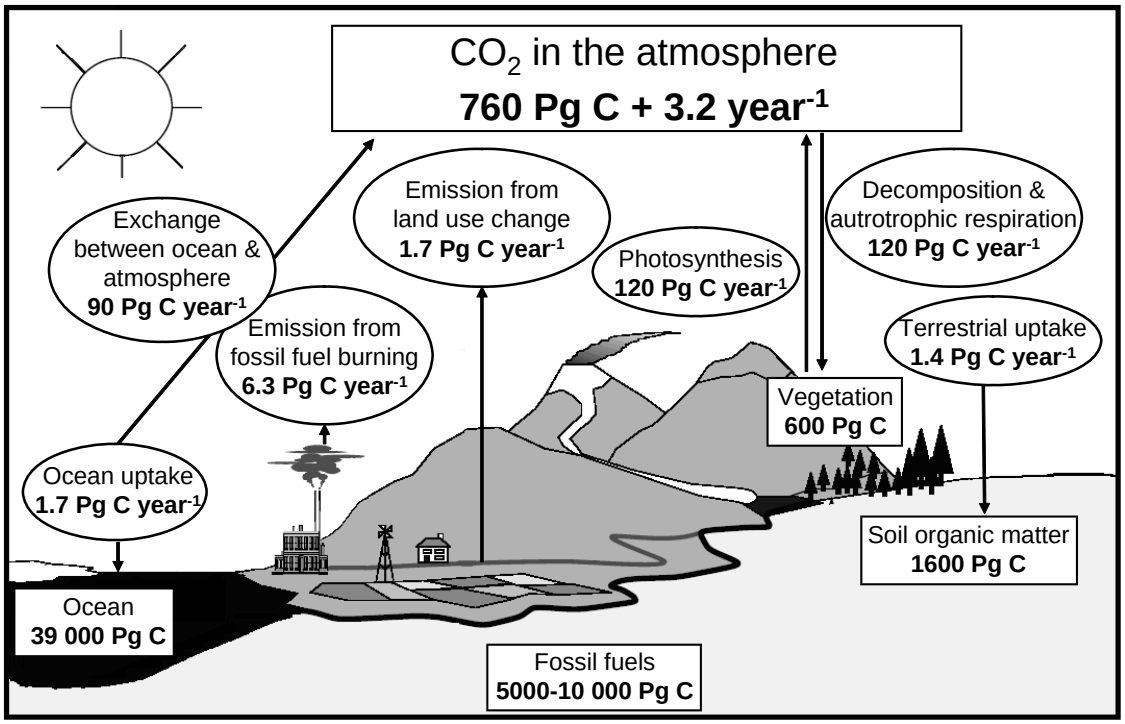

Figure 1.1: Global carbon reservoirs and major CO_2 exchanges per year between reservoirs (Schlesinger & Andrews, 2000; IPCC, 2001; Schimel *et al.*, 2001).

It is crucial to preserve the strength of the terrestrial sink, and SOM is supposed to play a key role in this, because more than 70% of the terrestrial C stocks that take part in the active C cycle are stored in the soil while less than 30% are bound in the vegetation (Figure 1.1). Thus, the selective accumulation of SOC offers a direct way of counteracting the greenhouse effect. How much SOC is stored and for how long this C remains in the soil depends upon SOC pools and their recycling. Therefore, direct control of SOC stocks implies a fundamental knowledge about the stabilization mechanisms of SOM.

1.2 Mechanisms of soil organic matter stabilization

Stabilization is defined as protection of organic matter from mineralization. Soil organic matter can be stabilized by (i) recalcitrance, i.e. selective preservation of organic matter as a function of its structural composition, (ii) spatial inaccessibility, e.g. by occlusion in aggregates, intercalation within phyllosilicates, hydrophobicity or encapsulation in organic macromolecules, and/or (iii) interaction with soil minerals and metal ions (Skjemstad *et al.*, 1996; Sollins *et al.*, 1996; Six *et al.*, 2002; v. Lützow *et al.*, 2006b). Generally, stabilized organic matter has a higher apparent C turnover time than unprotected organic matter. A range of physical and chemical fractionation procedures have been developed to identify SOM pools of different chemistry and stability. Since the conventional SOM fractionation in humic acids, fulvic acids, and humin produce SOM pools that hardly differ with respect to turnover rates (Balesdent, 1996) and contents of functional groups (Krosshavn *et al.*, 1992), physical fractionation methods (e.g. particle size, aggregate or density fractionation) have been proposed to analyze the processes of organic matter stabilization in soils (Christensen, 1992; Golchin *et al.*, 1997). These physical fractionation methods mainly isolate SOM pools with an apparent C turnover time of decades to centuries. Therefore, several chemical fractionation methods have been proposed for the isolation of stable SOM with C turnover times of centuries to millennia (for a review, see v. Lützow *et al.*, 2006a).

1.2.1 Selective preservation due to structural composition

Plant residues are composed of a range of organic compounds of different structural composition. The main components are polysaccharides (50–60%) – such as starch, cellulose, hemicellulose or pectin – and lignin (15–20%). Proteins, polyphenols (e.g. tannins), chlorophyll, cutin, suberin, lipids and waxes make up the remaining 10–20% (v. Lützow *et al.*, 2006b). The biodegradability of these compounds differs due to differences in their structural composition. Simple monomers (e.g. glucose, amino acids) are decomposed quickly and even polymers such as polysaccharides or proteins turn over within weeks. Macromolecular substances are easily degradable when they contain hydrolytic bondings, because these can easily be hydrolyzed by a group of enzymes, so-called hydrolases. Polymers that comprise aromatic rings (e.g. lipids, waxes,

cutin and suberin) are more resistant to degradation (v. Lützow *et al.*, 2006b). Most microbial and faunal components are polysaccharide- or protein-type macromolecules, which are supposed to underlie similar degrees of stabilization as plant-derived macromolecules, but microbial residues also contain different substances, such as murein, chitin, certain lipids or fungal-derived melanins, which were found to accumulate in soils (e.g. Guggenberger *et al.*, 1994; Kögel-Knabner, 2002; Kiem & Kögel-Knabner, 2003).

A means for investigating the structural composition of SOM is solid-state ^{13}C CPMAS NMR spectroscopy. It has successfully been applied in studies on changes of SOM structure during organic matter decomposition (Golchin *et al.*, 1994a; Baldock *et al.*, 1997; Conte *et al.*, 1997; Kögel-Knabner, 1997; Skjemstad *et al.*, 1997; Kölbl & Kögel-Knabner, 2004). These studies have shown that polysaccharides are affected first by decomposition, leading to a decrease in O-alkyl-C. Additionally, in many studies a concomitant increase in the alkyl-C content has been observed which was explained by selective preservation and in situ synthesis (Golchin *et al.*, 1994b; Baldock *et al.*, 1997; Kölbl & Kögel-Knabner, 2004). Further, ^{13}C CPMAS NMR spectroscopy as well as pyrolysis techniques revealed that lignin, which was considered to be fairly resistant to decomposition due to its aromatic rings (Derenne & Largeau, 2001), is not stabilized in the long-term in any soil fraction (Kögel-Knabner, 2000; Poirier *et al.*, 2003, 2005a,b).

In summary, structural composition is a stabilizing effect, which causes different degrees of selective preservation, but this stabilizing effect has to be considered as relative, because the microbial community is omnipotent and thus may degrade any natural organic matter in the soil. For example, according to radiocarbon data, Rethemeyer *et al.* (2005) found that microbial phospholipid fatty acids (PLFA) in the topsoil derived largely from the consumption of fresh plant residues, whereas a considerable contribution of more stabilized organic matter in PLFAs was found in deeper soil horizons. Another example is black carbon, which is considered highly recalcitrant due to its aromatic nature, but not inert. Degradation of black carbon to some extent was evidenced in several studies (e.g. Bird *et al.*, 1999; Krull *et al.*, 2006).

1.2.2 Spatial inaccessibility

The formation and stabilization of soil aggregates is influenced by a number of factors, e.g. the amount of clay, Fe- and Al-oxides (Lynch & Bragg, 1985) or the amount and quality of organic substances, especially plant and animal debris, fungi, bacteria and products of the microbial synthesis (Bossuyt *et al.*, 2001). At the clay microstructure level, a first stage of aggregation is induced by the flocculation of clay particles (Figure 1.2a).

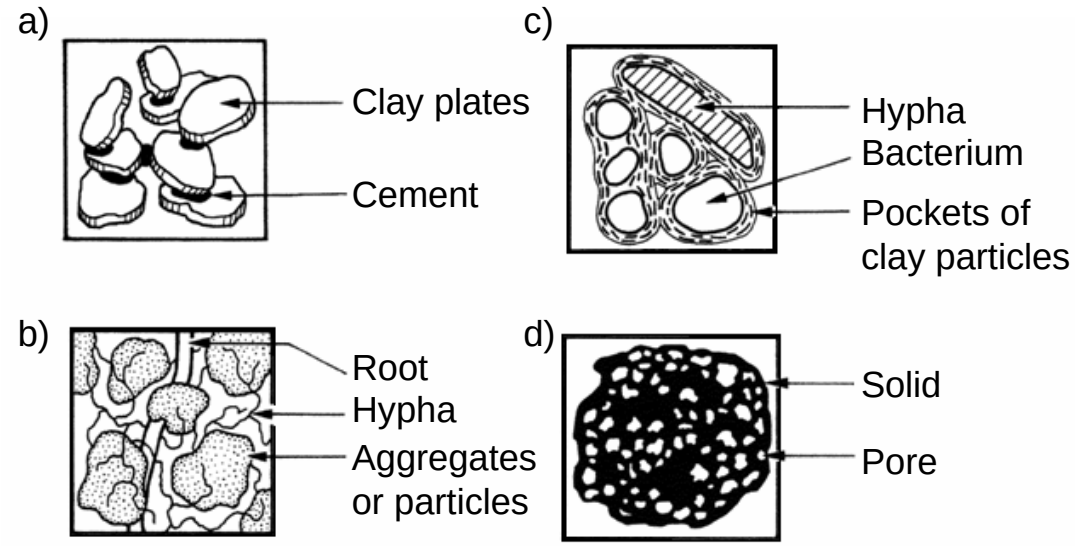

Figure 1.2 a–d: Stages of soil aggregation (according to Tisdall & Oades, 1982, modified).

Humic substances and biologically processed organic materials are involved in the formation of organo-mineral complexes by the binding of clay packets and silt particles < 20 µm into aggregates < 53 µm (Figure 1.2b). In clayey soils, abiotic aggregation may also be responsible for the creation of larger aggregates due to a high shrink-swell capacity, but usually, soil biota play the major role for aggregation at the larger scale. Golchin *et al.* (1997) suggested that particulate organic matter (POM) serves as a core for the formation of larger aggregates through processes of physical entanglement and the production of metabolic binding agents by soil microbes that attach primary particles and small micro-aggregates to the organic materials, leading to the formation of macroaggregates (Figure 1.2c). This protection from rapid microbial decomposition derives from reduced access for microorganisms and their enzymes and a restricted aerobic decomposition due to reduced oxygen diffusion, which is caused primarily by the

pore-size distribution created by the formation of soil aggregates (Figure 1.2d) (Hassink *et al.*, 1993; Oades, 1993). For example, Hassink *et al.* (1993) found strong correlations between the bacterial biomass and the volume of soil made up by pores between 0.2 and 1.2 µm ($r^2 = 0.9$) and between the biomass of nematodes and the volume of pores between 30 and 90 µm ($r^2 = 0.8$). Penetration of aggregates by the soil fauna is not only restricted to the pore size, but also to the air and water status they provide. Fungal hyphae, roots or root hairs are obligatory aerobes and therefore are restricted to the outer regions of macroaggregates, bacteria are also found in the centre of soil aggregates (Foster, 1988). On the other hand, soil bacteria are dependent on the presence of water, while fungal hyphae can extend through air-filled pores (Chenu & Stotzky, 2002).

Macro- and microaggregates offer protection of SOM at different time scales: Macroaggregation is a transient process – since the size of the macroaggregate is a function of the size of the plant material and due to rapid decomposition and subsequent fragmentation of organic cores of macroaggregates by soil organisms, the stability of macroaggregates declines at about the same rate at which plant material decomposes in the soil (Golchin *et al.*, 1997). In an incubation study using silty soil, Gale (2000a,b) and Gale & Cambardella (2000) observed increasing macroaggregation between days 0 and 180. After the maximum aggregation was reached (day 180), the amount of stable macroaggregates decreased continuously until the end of the experiment at day 360. This faster turnover of macroaggregates compared to smaller ones was also reported in a range of field studies in which macroaggregates were found to be enriched in young C compared to microaggregates or the non-aggregated fraction (e.g. Puget *et al.*, 2000; John *et al.*, 2005). Both, the organic matter occluded in these aggregates and the binding agents get decomposed, and unless the decomposed SOM is replaced by fresh POM, the binding effects are lost and aggregates break down. Therefore, macroaggregates were found to be very sensitive to changes in land use and cultivation practice, whereas microaggregates are supposed to be characteristic of a particular soil and unaffected by management in the short-term. While macroaggregation may be enhanced by the addition of fresh organic matter, the only way to increase microaggregation is usage of long-term conservation practices (Oades, 1990; Piccolo, 1996).

1.2.3 Stabilization of organic matter by interaction with mineral surfaces and/or metal ions

A major part of SOM is associated with the mineral matrix (Christensen, 1992; Golchin *et al.*, 1994a; John *et al.*, 2005). The association of SOM with soil minerals or metal ions results in a stabilized SOM pool as evidenced by an older mean age (Scharpenseel & Becker-Heidmann, 1989; Eusterhues *et al.*, 2003) and high apparent C turnover times (Balesdent, 1996; Ludwig *et al.*, 2003). Therefore, this stable pool of SOM is thought to play a significant role as a C sink and thus as a storage compartment for nutrients (Woodmansee, 1984; Piccolo, 1996).

The main inorganic soil constituents involved into interactions between organic matter and the mineral soil are of the clay-sized class, e.g. clay minerals or amorphous hydroxides of iron and aluminum because of their reactive hydroxyl groups (Cheshire & Hayes, 1990; Zech & Guggenberger, 1996). Humic substances and polysaccharides with their carboxyl and phenolic (aromatic) functional groups are major organic components involved into organo-mineral interactions (Newman & Hayes, 1990). A possible form of such an interaction is the organo-mineral complex, i.e. sorption of an organic macromolecule to the mineral surface. Besides the organo-mineral complex other interactions occur between organic matter and the mineral soil, e.g. ligand exchange, polyvalent cation bridges, or week interactions, such as van der Waals forces or hydrogen bonding (Zech & Guggenberger, 1996; Baldock & Skjemstad, 2000).

The stabilizing effect is assumed to derive from the fact that the substrate cannot be transported into the cell when in association with minerals or metal ions, rendering utilization by microorganisms impossible (Chenu & Stotzky, 2002). The strength of protection of the organic molecule is assumed to be linked to the surface load, i.e. a more intimate association of SOM with mineral surfaces occurs at lower SOC loadings, because of multiple ligand attachments, whereas at larger surface loadings, fewer ligand attachments are involved (Kaiser & Guggenberger, 2003).

1.3 Use of C isotopes for the investigation of C dynamics in the soil

Isotopes are atoms whose nuclei contain the same number of protons, but a different number of neutrons. Isotopes may be stable (about 1/5 of the isotopes discovered so far) or unstable, i.e. radioactive (about 4/5 of the isotopes discovered so far) (Hoefs, 1997). Two naturally occurring stable C isotopes (^{13}C and ^{12}C) and one radioactive isotope (^{14}C) were used in this study. With ~ 98.9% of all naturally occurring C, the ^{12}C isotope is the most abundant. Only 1.1% is ^{13}C and the ^{14}C isotope is present in very low amounts of 1.176×10^{-12} (Hoefs, 1997; Karlén *et al.*, 1968).

1.3.1 ^{13}C natural abundance

The stable isotope abundance is reported as the deviation in the $^{13}C/^{12}C$ ratio of a sample from that of an international standard – the carbonate skeleton of a crustacean mollusk named *Bellemnitella americana*, which was obtained from the Cretaceous Pee Dee formation in South Carolina (PDB, $\delta^{13}C$ = 0 ‰) – and expressed as $\delta^{13}C$ in ‰ V-PDB:

$$\delta^{13}\mathrm{C}\,(‰\ \mathrm{V-PDB}) = \left[\frac{\left({}^{13}C/{}^{12}C_{sample} \right)}{\left({}^{13}C/{}^{12}C_{reference} \right)} - 1 \right] \times 1000 \qquad [1]$$

Since the original material no longer exists, it has been replaced by assigning exact $\delta^{13}C$ values to another carbonate (NBS-19) relative to PDB. The new scale is termed Vienna PDB (V-PDB), because the International Atomic Energy Agency (IAEA), located in Vienna, played a major role in defining this new scale (Werner & Brand, 2001). Due to a ^{13}C depletion compared to the reference carbonate, $\delta^{13}C$ values of SOM and atmospheric CO_2 are negative. Soil organic matter has an isotopic C composition comparable to that of the vegetation from which it originates (Dzurec *et al.*, 1985). Plants discriminate $^{13}CO_2$ during photosynthesis as a result of biochemical properties of the primary C fixing enzymes and limitations to CO_2 diffusion into the leaf (Boutton, 1996). The extent of the discrimination varies in plants with differing photosynthetic pathways. For example, plants using C_3 photosynthesis (Calvin-cycle) incorporate less ^{13}C than plants with C_4 photosynthesis (Hatch-Slack-cycle).

Therefore, $\delta^{13}C$ values of C_3-plants range from –32 to –22‰ V-PDB, with a most frequent value of about –27‰ V-PDB whereas C_4 plants have $\delta^{13}C$ values between –17 and –9‰ V-PDB with a most frequent value of –13‰ V-PDB (Boutton, 1996). This difference in $\delta^{13}C$ values provides a useful means for assessing the dynamics of organic matter from vegetation into SOM pools in soils after a C_3-/C_4-vegetation change or vice versa (Balesdent & Mariotti, 1996).

1.3.2 Radiocarbon dating

Radiocarbon contents are calculated from the measured $^{14}C/^{12}C$ ratio of the sample compared to that of an international standard (95% of the activity of an oxalic acid standard "Ox I", measured in 1950 corrected to a $\delta^{13}C$ of –19‰ V-PDB) and commonly expressed in percent modern carbon (pMC). The correction further adjusts the isotope ratio of "Ox I" (105.26 pMC) to that of pre-industrial wood from 1850 A.D., which contains no fossil fuel-derived C. "Modern carbon" is defined as the $^{14}C/^{12}C$ ratio in 1950 A.D. which is by convention 100 pMC. Since the original material no longer exists, it has been replaced by other standards provided by IAEA, which additionally have to be corrected to the isotope ratio of the "Ox I" standard.

Plants have a $^{14}C/^{12}C$ ratio similar to that of the CO_2 in the atmosphere due to continuous exchange via photosynthesis. At the time of death, the exchange between the plant and the atmosphere is stopped, so the radiocarbon concentration begins to decrease due to radioactive decay (Godwin, 1962). Thus, radiocarbon dating can be used for the calculation of SOM turnover. It cannot be used as a means for calculating the absolute soil age, because soils are open systems which continuously receive organic C in the form of plant residues and loose C by mineralization of SOM and leaching. Further, SOM is a heterogeneous mixture of organic components, which accumulate and decompose at different rates and thus, ^{14}C ages between modern (pMC $\geq$ 100) and more than 20 000 years BP exist in SOM (Scharpenseel & Becker-Heidmann, 1992). Therefore, radiocarbon data of SOM reflect the average age of the different organic components in SOM, the mean ^{14}C age (Scharpenseel & Becker-Heidmann, 1992; Trumbore, 1996).

Radiocarbon dating can be conducted at two time scales: (i) natural ^{14}C, which is produced in the upper atmosphere, provides a tracer for the evaluation of long-term C dynamics, because it reflects the turnover of stabilized SOM residing in soils long enough for the occurrence of significant radioactive decay while (ii) the so-called "bomb ^{14}C", an enrichment of ^{14}C in the atmosphere due to atmospheric testing of nuclear weapons in the 1950s and 1960s, acts as a tracer for the evaluation of C exchanges that occur within years to decades, because the bomb peak was progressively incorporated in SOM by the addition of plant residues that are in equilibrium with the atmospheric $^{14}CO_2$ via photosynthesis. Both approaches have their limitations. The first one assumes SOM as a homogeneous pool with a single decay rate and thus, the calculated mean ages may drastically underestimate the C turnover (Trumbore & Druffel, 1995). The second approach requires archived soil samples from before the emergence of the bomb peak, because a direct documentation of SOM incorporation over a certain time period is necessary (O'Brien & Stout, 1978; O'Brien, 1984).

1.4 Use of physical and chemical fractionation techniques for obtaining functional SOM pools

1.4.1 Physical fractionation

Physical fractionation methods are considered less destructive than chemical fractionation procedures and results obtained from physical soil fractions are anticipated to relate directly to the structure and function of SOM in situ (Christensen, 1992). Physical fractionation methods established in soil science are, for example, (i) particle size, (ii) aggregate or (iii) density fractionation.

Particle size fractionation involves complete dispersion of a soil so that its primary organo-mineral complexes are released. This fractionation technique relies on the concept that SOM associated with particles of different size differs in its structure and function (Christensen, 1992). Generally, the C accumulation was found to increase in the order sand < silt < clay (Christensen, 2001). Besides differences in SOM contents, the chemical composition of SOM changed with particle size: results from ^{13}C CPMAS NMR spectroscopy and CuO oxidation showed that plant derived carbohydrates and lignin contribute to SOM in the sand-sized fractions, while the clay-sized fraction was dominated by products of the microbial synthesis (Guggenberger *et al.*, 1994, 1995; Chen & Chiu, 2003),

which corroborates the hypothesis that the degree of decomposition increases with decreasing particle size (Baldock *et al.*, 1992). Further, a general trend of increasing apparent C turnover times with decreasing particle size was observed (Balesdent *et al.*, 1987; Ludwig *et al.*, 2005).

Aggregate fractionation is used to isolate different aggregate size classes from a soil. In combination with density fractionation, the free organic matter can be separated from that occluded in aggregates of the separated size classes. Stability of soil aggregates in the field is generally related to (i) disruption through the action of water or (ii) the action of external mechanical stresses (Dexter, 1988). Because water is considered to be the main agent for aggregate breakdown (Lynch & Bragg, 1985), most studies refer to water-stable aggregation.

Up to 90% of SOC in surface soils were found to be associated with aggregates (Jastrow *et al.*, 1996). As described in chapter 1.2.2, aggregation may be controlled by abiotic (prevalently in clayey soils) as well as biotic factors. In soils where SOM is the main agent for soil aggregation, aggregates were found to break down hierarchically (Tisdall & Oades, 1982) and soil macroaggregates (> 250 µm) were generally richer in SOC than microaggregates (< 250 µm) (Aoyama *et al.*, 1999a,b; Jastrow *et al.*, 1996; John *et al.*, 2005). Decreasing C/N ratios with decreasing aggregate size suggest an increasing degree of decomposition with decreasing aggregate size (Aoyama *et al.*, 1999a,b; Gregorich *et al.*, 2003; John *et al.*, 2005). Further, Angers & Recous (1997) traced C and N deriving from $^{13}C^{15}N$-labelled wheat straw and found a redistribution of the ^{13}C from macro- to microaggregates with time, indicating the progressive incorporation of C from the larger to the finer aggregate fractions. This is further corroborated by findings of several studies that the apparent C turnover time increases from several decades in macroaggregates to several centuries in microaggregates (Besnard *et al.*, 1996; Puget *et al.*, 2000; John *et al.*, 2005; Yamashita *et al.*, 2006).

Density fractionation differentiates organic matter pools by the strength of organic matter association to the soil mineral matrix through flotation / sedimentation in heavy organic liquids or inorganic salt solutions with specific densities (generally in the range of 1.6–2.2 g cm^{-3}) (Christensen, 1992). The

preferred density agent in recent times is sodium polytungstate ($Na_6(H_2W_{12}O_{40})$; SPT), because it allows the creation of heavy liquids of various densities (between 1.0 and 3.1 g cm^{-3}) (Six *et al.*, 1999). Organic matter without strong interaction with the soil mineral matrix, which generally consists of particulate plant residues, belongs to the light fraction with a density < 1.6–2.0 g cm^{-3}. When this particulate organic matter (POM) exists free in the soil, it turns over within years (Christensen, 1992; Baisden & Amundson, 2002; John *et al.*, 2005) while POM occluded in aggregates was found to turn over more slowly (John *et al.*, 2005; Yamashita *et al.*, 2006). This slower turnover can result from differences in recalcitrance (cp. chapter 1.2.1) and/or spatial inaccessibility (cp. chapter 1.2.2). Organic matter strongly associated with soil minerals belongs to the heavy fraction, because it is most often characterized by a density > 1.6–2.0 g cm^{-3}.

Due to its short turnover time and to correlations between SOC in the light fraction and soil respiration rates (Janzen *et al.*, 1992), the light fraction seems to accurately represent the active SOM pool. An exception are soils with high black carbon contents, because black carbon has turnover times up to several thousands of years and thus likely belongs to the stable SOM pool, but is mainly associated with the light fraction due to few interactions with soil minerals (Glaser *et al.*, 2000). The passive pool is too heterogeneous to be represented by the heavy fraction (v. Lützow *et al.*, 2006b). However, density fractionation as pre-treatment is a useful tool for a further differentiation of functional fractions, e.g. by chemical fractionation.

1.4.2 Chemical fractionation

Stable or passive SOM pool(s) are considered to have a very slow carbon turnover of several centuries to millennia and thus are almost unaffected by short-term effects of land use, but remain relevant as a long-term sink for SOC (Wang & Hsieh, 2002). Knowledge about the contribution of stable to total SOM would be helpful in modeling SOC dynamics (Ludwig *et al.*, 2003, 2005) as well as in estimating long-term effects of changes in land use or climate on the global C cycle (Skjemstad *et al.*, 1996; Falloon & Smith, 2000).

Many fractionation procedures have been used for the isolation of stable SOM, e.g. stepwise hydrolysis using trifluoroacetic acid (TFA) and hydrochloric acid

(HCl) (e.g. Poirier *et al.*, 2003, Quénéa *et al.* 2006) or hydrolysis using HCl in a single step (e.g. Paul *et al.*, 2001; Six *et al.*, 2002; Plante *et al.*, 2006), oxidative treatments using several oxidants like hydrogen peroxide (H_2O_2) (e.g. Theng *et al.*, 1999; Plante *et al.*, 2004; Eusterhues *et al.*, 2005), disodium peroxodisulphate ($Na_2S_2O_8$) (e.g. Eusterhues *et al.*, 2003), or sodium hypochlorite (NaOCl) (e.g. Kleber *et al.*, 2005; Mikutta *et al.*, 2005a). Furthermore, in acid subsurface horizons treatment with NaOCl was coupled with a subsequent demineralization step using 10% HF (NaOCl+HF) in order to retain a recalcitrant SOM fraction (Mikutta *et al.*, 2006).

Hydrolysis removes compounds that are supposed to be potentially biodegradable, e.g. proteins, nucleic acids or polysaccharides, leaving behind a fraction of recalcitrant biomacromolecules (e.g. cutans, suberans). Consequently, Six *et al.* (2002) suggested that hydrolysis using 6 M HCl in a single step is the simplest and best available technique for defining a stable organic matter fraction in soil. In order to avoid possible artifacts by Maillard-type reactions, i.e. random condensation of labile macromolecules, mainly polysaccharides and proteins, Poirier *et al.* (2003) proposed a stepwise hydrolysis using TFA and HCl in increasing concentrations and reaction times.

Treatments using oxidizing reagents (H_2O_2, $Na_2S_2O_8$, NaOCl) have been suggested to mimic biodegradation to the extent that treatments preferentially remove SOM in weak association with the mineral matrix with a prevailing modern isotopic signature from various soils and subsequently cause the relative enrichment of older, ^{14}C-depleted SOM components (Eusterhues *et al.*, 2003; Plante *et al.*, 2004; Mikutta *et al.*, 2006) more intimately associated with mineral surfaces (Kaiser & Guggenberger, 2007). Results of several studies on the size and stability of oxidation-resistant SOC vary greatly. Whereas Eusterhues *et al.* (2005) found the proportion of H_2O_2-resistant SOC to increase with soil depth and hence with C depletion, Plante *et al.* (2004) found no correlation between oxidation-resistant SOM and C depletion in the clay fraction of a silt loam derived from a cultivation chronosequence. Furthermore, mean ^{14}C ages of oxidation resistant SOM reported in the literature vary from modern to several thousands of years (e.g. Balesdent, 1996; Theng *et al.*, 1992; Eusterhues *et al.*, 2005, Kleber *et al.*, 2005).

2 Objectives

Summarizing chapter 1, despite many efforts, a quantitative understanding and a prognosis of the development of different C pools in soils under different conditions is not yet possible due to a shortage of information about the prevailing factors controlling the stabilization processes. Therefore, the objective of the present work was to evaluate the capability of present fractionation methods for obtaining functional SOM pools and to get a better understanding of the mechanisms of SOM stabilization and their controls.

Investigations of SOM in density and aggregate size fractions

Cultivation of virgin soils results in a decrease in SOC and total nitrogen (N_t) contents. Maximum losses of SOC after cultivation affect the POM fraction, because tillage breaks up large macroaggregates and exposes some of the labile organic matter to microbial attack (Cambardella & Elliott, 1992; Besnard *et al.*, 1996). The type of land use controls the magnitude of SOC stocks, but it also influences the composition and quality of organic matter in soils. However, only few studies focus on the effect of land use on the composition of different SOM fractions (Preston *et al.*, 1994; Guggenberger *et al.*, 1994, 1995; Golchin *et al.*, 1995; Gregorich *et al.*, 1996).

In this part of the study, ^{13}C CPMAS NMR spectroscopy was used to analyze the chemical structure of SOM in density and aggregate fractions isolated by John *et al.* (2005). The main objectives were (i) to analyze the changes in composition that organic matter undergoes when passing from plant litter to SOM in different density and aggregate fractions in silty soils, (ii) to determine the effect of land use (spruce, grassland, maize) and related litter composition on the composition of SOM in different fractions, and (iii) to examine whether the composition of SOM contributes to the different stability of SOM in density and aggregate fractions.

Suitability of chemical fractionation procedures for obtaining stable pools of soil organic C

A range of different chemical fractionation procedures has been applied for obtaining stable SOC fractions (cp. chapter 1.4.2), but still there is no general

accepted method for isolating SOC fractions with apparent C turnover times of centuries to millennia. Moreover, the different methods have not yet been tested and compared systematically using different soil types and land use regimes.

Therefore, the objective of this part of the study was to evaluate the capability of different chemical fractionation methods (treatment with H_2O_2, $Na_2S_2O_8$, NaOCl, NaOCl+HF and stepwise hydrolysis) to isolate stable SOC. The fractionation methods were applied to soils comprising different texture and land use regimes and compared on the basis of their efficiency to remove SOC and the stability of the residual SOC fractions as determined by their isotopic contents (^{13}C, ^{14}C).

Comparison of the SOC fractions obtained by chemical fractionation with the inert organic matter pool of the Rothamsted Carbon Model

As a contemporary model of SOC turnover, the Rothamsted Carbon Model contains five pools, four of which are assumed to consist of SOM underlying first-order decay, i.e., decomposable and resistant plant material, microbial biomass, and humified organic matter (Jenkinson & Rayner, 1977; Jenkinson *et al.*, 1992). The fifth pool, termed inert organic matter (IOM) pool, is supposed to underlie no decomposition during the modeled time-period. The amount of IOM can significantly contribute to the total C stock and hence uncertainties in the size of the IOM pool may be a major source of error in modeling SOC (Falloon & Smith, 2000). Consequently, an operational fractionation procedure capable of isolating a stable SOC fraction would strongly improve the modeling of long-term C dynamics. Therefore, the size of the residual SOC fractions was compared with that of the IOM pool calculated using the Rothamsted Carbon Model and isotope data.

Assessing the suitability of thermal oxidation for obtaining stable SOC pools

Close correlations between thermally labile organic matter and the amount of O-alkyl-C determined by ^{13}C NMR analysis and between the thermally resistant organic matter and the amount of aromatic C determined by ^{13}C NMR analysis were observed in an investigation on density fractions of a silty clay loam under bare fallow, arable rotation and grassland (Lopez-Chapel *et al.*, 2005). A correlation of thermal stability with the proportion of aromatic structures of SOM was also observed by other authors (Ahmed *et al.*, 2002; Czimczik *et al.*, 2002).

Further, thermal stability of organic matter correlated with CO_2 evolution rates during soil incubation (Siewert, 2001) and was found to be higher in the heavy, mineral-associated SOM fraction compared to the light fraction (Schulten & Leinweber, 1999; Lopez-Chapel *et al.*, 2005). Consequently, thermal oxidation with increasing temperature causes progressive degradation of organic compounds according to their thermal stability, which in turn relates to the nature of the organic compounds and binding on mineral particles. This led to the hypothesis that thermal stability of SOM may be related to stability against degradation.

In this part of the study, the suitability of thermal oxidation for obtaining stable SOM pools was tested by investigating two soils with different texture and land use regimes including a C_3-/C_4-vegetation change. If thermal oxidation was able to isolate stable SOM, the thermally stable fraction should (i) be a larger proportion of initial, mineral-bound SOC in more C-depleted soils (agricultural Ap and E horizon) compared to C-rich soils (grassland, forest soil), because the more labile components of SOM are consumed first when C inputs decrease, and (ii) be of higher stability as defined by decreasing proportions of young, maize-derived SOC in thermally stable fractions and hence a higher apparent C turnover time determined by natural ^{13}C abundance.

<u>Effect of residue decomposability and the structure and activity of the microbial biomass on the formation of macroaggregates and C sequestration in aggregate fractions</u>

Only few studies which deal with aggregate dynamics involve direct investigations on the factors controlling aggregation. Thus, many conclusions rely on indirect observations (e.g. land use effects on aggregation; increasing SOC losses after disruption of aggregates) and no insight into the mechanisms controlling aggregate formation is provided. The relevance of bacterial and fungal biomass and their activity for macroaggregate formation was interpreted controversially. Further, the impact of residue decomposability on the formation of stable macroaggregates or the relation between the dynamics of macroaggregate stability and that of the activity and composition of the microbial biomass are largely unknown.

To make a contribution in this context, the aim of this part of the present work was to investigate the role that (i) the residue decomposability and (ii) the size, activity and composition of the microbial biomass pool play in the formation of macroaggregates. Further, (iii) the sequestration and stabilization of SOC and N_t during the processes of soil aggregation was investigated. A silty loam under wheat was sampled for incubation. After the destruction of all macroaggregates, the soil was incubated with and without addition of ^{15}N labeled maize residues of different decomposability (leaf and coarse root material) with and without application of fungicide for a three month period. Soil respiration, microbial properties, soil aggregation and partitioning of the added C and N were investigated at regular time intervals.

3 Materials and methods

3.1 Study sites

3.1.1 Rotthalmünster

This site is a long-term experimental field of the “Höhere Landbauschule” in Rotthalmünster, Germany (48°21´N/13°11´E (Figure 3.1), elevation: 360 m A.S.L.). The mean annual precipitation and temperature are 886 mm and 8.7°C, respectively. The soil is a Stagnic Luvisol derived from loess; soil texture is silty loam with 11% sand, 72% silt and 17% clay. Soils under the following land uses were chosen for investigation: (i) continuous wheat (*Triticum aestivum L.*) since 1969 on former grassland, (ii) continuous maize (*Zea mays L.*) since 1979, (iii) continuous grassland since 1961, and (iv) a nearby Norway spruce stand (*Picea abies L.*) since 1920.

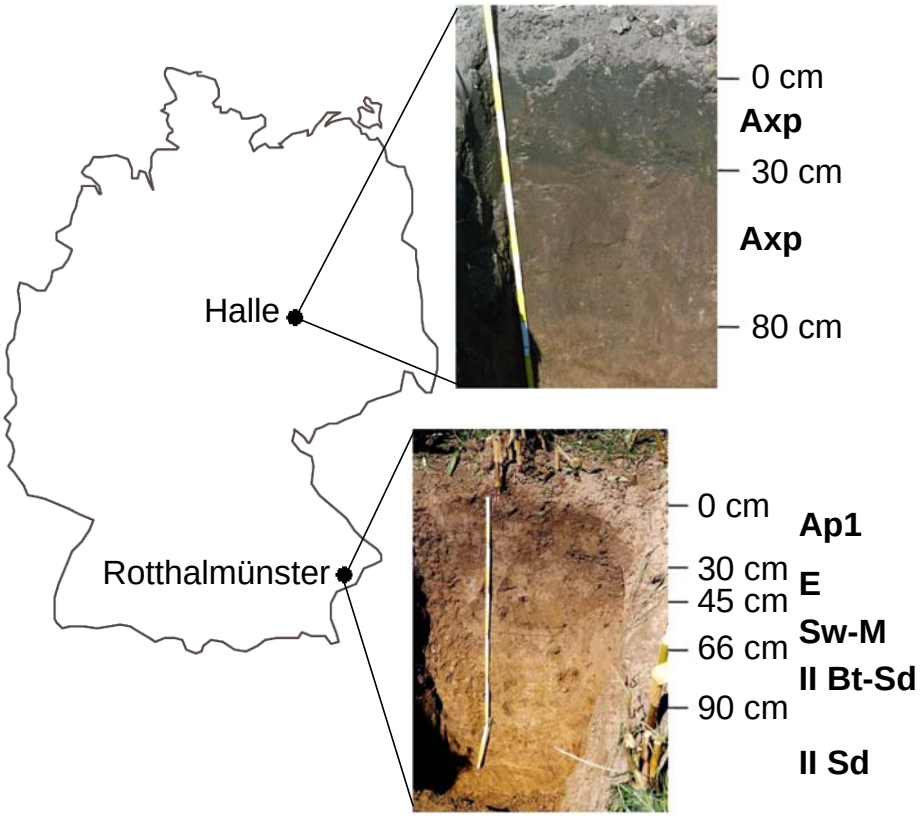

Figure 3.1: Location of the long-term field experiments and soil profiles from the arable soils of the study sites (Photographs by H. Flessa).

On the agricultural fields, tillage depth was 30 cm. Since 1998, conservation tillage (grubbing instead of plowing) took place on the wheat field, and tillage

depth was reduced to 20 cm. Maize and wheat straw was incorporated into the soil after harvest. The mean yield of straw C (aboveground) was 0.46 kg m^{-2} $year^{-1}$. The mean N-fertilization rate was 180 kg N ha^{-1} $year^{-1}$. Liming was done every few years – the last time in 2001 (Schnellhammer & Sirch, 2001). The vegetation of the grassland was dominated by the grass species *Trisetum flavescens* and *Alopecurus pratensis*. It was cut four times a year and the nitrogen fertilization rate (physiologically alkaline fertilizers) was 160 kg N ha^{-1} $year^{-1}$. The grassland was limed regularly – the last time in 1991. General soil properties are listed in Table 3.1.

3.1.2 Halle

This site belongs to the long-term field experiment "Eternal rye" in Halle, Germany (55°30.8´N/11°59.9´E (Figure 3.1), elevation: 110 m A.S.L.), which was established in 1878 as a continuous rye cropping (*Secale cereale L.*). In 1961, after harvest, continuous maize (*Zea mays L.*) cropping was started on one plot of the continuous rye field. The soil is located in the central German arid region and the mean annual precipitation and temperature are 465 mm and 9.2°C, respectively. The soil is a Haplic Phaeozem; soil texture is loamy sand with 70% sand, 20% silt and 10% clay (Garz *et al.*, 1996). Halle is located in an industrial area where soot and coal dust inputs from the nearby chemical industry, steam locomotives, thermal power stations, coal loading, and domestic fuel took place (Schmidt *et al.*, 2000). This resulted in a large amount of black carbon (~2 g kg^{-1} soil) compared to the Rotthalmünster soils (0.5–1.2 g kg^{-1} soil) (Table 3.1). The amount of fossil C calculated using ^{14}C data was even larger (~50% at the Halle site compared to a small amount of fossil C at the Rotthalmünster site, Rethemeyer, 2004). We investigated the following treatments of the field experiment: (i) continuous maize since 1961 with mineral NPK fertilization, and (ii) continuous rye since 1878 with mineral NPK fertilization. The rye was threshed and straw removed from the field. Maize was used for silage making and only short maize stubbles were ploughed in. The depth of the plough horizon was 20 cm until 1969. From 1970 onwards, depth of tillage increased to 25 cm and further to 30 cm in 2000. Liming was done every few years to avoid acidification – the last time in 1985 (Schliephake *et al.*, 2000). Table 3.1 shows general soil properties of this study site.

Table 3.1: Bulk density (BD), pH (in 0.01 M $CaCl_2$), soil organic carbon (SOC), total nitrogen (Nt), black carbon (BC), $\delta^{13}C$, apparent C turnover time determined by ^{13}C natural abundance, ^{14}C activity, and mean ^{14}C age of the soils in Rotthalmünster ("R") and Halle ("H") (means and standard errors, n = 4).

Site	Soil depth	BD	pH	SOC	N_t	BC	$\delta^{13}C$	Apparent C turnover time	^{14}C activity	Mean ^{14}C age
	/cm	/g cm^{-3}		———	/g kg^{-1}	———	/‰ V-PDB	/years	/pMC	/years BP
R-Wheat	0–30	1.45	6.5	12.0 (0.7)	1.3 (0.1)	0.58 (0.1)[1)]	-26.5 (0.1)		n.d.	n.d.
	30–45	1.58	6.6	4.5 (0.1)	0.6 (0.0)	0.47 (0.0)[1)]	-25.6 (0.1)		n.d.	n.d.
R-Maize	0–30	1.38	6.9	13.0 (0.1)	1.4 (0.0)	0.59 (0.0)[1)]	-21.6 (0.4)	54 (+4/-4)	102.7[3)]	modern[3)]
	30–45	1.53	6.5	6.8 (0.2)	0.8 (0.0)	0.48 (0.0)[1)]	-23.4 (0.2)	144 (+9/-8)	n.d.	n.d.
R-Grass	0–10	1.23	5.9	24.5 (0.3)	2.4 (0.0)	0.67 (0.1)[1)]	-28.0 (0.1)		111.5	modern
R-Forest	L (+12–+8)	0.20	3.4			n.d.	-26.0 (0.2)		n.d.	n.d.
	Of (+8–+3)	0.23	3.0			n.d.	-25.8 (0.1)		n.d.	n.d.
	Oh (+3–0)	0.36	2.9			n.d.	-26.0 (0.0)		n.d.	n.d.
	0–7	0.91	3.2	40.5 (0.7)	1.9 (0.0)	1.2 (0.1)[1)]	-25.7 (0.0)		108.3	modern
H-Rye	0–20	1.40	5.7	10.7 (0.5)	0.9 (0.1)	1.8 (0.2)[2)]	-25.8 (0.1)		n.d.	n.d.
H-Maize	0–20	1.45	5.7	10.6 (0.3)	0.8 (0.0)	1.9 (0.0)[2)]	-23.6 (0.1)	282 (+28/-23)	52.0	5248

[1)] Brodowski *et al.* (2006); Quantification of BC using benzene polycarboxylic acids as specific markers
[2)] Brodowski (2005); Quantification of BC using benzene polycarboxylic acids as specific markers
[3)] Rethemeyer (2004)
n.d.: not determined

3.2 Soil and plant sampling

The **Rotthalmünster** soil was sampled in September 2002 with four replicates per soil horizon. Undisturbed (250 cm^{-3}) and mixed soil samples were taken from the Ap (0–30 cm) and E horizon (30–45 cm) of the wheat and maize site, the Ah horizon (0–7 cm) of the forest stand, and the Ah horizon (0–10 cm) of the continuous grassland. Additionally, samples of the humus layer (moder) of the forest soil representing the different stages of litter decomposition (horizons: L, Of, Oh) were taken (small plots of 0.25 m^2). Shoot and root material was collected from the continuous wheat, maize and grassland plots as well as needles, twigs and cones from the spruce site. The **Halle** soil was sampled in September 2000 with four replicates per soil horizon. The Ap horizon (0–20 cm) of the maize and rye fields with NPK fertilization was sampled as well as shoot and root material from the rye and maize plots. Samples were oven-dried at 40ºC (soil samples) or 60°C (plant materials), ball-milled and stored until use.

3.3 Fractionation of water-stable aggregates

Water-stable aggregates of the surface soils were isolated using the method described by John *et al.* (2005). Briefly, one hundred grams of soil were soaked in distilled water for 10 minutes to allow slaking. The mixture was poured on a 2000-µm sieve, which was moved up and down in water by about 3 cm with 50 repetitions. The aggregates > 2000 µm were collected and sieving was repeated using a 1000 µm-sieve. Then, the 250–1000 µm and 53–250 µm fractions were collected as described above. The supernatant water of all aggregate fractions was collected and combined. Clay particles in the supernatant were precipitated with 0.5 M $AlCl_3$ and the precipitate was combined with the fraction < 53 µm. All aggregate size classes were air dried at 25°C.

Since there is little or no binding of OM with sand particles, a correction for the sand content is necessary. Carbon concentration in sand-free aggregates was calculated using equation [2] (Elliott *et al.*, 1991):

$$C_{sand\text{-}free}\ [g\ kg^{-1}\ fraction] = C_{fraction} / (1 - sand\ proportion_{fraction} / 100) \qquad [2]$$

where $C_{fraction}$ was the C concentration (g kg^{-1} fraction) in the respective aggregate fraction and sand $proportion_{fraction}$ was the relative proportion of sand (%) in the respective aggregate fraction.

3.4 Density fractionation

3.4.1 Density fractionation for obtaining free and occluded particulate organic matter fractions

The density fractionation of the surface soils was carried out by Bettina John at the Institute of Soil Science and Forest Nutrition. Figure 3.2 shows the fractionation scheme as given in John *et al.* (2005).

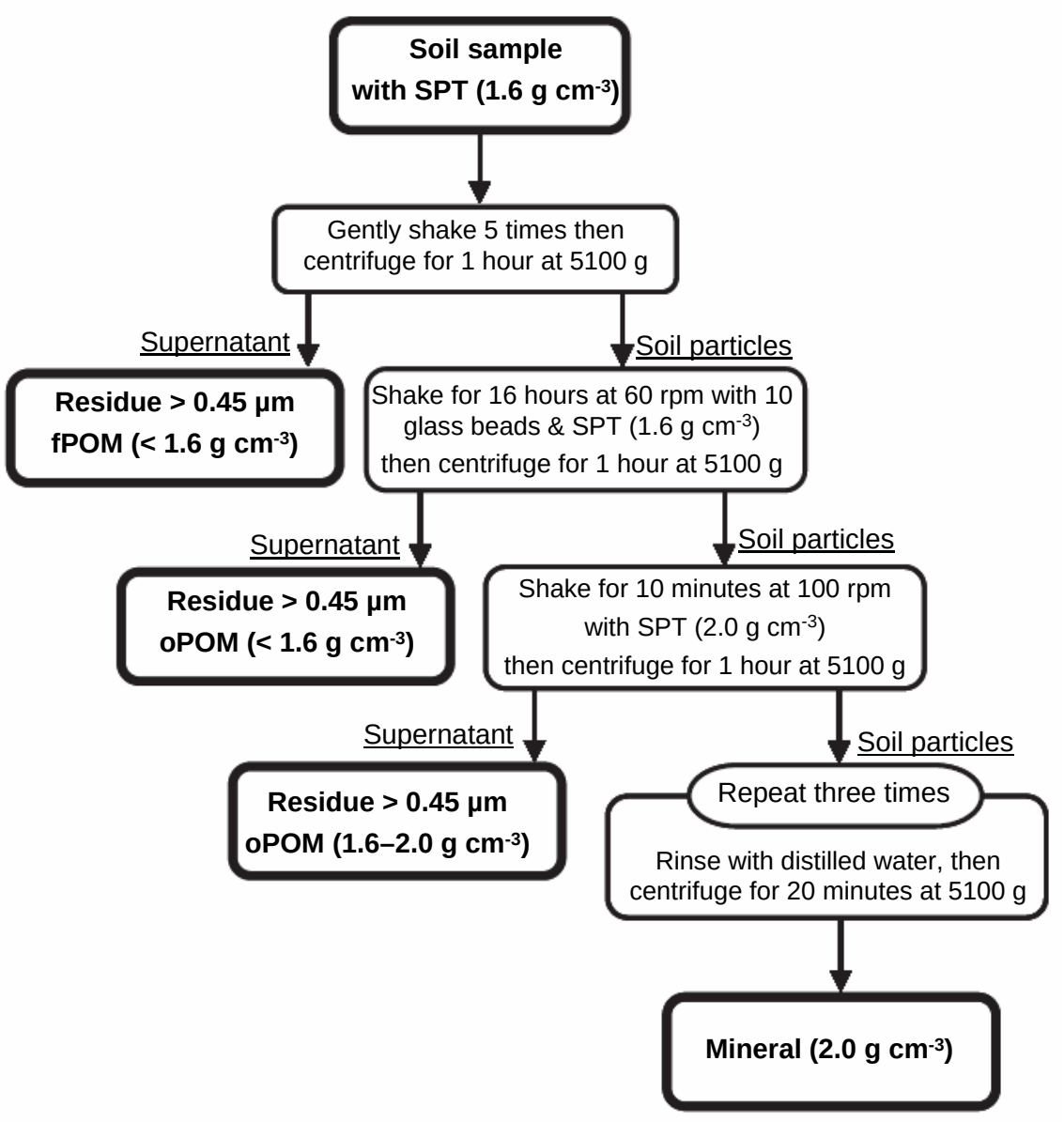

Figure 3.2: Density fractionation scheme (from John *et al.*, 2005).

Ten grams of air dried soil (< 2 mm) were placed in a centrifuge tube. Forty millilitres of sodiumpolytungstate solution (Sometu, Berlin, Germany) with a density of 1.6 g cm^{-3} was added. After shaking the tube gently five times by hand, the solution was allowed to settle for 30 minutes. Thereafter, it was centrifuged at 5100 g for 1 hour. The supernatant with floating particles was vacuum filtered (0.45 µm) and washed with distilled water to gain the free POM with a density of 1.6 g cm^{-3} ($fPOM_{<1.6}$). The sediment was dispersed with 40 ml of sodiumpolytungstate solution (1.6 g cm^{-3}) using a test tube shaker. To break up the aggregates, the solution was shaken for 16 hours at a frequency of 60 oscillations per minute after addition of 10 glass beads of 5 mm diameter (Balesdent *et al.*, 1991). Then, the soil suspension was centrifuged for 1 hour at 5100 g. The supernatant with floating particles (occluded POM with a density of 1.6 g cm^{-3}, $oPOM_{<1.6}$) was vacuum filtered (0.45 µm) and washed with distilled water. The sediment was dispersed as described above but using sodium-polytungstate solution of a density of 2.0 g cm^{-3}. The supernatant with floating particles (occluded POM with a density of 1.6–2.0 g cm^{-3}, $oPOM_{1.6\text{-}2.0}$) was filtered under vacuum and washed. To remove the salt, the sediment containing the mineral-associated SOM fraction > 2.0 g cm^{-3} ($Mineral_{>2.0}$) was washed three times with distilled water. Finally, the sample was centrifuged and the supernatant was discarded. All fractions were dried at 40°C and the POM fractions were ground using a mortar and pestle.

3.4.2 Density fractionation for removal of fresh plant debris prior to chemical fractionation and thermal oxidation

Before chemical fractionation, particulate organic matter was removed by density fractionation with sodium polytungstate solution (Sometu, Berlin, Germany) adjusted to a density of 1.8 g cm^{-3} to avoid erroneous results, because POM mainly consists of young and more easily degradable organic matter (Trumbore & Zheng, 1996; Six *et al.*, 2001; John *et al.*, 2005), which may potentially survive some of the tested protocols, e.g. treatment with H_2O_2 or hydrolysis (v. Lützow *et al.*, 2006a). Following density fractionation, samples were washed three times with 40 ml distilled water and freeze-dried. Thus, subsequent chemical fractionation procedures were applied to mineral-associated SOM.

Since black carbon in the Halle soil likely contributes to stable SOM but is mainly associated with soil fractions of a density < 1.8 g cm^{-3}, treatments with H_2O_2 and $Na_2S_2O_8$ were additionally carried out without preliminary density fractionation.

3.5 ^{13}C CPMAS NMR spectroscopy

^{13}C CPMAS NMR spectroscopy was carried out on a set of samples of the Rotthalmünster soil. ^{13}C NMR spectra of water-stable aggregate size fractions (53–250 µm, 250–1000 µm, 1000–2000 µm, > 2000 µm) were obtained for the spruce forest and the continuous grass plot. Density fractions were analyzed for the forest site, the continuous grassland and the continuous maize plot. Because of the small yield of the $oPOM_{<1.6}$ fraction in the grassland and maize soil, this fraction was analyzed only at the spruce site. At the spruce site, spruce needles, twigs, cones and the different horizons of the humus layer (L, Of, Oh) were investigated. At the grassland and maize plot, root and shoot material were analyzed. To remove paramagnetic compounds and to increase the C content of the mineral soil fractions, these were treated with 10% hydrofluoric acid (HF) prior to ^{13}C CPMAS NMR spectroscopy (Kögel-Knabner, 1997).

^{13}C CPMAS NMR spectra were obtained using a Bruker AMX 300 spectrometer with a MAS probe for 7 mm Bruker BL, double bearing Zirkonia spinner with Kel-F cap. This spectrometer operates at a ^{13}C frequency of 75.47 MHz and a ^{1}H frequency of 300 MHz. Cross-polarization with magic angle spinning was applied using a spin speed of 4950 Hz. The 90° pulse length was 4.5 µs. An acquisition time of 33 ms and a 5.7 µs pre-scan delay with a spectral width of 125 kHz was used. The recycle time was 1 s to obtain fully relaxed spectra. The Hartmann-Hahn condition was determined using glycine as standard. Based on variable contact time (VCT) experiments with back-extrapolation to zero that were carried out on plant material, litter and density fractions, a contact time of 1 ms was found to be optimal and used for all samples in this study. Shoot and root material as well as spruce needles, cones and branches were analyzed in triplicate (real replicates). Numbers of scans varied between 1000 and 200 000 depending on the organic carbon content of the samples. The spectra were processed with Bruker's 1D WINNMR software package version 6.

The spectra obtained were subdivided into four regions (Figure 3.3): (i) aliphatic or alkyl-C (0–45 ppm) of lipids, fatty acids, and plant aliphatic polymers; (ii) O-alkyl-C (45–110 ppm) deriving primarily from polysaccharides (cellulose and hemicelluloses), but also from proteins and side chains of lignin; (iii) aromatic or aryl-C (110–162 ppm), deriving from lignin, proteins, tannins and other non-oxygen substituted aromatic structures or from charcoal; and finally (iv) carbonyl-C (162–190 ppm) from aliphatic esters, carboxyl groups and amide carbonyls. Integration of the peaks within each of the chemical shift regions allowed estimation of the relative C contents expressed as percentages of total area.

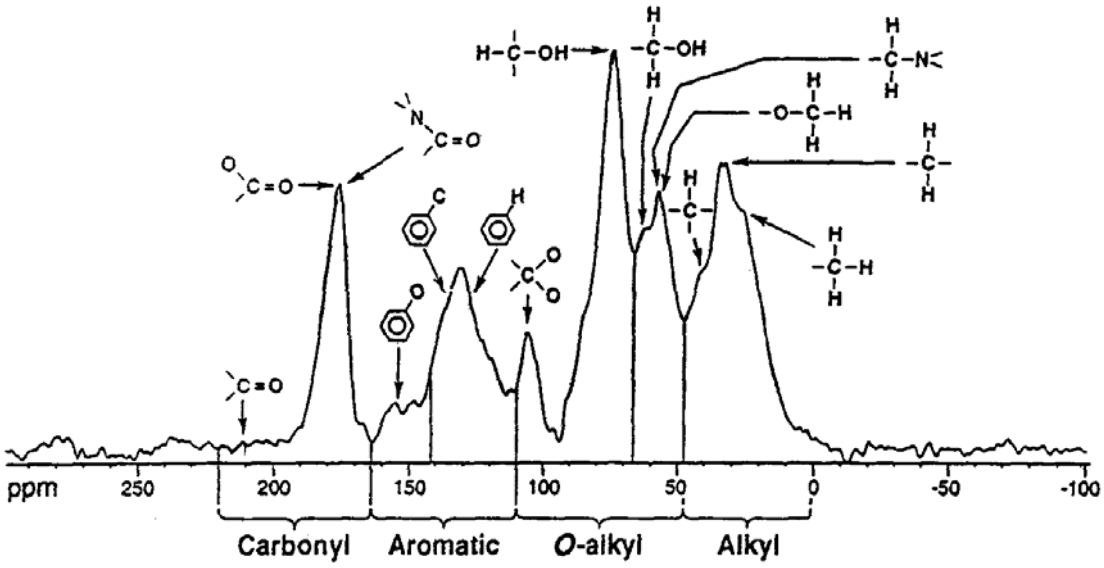

Figure 3.3: The chemical shift (adopted from Skjemstad *et al.*, 1997).

The aromaticity of the organic matter was calculated from equation [3] (Hatcher *et al.*, 1981; Guggenberger *et al.*, 1995):

$$\text{Aromaticity} = \text{Aromatic C} / (\text{alkyl-C} + \text{O-alkyl-C} + \text{aromatic C}) \times 100 \qquad [3]$$

The given relative C contents have to be interpreted with care due to some limitations that solid-state ^{13}C NMR has according to its quantitative reliability, because (i) different types of C respond differently well to CP. Thus, when a single contact time is used, not all C atoms may have cross-polarized completely. Variable contact time experiments with backextrapolation to intensity at time t = 0 as done in this study can be used to overcome this problem and enhances

the quality of the obtained data (Wilson, 1987). As mentioned above, (ii) C atoms close to a paramagnetic centre may not be detected, a problem that may be overcome to some degree by treatment with HF prior to NMR analysis (Kögel-Knabner, 1997) as done in this study, and (iii) some signals may cause spinning sidebands if the spinning speed is low. In well-resolved spectra, they may be detected and can be added to the peak they belong to, but if the spectrum has broad peaks, they may even be hidden by other signals (Kinchesh *et al.*, 1995). The whole chemical shift region (–50–300 ppm) is shown in this study to point out that spinning side bands affected the quantification only marginally due to the low aromaticity of the investigated samples.

3.6 Chemical fractionation

Chemical fractionation was carried out using the mineral-associated SOM fraction after density fractionation. The Ap horizon of the maize site at Halle and the Ap and E horizons of the maize site, the Ah horizon of the continuous grassland and the spruce forest site of the Rotthalmünster soil were used for the investigations. The continuous rye plot at Halle and the continuous wheat plot at Rotthalmünster were used as C_3-reference soils for the determination of proportions of young, maize-derived C in the obtained fractions and calculation of apparent C turnover times using ^{13}C analysis as described in chapter 3.9.

3.6.1 Treatment with hydrogen peroxide

The procedure was slightly modified from Plante *et al.* (2004). Here, 10% H_2O_2 instead of 30% H_2O_2 was used, because 30% H_2O_2 may be too concentrated for obtaining a stable SOC fraction. Briefly, one gram of soil was wetted with 10 ml distilled water for 10 minutes. Then, 90 ml H_2O_2 were added. The treatment was performed for 7 days at 50°C on a heatable magnetic stirrer using a glass coated magnetic stir bar to avoid C contamination due to abrasion. The sample was then washed three times with 40 ml distilled water and freeze-dried.

3.6.2 Treatment with disodium peroxodisulphate

The procedure was carried out as described by Menegatti *et al.* (1999). Briefly, half a gram of soil was dispersed in 250 ml distilled water by ultrasound (4 minutes, 440 J ml^{-1}). Then, 20 g $Na_2S_2O_8$ buffered with 22 g $NaHCO_3$ were

added. The experiment was run for 48 hours at 80°C on a heatable magnetic stirrer. The sample was washed twice with 40 ml distilled water. Since traces of carbonate from the buffer remaining in the sample may interfere with ^{14}C analysis of $Na_2S_2O_8$-resistant SOC (Mikutta *et al.*, 2005b; v. Lützow *et al.*, 2006a), 20 ml 0.01 M HCl were added to the sample dispersed in 20 ml distilled water in order to remove traces of carbonate left after the treatment as suggested by Meier & Menegatti (1997). After removal of carbonates, the sample was washed until neutral pH and freeze-dried.

3.6.3 Treatment with sodium hypochlorite and hydrofluoric acid

NaOCl. The procedure was carried out as given by Mikutta *et al.* (2006). In brief, 5-g samples were placed in 100 ml bottles and reacted three times with 50 ml of 6% NaOCl (pH 8) for a total of 18 hours. Afterwards, the samples were washed twice with 50 ml 1 M NaCl and twice with distilled water, dialyzed until the electric conductivity was < 40 µS cm^{-1}, and freeze-dried.

NaOCl+HF. Hypochlorite-treated subsamples of 3 g were weighed into centrifuge bottles and shaken four times with 20 ml of 10% HF for 2 hours. Finally, the samples were washed five times with 20 ml bi-distilled water and freeze dried.

3.6.4 Stepwise hydrolysis

The procedure was adopted from Poirier *et al.* (2003). Briefly, humic and fulvic acids were extracted in a first step using 0.1 M NaOH. The sample was then exposed to stepwise hydrolysis under reflux at 100°C using trifluoroacetic acid (TFA) and hydrochloric acid (HCl) with increasing concentrations (2, 4, and 6 M) and reaction times (2, 4, 8, 16, and 24 hours) with a ratio of sample to acid of one to three. After each reaction step, the sample was washed three times using 40 ml distilled water. After the final treatment, the sample was washed five times with 40 ml distilled water and freeze-dried.

3.7 Thermal oxidation

The same set of samples as used for chemical fractionation was used for assessing the suitability of thermal oxidation for obtaining stable SOM. The mineral-associated SOC fraction after density fractionation was exposed to

thermal oxidation for 24 hours in a chamber furnace at temperatures of 200, 225, 250, 275, 300, 400, 500 or 600°C. The temperature range was chosen based on findings of previous studies, which indicated that weight losses below 200°C derive mainly from losses of water as indicated by an endothermic DSC-curve (Leinweber *et al.*, 1992). Consequently, they were not accompanied by significant SOC degradation or changes in ^{13}C signature (Kuzyakov *et al.*, 2006). Exothermic reactions, pointing to oxidation of SOM, start at around 200°C and generally have two main peaks: The first one at around 300°C corresponds to oxidation of labile aliphatics and decarboxylation, while exothermal oxidation of more refractory aromatic C and of aliphatic C bound to clay minerals occurs at higher temperature (around 450°C) (Leinweber *et al.*, 1992). Thermal degradation of SOM was found to be largely completed at ~ 600°C (Leinweber & Schulten, 1992; Kuzyakov *et al.*, 2006). After thermal oxidation, the soil was weighed and stored for further analysis.

3.8 The incubation experiment

3.8.1 Experimental design

In September 2005, soil from the Ap horizon (0–30 cm) of the wheat site of the long-term experimental field of the Höhere Landbauschule in Rotthalmünster was collected for the incubation experiment. The soil was air-dried and sieved < 250 µm to destroy all macroaggregates at the start of the experiment. A hundred and fifty grams of dry, sieved soil was filled into 500-ml jars adjusted to a bulk density of 1 g cm^{-3} and brought to 60% of the maximum water holding capacity using distilled water. During incubation, the humidity of the soil was controlled by weight and corrected if necessary.

The following six treatments were applied with four replicates each:

(1) "Control" (soil without straw application without fungicide),
(2) "Control Captan" (soil without straw application with fungicide),
(3) "Leaf" (soil with maize leaf residues without fungicide),
(4) "Leaf Captan" (soil with maize leaf residues with fungicide),
(5) "Root" (soil with maize root residues without fungicide), and
(6) "Root Captan" (soil with maize root residues with fungicide).

For the treatments with incorporation of maize residues, the 150 g of dry and sieved soil were thoroughly mixed with 0.75 g (DW) of maize leaves (2105 µg C g^{-1} soil; C/N 27.4) or coarse maize roots (2110 µg C g^{-1} soil; C/N 86.4) and then filled into the jars and wetted. Maize leaves and roots were chosen due to their different degradability (roots have a larger C/N ratio and higher amounts of persistent components). General properties of the soil and plant materials used for the incubation experiment are given in table 8.1 and 8.2 (Appendix).

The added residues derived from maize plants which were grown in pots with coarse sand during the vegetation period 2005. The maize plants were watered with a nutrient solution that contained 98% labeled ammonium nitrate ($^{15}NH_4^+$ $^{15}NO_3^-$) in order obtain two isotope tracers (^{15}N from the nutrient solution and ^{13}C naturally enriched in the maize plant during photosynthesis) for the incubation experiment (Figure 3.4). Leaf, stem and root material were harvested separately and oven-dried at 40°C. Before application, plant materials (leaf material and coarse roots) were ground < 500 µm.

Figure 3.4: Breeding and harvesting of ^{15}N-labelled maize plants for the incubation experiment.

The fungicide application for treatments (2), (4) and (6) was carried out as given by Denef *et al.* (2001) and Bossuyt *et al.* (2001) by adding 0.3 g fungicide (Captan 50W wettable powder, 89% active ingredient) per 100 g soil.

The incubation was run for 84 days at 15°C. At the start of the experiment, the experiment consisted of 104 samples (Table 3.2).

Table 3.2: Overview of samples taken for analysis after 0, 14, 28, 56 and 84 day of incubation.

	Number of samples at day				
Treatment	**0**	**14**	**28**	**56**	**84**
Control	4	4	4	4	4
Control Captan	4	4	4	4	4
Leaf		4	4	4	4
Leaf Captan		4	4	4	4
Root		4	4	4	4
Root Captan		4	4	4	4
	8	24	24	24	24

The experiment was sampled destructively. The control with and without addition of fungicide was taken for analysis at day 0 (n = 8) and a complete set of treatments (n = 24) was taken for analyses at days 14, 28, 56 and 84.

3.8.2 Gas measurements

Carbon dioxide was measured continuously using an automated gas chromatographic (GC) system for CO_2 analysis (Flessa & Beese, 1995) of input air, exhaust air and calibration gases consisting of a ^{63}Ni-Electron Capture Detector (GC-ECD, Shimadzu). The headspace of each soil column was continuously flushed with a controlled flow (10 ml min^{-1}) of fresh air. The $\delta^{13}C$ values of the produced CO_2 was determined twice a week during the first five weeks of incubation and once a week thereafter by collecting gas samples in 2 ml vials at the outlet of the soil columns.

After day 56, the difference between the CO_2 concentrations of the fresh air input and the exhaust air of the jars was too low for accurate determination of the $\delta^{13}C$ values in the respired CO_2. Thus, columns were closed for at least 16 h for CO_2-enrichment before sampling with a syringe (50 ml) from the headspace. From the 50 ml syringe, a subsample was flushed through a 2 ml gas sample vial (Figure 3.5).

The $\delta^{13}C$ values of the collected air sample (δ_{out}) vials were measured in a GC-IRMS (Finnigan MAT, Delta C). Additionally, the $\delta^{13}C$ values of the input air

(δ_{in}) were measured with 5 replicates. The $\delta^{13}C$ values of the CO_2 originating from the soil columns ($\delta_{resp.}$) were calculated using equation [4]:

$$\delta_{resp.} = \left(\delta_{out} - \delta_{in} \times \frac{CO_{2\,in}}{CO_{2\,out}} \right) \times CO_{2\,out} / \left(CO_{2\,out} - CO_{2\,in} \right) \qquad [4]$$

where $CO_{2\ in}$ was the CO_2-concentration of the fresh air input and $CO_{2\ out}$ the CO_2- concentration of the sample.

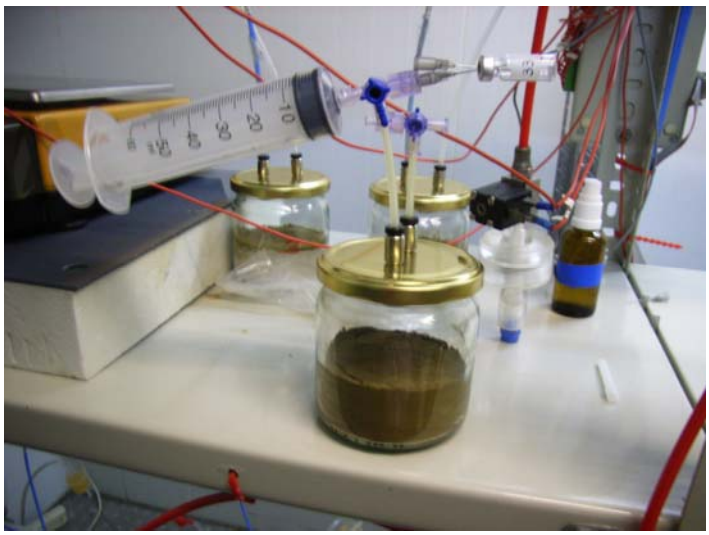

Figure 3.5: Collection of CO_2-enriched gas samples via syringe.

3.8.3 Soil microbial properties

Soil microbial properties were determined on the bulk soil after 0, 14, 28, 56 and 84 days of incubation. The properties of the bulk soil at each sampling day are listed in table 8.5 (Appendix).

3.8.3.1 Chloroform fumigation extraction (CFE)

Changes in the size of the microbial biomass pool were estimated using chloroform-fumigation-extraction (CFE) (Vance *et al.*, 1987). A K_2SO_4 solution of 0.05 M was used for the extraction instead of 0.5 M K_2SO_4, because large quantities of salt hamper adequate determination of isotopes using EA-IRMS (^{13}C, ^{15}N) by affecting the oxidation process – leading to irreproducible results (Potthoff *et al.*, 2003). Two portions equivalent to 15 g dry soil were taken from the soil sample, one portion was fumigated for 24 hours with ethanol-free $CHCl_3$. Following fumigant removal, the soil was extracted with 60 ml 0.05 M K_2SO_4 by

30 minutes horizontal shaking at 150 revolutions per minute and filtered. The non-fumigated portion was extracted similarly at the same time fumigation commenced. After determination of the C and N concentrations in the extract (Dohrmann DC 80; Cenco continuous flow), the microbial biomass C (respectively N) pool extractable by 0.05 M K_2SO_4 was calculated by subtracting the organic C (total N) extracted from non-fumigated soils from the organic C (total N) extracted from fumigated soils.

The $\delta^{13}C$ values of the extracts were determined on freeze-dried K_2SO_4-extracts by EA-IRMS (Finnigan Mat delta plus, Fissions EA, Bremen, Germany) and the $\delta^{13}C$ of the microbial biomass C pool ($\delta^{13}C_{MB}$) was calculated using equation [5] (Potthoff *et al.*, 2003):

$$\delta^{13}C_{MB} = (\delta^{13}C_{extr.fum} \times C_{extr.fum.} - \delta^{13}C_{extr.} \times C_{extr.}) / (C_{extr.fum} - C_{extr.}) \quad [5]$$

where $\delta^{13}C_{extr.fum}$ is the $\delta^{13}C$ value of the extract after fumigation, $\delta^{13}C_{extr.}$ is the $\delta^{13}C$ value of the non-fumigated extract, $C_{extr.fum}$ is the C concentration in the extract after fumigation and $C_{extr.}$ is the C concentration in the non-fumigated extract. The same equation was used for determination of the ^{15}N of the microbial biomass N pool.

The percentage of maize-derived C (or N) in the microbial biomass C (or N) pool *f* was calculated using equation [6]:

$$f = (\delta_{treatment} - \delta_{control}) / (\delta_{maize\ plant} - \delta_{soil}) \quad [6]$$

where $\delta_{treatment}$ is the $\delta^{13}C$ value (‰ V-PDB) or the ^{15}N value (atom%) of the C, respectively N, extract of the soil with added maize residue (either leaf or root), $\delta_{control}$ is the $\delta^{13}C$ (^{15}N) value of the C (N) extract of the control soil, $\delta_{maize\ plant}$ is the $\delta^{13}C$ (^{15}N) value of the maize residue itself (–12.7 ± 0.2‰ V-PDB and 7.4 ± 0.4 atom% for leaf; –11.9 ± 0.2‰ V-PDB and 7.9 ± 0.5 atom% for root material) and δ_{soil} is the $\delta^{13}C$ (^{15}N) value of the control soil (–26.5 ± 0.1‰ V-PDB; 0.369 ± 0.0 atom%).

3.8.3.2 Ergosterol

Ergosterol (ergosta-5,7,22-trien-3β-ol), which is the predominant fungal sterol (Newell *et al.*, 1987), was measured in 2 g moist soil or 5 g dry material of maize leaves / roots at the Department of Soil Biology and Plant Nutrition in Witzenhausen (Germany). The sample was extracted with 100 ml ethanol for 30 minutes by oscillating shaking at 250 revolutions per minute (Djajakirana *et al.*, 1996). Quantitative determination of ergosterol was performed by reversed-phase HPLC analysis using a column of 125 × 4 mm Sphereclone 5 µ ODS II with a Phenomenex guard column (4 × 3 mm). The chromatography was performed isocratically with 100% methanol as the mobile phase and a resolution in detection of 282 nm (Dionex UVD 170 S).

3.8.4 Aggregate fractionation

The fractionation of water-stable aggregates was carried out according to John *et al.* (2005) as described in chapter 3.3. Maize-residues that were swimming at the water surface and thus not protected within aggregates or in other association with the soil mineral matrix were carefully siphoned off.

3.9 General soil analyses

Soil fractions were finely ground, and SOC and N_t contents were determined using an automated C and N analyzer (Heraeus Vario EL). The $^{13}C/^{12}C$ isotope ratio of SOM was determined at the Centre for Stable Isotope Research and Analysis Göttingen (Germany) by an isotope ratio mass spectrometer (Finnigan MAT, DELTA^{plus}) and expressed as $\delta^{13}C$ (cp. chapter 1.3.1, equation [1]):

Under the assumption that the maize and reference site have a similar history and similar C dynamics, the proportion of C derived from maize in a sample was calculated using equation [7] (Balesdent & Mariotti, 1996):

$$f = (\delta_{sam} - \delta_{ref}) / (\delta_{maize} - \delta_{ref.plant}) \quad [7]$$

where f corresponds to the proportion of maize-derived C in the sample, δ_{sam} to the measured $\delta^{13}C$ of the sample, δ_{ref} stands for the $\delta^{13}C$ of the corresponding sample from the C_3 reference soil, and δ_{maize} and $\delta_{ref.plant}$ for the $\delta^{13}C$ values of the vegetation from the maize and the reference plot (Rotthalmünster: wheat; Halle:

rye). The $\delta^{13}C$ values (mean ± standard deviation) for the maize and wheat vegetation of the Rotthalmünster site were -12.7 ± 0.2‰ V-PDB and -26.8 ± 0.1‰ V-PDB (root and shoot material), respectively. For Halle, they were -11.6 ± 0.0‰ V-PDB and -28.4 ± 0.1‰ V-PDB for maize and rye, respectively (root and shoot material). The standard deviation s_f of maize-derived C proportions was calculated from the standard deviations of δ_{ref} and δ_{sam} under the assumption that contributions of uncertainties in δ_{maize} and $\delta_{ref.plant}$ were negligible (Ludwig *et al.*, 2003).

The apparent C turnover time T was calculated according to equation [8], assuming steady state conditions and homogeneous soil fractions that can be described with a single pool model:

$$T\ (\text{years}) = 1 / k = (t - t_0) / \ln(C_t - C_{t0}) \qquad [8]$$

where *k* is the rate constant of the first-order decay equation, *t* gives the time of sampling (year), t_0 the time of vegetation change (year), C_t is the proportion of remaining SOC derived from C_3-vegetation at the time of sampling (%) and C_{t0} is the percentage of SOC derived from C_3 plants at t_0 (%). For the continuous maize cropping plots *t* was 2002 (Rotthalmünster) and 2000 (Halle), t_0 was 1979 (Rotthalmünster) and 1961 (Halle), C_{t0} was 100%, and C_t was calculated as 100% – (*f* × 100%).

3.10 AMS ^{14}C measurements

The AMS measurements were carried out at the Leibniz-Laboratory in Kiel (Germany). Ground soil samples were transferred into pre-combusted quartz tubes with 450 mg copper oxide and 150 mg silver wool. The tubes were evacuated and subsequently flame sealed. The samples were combusted at 900°C for 4 hours, and the resulting CO_2 was subsequently trapped in liquid nitrogen and reduced at 600°C with H_2 over about 2 mg of iron powder as catalyst. The resulting carbon/iron mixture was pressed into a pellet in the target holder (Nadeau *et al.*, 1997, 1998).

The precision of measurements were about 0.3 pMC for modern, standard sized (1 mg C) samples (Nadeau *et al.*, 1998). ^{14}C concentrations were calculated from

the measured $^{14}C/^{12}C$ ratio of the samples compared to the NIST-oxalic acid 2 standard (NIST, National Institute of Standards and Technology, Gaithersburg, MD), both corrected for isotopic fractionation using the simultaneously measured $^{13}C/^{12}C$ ratios. The ^{14}C activity is expressed in pMC with 1-σ measurement uncertainty according to Stuiver & Polach (1977).

Since soils are open systems which continuously receive organic carbon from plant residues and loose gaseous and dissolved carbon, SOM is a heterogeneous mixture of organic compounds with ^{14}C ages ranging from recent to more than 20 000 years (Scharpenseel & Becker-Heidmann, 1992). In consequence, conventional ^{14}C ages of SOM do not represent the soil age but reflect the average age of different organic compounds, the mean age (Wang *et al.*, 1996). This can be calculated using equation [9]:

$$\text{Mean } ^{14}C \text{ age [years BP]} = -8033 \text{ years BP} \times \ln(^{14}C \text{ activity} / 100) \quad [9]$$

3.11 Statistical analysis

All statistical analyses were carried out using the STATISTICA 6.1 software package (StatSoft Inc., Tulsa, Oklahoma, USA). The data were analyzed by one-way analysis of variance followed by the Student-Newman-Keuls test. The Mann-Whitney U test was carried out if only two sample groups were compared. The statistical significance was evaluated at the $p < 0.05$ level.

4 Results

4.1 Investigations of organic matter composition in litter, aggregate and density fractions

4.1.1 Spruce site

The dominant peaks in the ^{13}C CPMAS NMR spectra of needle, twig and cone samples, organic layers and different fractions of the mineral soil (0–7 cm) of the spruce site were detected in the alkyl-C and O-alkyl-C region (Figure 4.1a,b).

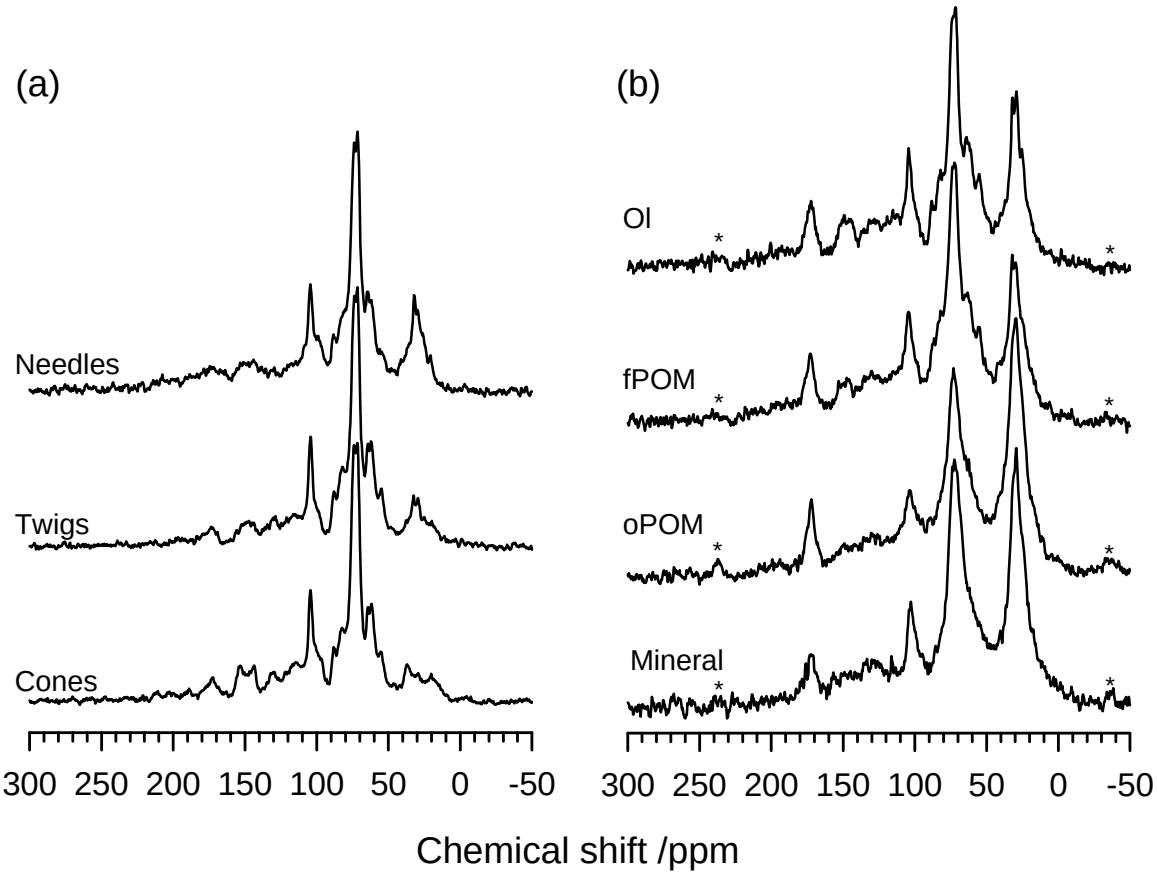

Figure 4.1: ^{13}C NMR spectra of (a) forest litter materials (needles, twigs and cones) and of the (b) L horizon of the moder humus and of density fractions from the Ah horizon ($fPOM_{<1.6}$, $oPOM_{1.6\text{-}2.0}$, $Mineral_{>2.0\ g\ cm\text{-}3}$) of the spruce site. Spinning side bands are marked with "*".

For all samples, the dominant band in the alkyl-C chemical shift was that of methylene C (25–35 ppm) derived from long-chained aliphatic compounds. Broad resonances at 30–50 ppm also derive from proteins and peptides. The chemical shift region of O-alkyl-C had two distinct peaks (72–75 ppm: mainly cellulose; 105 ppm: hemicelluloses and other carbohydrates). Other components

that cause peaks in the region between 60–90 ppm are aliphatic side chains of lignin. Peaks in the aryl-C chemical shift region derive from lignin, tannin and other non-oxygen substituted aromatic structures. The carbonyl-C peak at 175 ppm derives from aliphatic esters, carboxyl groups and carbonyl-C of lignin, hemicelluloses, lipids and proteins. The O-alkyl-C content of spruce needles (64.4%, Table 4.1) was higher than that of the L horizon (52.7%), and the alkyl and the aryl-C contents were lower (Table 4.1).

Table 4.1: Organic carbon (C_{org}), total N (N_t), relative contents of alkyl-C, O-alkyl-C, aryl-C, and carbonyl-C, alkyl-C/O-alkyl-C and aryl-C/O-alkyl-C ratios of the horizons of the humus layer (L, Of, Oh) of the spruce site and of litter materials of the spruce site, the continuous grass and maize plots (means and standard deviation, n = 3).

Material	C_{org}	N_t	Alkyl-C	O-alkyl-C	Aryl-C	Carbo-nyl-C	Alkyl-C/ O-alkyl-C	Aryl-C/ O-alkyl-C
	/g kg^{-1} dry matter		/%					
Spruce forest site								
Spruce needles	470 (2.9)	12.8 (1.0)	18.7 (2.0)	64.4 (2.2)	12.1 (2.6)	4.8 (1.5)	0.29 (0.03)	0.19 (0.05)
Spruce cones	477 (6.2)	5.2 (0.7)	10.7 (1.0)	66.6 (1.6)	18.3 (1.0)	4.5 (0.2)	0.16 (0.02)	0.27 (0.02)
Spruce branches	480 (7.1)	8.9 (0.1)	14.2 (1.4)	66.3 (1.6)	15.4 (0.7)	4.2 (0.5)	0.21 (0.03)	0.23 (0.01)
L	458 (84)	17.9 (1.8)	23.5	52.7	17.3	6.5	0.45	0.33
Of	430 (7.5)	16.6 (0.2)	26.9	50.0	16.8	6.2	0.54	0.34
Oh	302 (18)	12.0 (0.3)	28.0	49.1	15.7	7.3	0.57	0.32
Grassland site								
Grass shoots	387 (13)	37.9 (3.0)	19.1 (0.7)	63.6 (1.5)	9.5 (0.9)	7.9 (1.4)	0.30 (0.01)	0.15 (0.02)
Grass roots	258 (79)	11.7 (1.1)	13.4 (0.3)	68.0 (3.9)	13.6 (2.4)	4.9 (1.7)	0.20 (0.01)	0.20 (0.05)
Maize site								
Maize shoots	424 (1.3)	7.0 (1.0)	11.1 (0.9)	79.0 (1.5)	7.0 (0.7)	2.9 (0.1)	0.14 (0.01)	0.09 (0.01)
Maize roots	387 (15)	4.1 (0.4)	9.5 (0.9)	77.8 (0.3)	9.4 (0.7)	3.3 (0.4)	0.12 (0.01)	0.12 (0.01)

With increasing degree of decomposition from the L to the Oh horizon, a further decrease in carbohydrates (from 52.7–49.1%) was evident, accompanied by an

increase in the alkyl-C content (from 23.5–28.0%). This resulted in an increasing alkyl-C/O-alkyl-C ratio from 0.45 (L) to 0.57 (Oh) (Table 4.1). Also, a slight decrease in the aromatic region from 17.3% (L) to 15.7% (Oh) was observed. No clear trend was found for the carbonyl-C content. The trend of increasing contents of alkyl-C and decreasing contents of O-alkyl-C and aryl-C persisted in the mineral soil (Ah horizon) as can be seen in aggregate size fractions with an average alkyl-C content of 30% alkyl-C, 48% O-alkyl-C, 14% aryl-C and 7% carbonyl-C (Figure 4.2a).

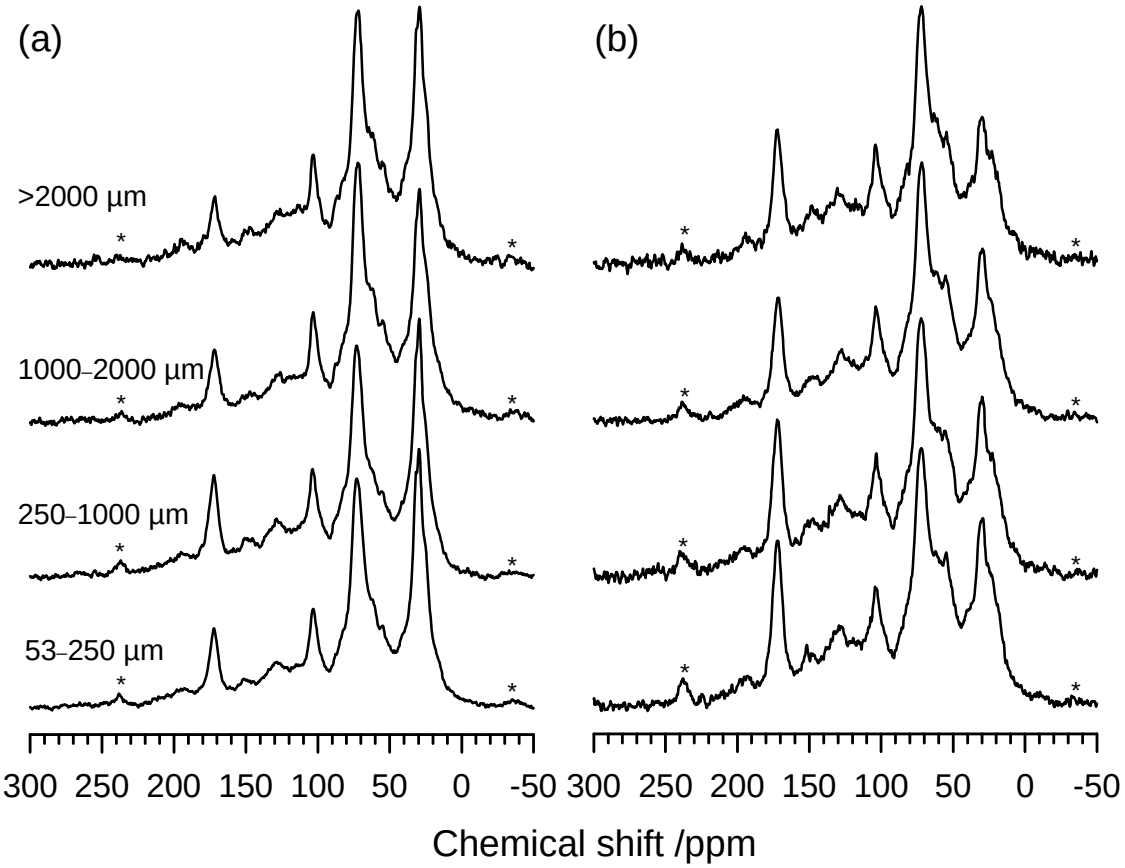

Figure 4.2: ^{13}C NMR spectra of aggregate size fractions (> 2000 µm, 1000–2000 µm, 250–1000 µm, 53–250 µm) of the Ah horizon of the (a) spruce forest and (b) grassland site. Spinning side bands are marked with "*".

The C/N ratios (19.1–21.4) suggested that the degree of decomposition was similar in all POM fractions. However, the ^{13}C CPMAS NMR analyses revealed differences in the composition between these POM fractions. The O-alkyl-C content decreased in the order $fPOM_{<1.6}$ (54.0%) > $oPOM_{1.6\text{-}2.0}$ (44.9%) > $oPOM_{<1.6}$ (40.2%), while the alkyl-C content increased in this order from 22.7%

Table 4.2: Organic carbon (C_{org}), total N (N_t) (means and standard deviation, n = 4), relative contents of alkyl-C, O-alkyl-C, aryl-C, and carbonyl-C, alkyl-C/O-alkyl-C and aryl-C/O-alkyl-C ratios of the density fractions (free particulate organic matter with a density < 1.6 g cm^{-3} ($fPOM_{<1.6}$) occluded particulate organic matter with densities < 1.6 g cm^{-3} ($oPOM_{<1.6}$) and 1.6-2.0 g cm^{-3} ($oPOM_{1.6-2.0}$) and mineral associated SOM > 2.0 g cm^{-3} ($Mineral_{>2.0}$) of the surface soils from the spruce site (0–7 cm), the grassland plot (0–10 cm) and the maize plot (0–30 cm).

Material	C_{org}	N_t	C_{org}	N_t	Alkyl-C	O-alkyl-C	Aryl-C	Carbonyl-C	Alkyl-C/ O-alkyl-C	Aryl-C/ O-alkyl-C
	/g kg^{-1} fraction		/g kg^{-1} soil		/%					
Spruce forest site (0–7 cm)										
$fPOM_{<1.6}$	214 (21)	10.8 (1.0)	13.4 (2.0)	0.68 (0.1)	22.7	54.0	16.5	6.8	0.42	0.31
$oPOM_{1.6-2.0}$	371 (22)	17.3 (0.8)	6.6 (1.0)	0.35 (0.1)	37.0	44.9	12.2	5.9	0.82	0.27
$oPOM_{<1.6}$	216 (31)	11.3 (1.4)	0.56 (0.1)	0.03 (0.0)	40.1	40.2	13.5	6.3	1.00	0.34
$Mineral_{>2.0}$	21.8 (1.0)	1.6 (0.1)	19.3 (0.8)	1.4 (0.1)	32.2	49.6	13.0	5.2	0.65	0.26
Continuous grass plot (0–10 cm)										
$fPOM_{<1.6}$	228 (22)	14.3 (0.3)	0.86 (0.1)	0.06 (0.0)	16.6	70.3	9.4	3.7	0.24	0.13
$oPOM_{1.6-2.0}$	191 (8)	15.8 (0.7)	2.1 (0.8)	0.17 (0.1)	19.7	55.4	18.0	6.9	0.36	0.33
$Mineral_{>2.0}$	20.8 (1.7)	3.1 (0.2)	20.0 (1.7)	3.0 (0.2)	20.7	56.4	15.0	7.9	0.37	0.27
Continuous maize plot (0–30 cm)										
$fPOM_{<1.6}$	320 (10)	16.8 (2.5)	0.51 (0.2)	0.03 (0.0)	16.2	64.1	14.8	4.9	0.25	0.23
$oPOM_{1.6-2.0}$	197 (9)	14.2 (n.d.)	1.0 (0.2)	0.07 (n.d.)*	21.7	52.3	18.6	7.4	0.41	0.36
$Mineral_{>2.0}$	11.3 (1.0)	1.5 (0.2)	10.8 (1.0)	1.4 (0.2)	17.4	48.1	23.7	10.8	0.36	0.49

*n.d. denotes not determined

in the $fPOM_{<1.6}$ fraction to 40.1% in the $oPOM_{<1.6}$ fraction (Table 4.2). The heavy mineral-associated SOM ($Mineral_{>2.0}$), which had the lowest C/N ratio of the density fractions, showed a considerably higher proportion of O-alkyl-C (49.6%) and a smaller content of alkyl-C (32.2%) than the oPOM fractions (Table 4.2).

The SOM of all aggregate size fractions consisted mainly of alkyl-C and O-alkyl-C which ranged from 30–32% and from 46–51%, respectively, and there was no clear trend with aggregate size (Figure 4.2a). The carbonyl-C contents ranged from 6–9% and the aryl-C contents varied between 12 and 16%, which was approximately the same as in the mineral-associated SOM fraction ($Mineral_{>2.0}$). There was no clear influence of the aggregate size on the alkyl-C/O-alkyl-C ratio or the aryl-C/O-alkyl-C ratio (Table 4.3).

Table 4.3: Organic carbon (C_{org}), total N (N_t) (means and standard deviation, n=4), alkyl-C/O-alkyl-C and aryl-C/O-alkyl-C ratios of water-stable aggregate size fractions isolated from the Ah horizon of the spruce site and the continuous grass plot.

Material	C_{org}	N_t	C_{org}	N_t	Alkyl-C/O-Alkyl-C	Aryl-C/O-Alkyl-C
	/g kg^{-1} fraction		/g kg^{-1} soil			
Spruce site (0–7 cm)						
$Forest_{>2mm}$	51.6 (3.5)	2.6 (0.3)	21.5	1.0	0.63	0.32
$Forest_{1-2mm}$	47.3 (12)	2.7 (0.6)	6.4	0.3	0.59	0.24
$Forest_{250-1000µm}$	43.2 (3.1)	2.7 (0.3)	8.6	0.4	0.65	0.34
$Forest_{53-250\ µm}$	49.7 (4.1)	2.9 (0.2)	8.3	0.4	0.66	0.28
Continuous grass plot (0–10 cm)						
$Grass_{>2mm}$	26.6 (2.5)	3.0 (0.2)	10.2	1.0	0.44	0.34
$Grass_{1-2mm}$	26.2 (1.2)	2.9 (0.03)	4.3	0.4	0.49	0.34
$Grass_{250-1000\ µm}$	26.9 (1.0)	3.2 (0.3)	7.2	0.7	0.50	0.37
$Grass_{53-250\ µm}$	24.3 (0.3)	2.8 (0.1)	3.7	0.4	0.47	0.31

4.1.2 Grassland site

The organic matter input in the grassland soil consisted mainly of aboveground and belowground grass biomass. The ^{13}C CPMAS NMR spectra of the root and shoot material of the sod showed that the fresh litter was dominated by O-alkyl-C (64–68%) and that the relative content of alkyl-C was lower (13% versus 19%) and that of aryl-C was higher (14% versus 10%) in the root biomass than in the shoot biomass (Figure 4.3a, Table 4.1). The higher proportion of aromatic C in the roots indicated higher lignin contents. Differences in the composition of aboveground and belowground plant litter were also evident from the C/N ratio (10.2 for the shoot biomass and 22.1 for the root biomass). Among the fresh litter analyzed in this study, grass shoots had the highest N content, and they were the only litter type showing a pronounced signal at 175 ppm in the ^{13}C CPMAS NMR spectrum. This sharp peak in the carbonyl region is made up of a combination of carboxyl, ester and amide groups. The high N content suggests that amides are the largest contributors to this peak.

The alkyl-C/O-alkyl-C ratio of the $fPOM_{<1.6}$ fraction (0.24) was between those of grass roots (0.20) and shoot material (0.30). This indicated that $fPOM_{<1.6}$ consisted mainly of fresh or slightly decomposed plant material. Upon decomposition of grass shoots and roots to oPOM and mineral-associated SOM, the O-alkyl-C contents decreased and the alkyl-C contents increased (Tables 4.1, 4.2). The proportion of aromatic C was higher in the oPOM than in the fPOM fraction. Together with the increase in the alkyl-C/O-alkyl-C ratio with decomposition / humification, there was an increase in the 175 ppm peak of the ^{13}C CPMAS NMR spectra indicating a relative accumulation of carboxyl and amide groups (Figure 4.3a), which was in line with the decrease in the C/N ratio in the order $fPOM_{<1.6} > oPOM_{1.6\text{-}2.0} > Mineral_{>2.0}$.

In the aggregate size classes of the grassland soil, O-alkyl-C contents made up 48–50% of the relative C contents (Figure 4.2b) and, as was found in the forest soil, there was no clear influence of aggregate size on the alkyl-C/O-alkyl-C or aryl-C/O-alkyl-C ratio, which suggests that the degree of SOM decomposition was similar in all aggregate size fractions (Table 4.3).

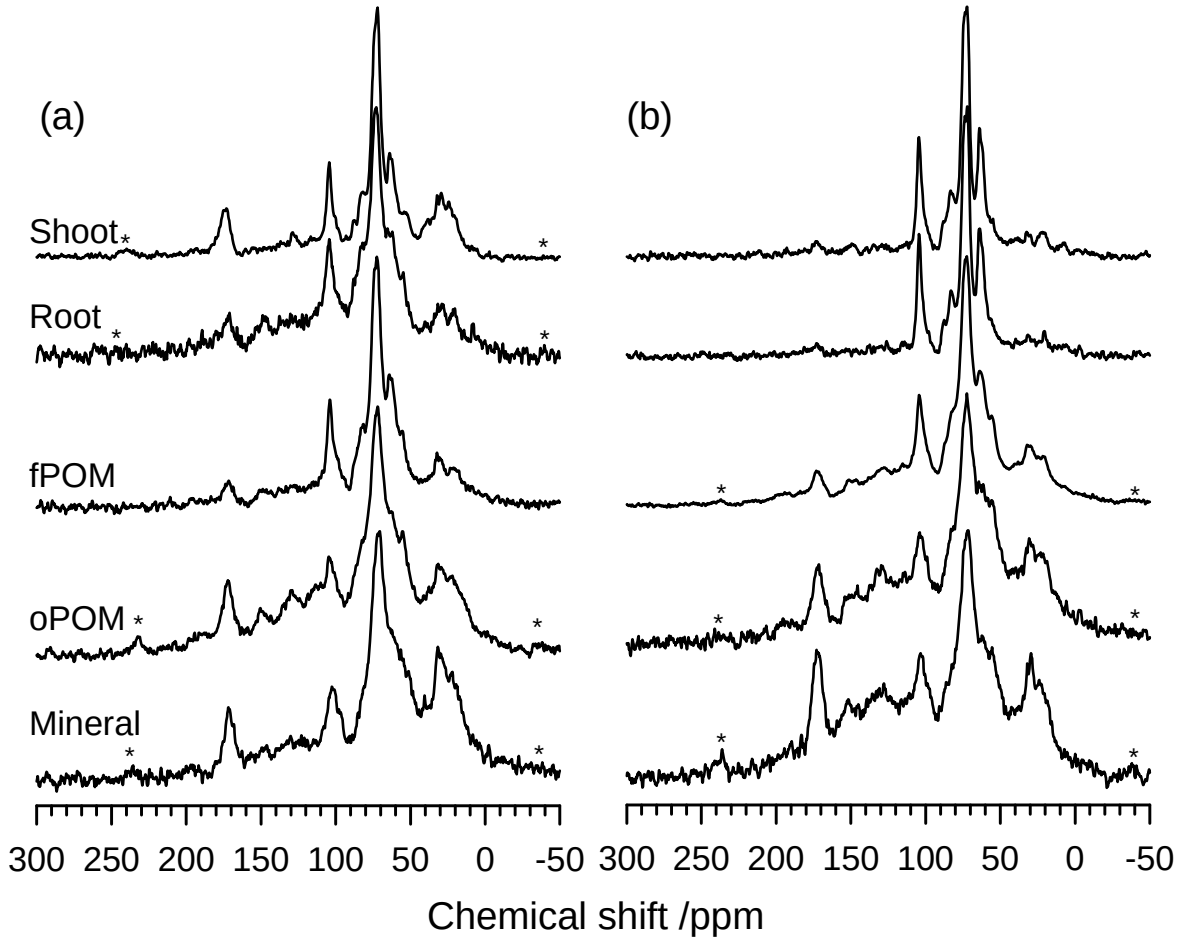

Figure 4.3: ^{13}C NMR spectra of root material (root), shoot material (shoot) and density fractions ($fPOM_{<1.6}$, $oPOM_{1.6\text{-}2.0}$, $Mineral_{>2.0}$) from (a) the Ah horizon of the grassland site and (b) the Ap horizon of the continuous maize plot. Spinning side bands are marked with "*".

4.1.3 Maize site

The ^{13}C CPMAS NMR spectra of the root and shoot material of maize showed distinct peaks in the O-alkyl-C region, but only small fractions of alkyl-C, aryl-C or carbonyl-C (Figure 4.3b). The dominating signals were from C_6-C and oxygenated C of carbohydrates (at 65–72 ppm) and from dioxygenated C of polysaccharides (at 105 ppm), mainly originating from cellulose and hemi-cellulose structures. The O-alkyl-C comprised 79% (shoot biomass) and 78% (root biomass) of the relative C content, indicating that, compared with the other litter types, the maize residues contained the largest proportion of easily decomposable carbohydrates (Table 4.1). The contribution of alkyl-C and aryl-C was only about 10–11% and 7–9%, respectively.

The O-alkyl-C content of the $fPOM_{<1.6}$ fraction (64.1%) showed that a considerable amount of carbohydrates decomposed during the initial stage of litter mineralization (Table 4.2). This loss of carbohydrates was also reflected by a decrease of the C/N ratio, which indicated a two fold (maize shoot) to about four fold (maize root) increase in the N concentration of the organic matter when passing from fresh litter to $fPOM_{<1.6}$. When progressing from the $fPOM_{<1.6}$ to the $oPOM_{1.6\text{-}2.0}$ fraction, the O-alkyl-C content decreased further to 52.3%, while the C/N ratio decreased from 19 to 14, and the relative contents of alkyl-C, aryl-C and carbonyl-C increased (Table 4.2).

The mineral-associated SOM fraction ($Mineral_{>2.0}$) had less O-alkyl-C and alkyl-C but more aromatic C and carbonyl-C than the $oPOM_{1.6\text{-}2.0}$ fraction (Table 4.2, Figure 4.3b). Thus, the alkyl-C/O-alkyl-C ratio was greater in the $oPOM_{1.6\text{-}2.0}$ fraction (0.41) than in the mineral-associated SOM (0.36). The aromaticity and the aryl-C/O-alkyl-C ratio increased in the order maize litter < $fPOM_{<1.6}$ < $oPOM_{1.6\text{-}2.0}$ < $Mineral_{>2.0}$.

4.2 Suitability of chemical fractionation methods for obtaining stable pools of soil organic C

4.2.1 Mineral-associated soil organic matter

After density fractionation, the Rotthalmünster soils covered a wide range of SOC contents (3.6–24.5 g kg^{-1} soil; Table 4.4); with significantly smaller amounts of SOC in the agricultural than in the grassland and forest soils. Carbon loss by density fractionation was highest in the forest soil (56%), which contained large amounts of particulate organic matter and amounted 9–28% in soils under the other land use regimes (Table 4.4).

The C/N ratio of SOM after density fractionation was lowest in the subsoils of the agricultural sites (4.4–5.9) and highest for the forest site (16.8) (Table 4.4). The apparent C turnover time was slightly higher for mineral-associated SOC than for bulk SOC, corresponding to the removal of young plant and animal debris by density fractionation. The ^{14}C content indicated a modern mean ^{14}C age of SOC in the maize soil. Density fractionation of the Halle soil caused higher SOC losses (~40%) compared to the agricultural sites in Rotthalmünster (20–30%) and resulted in a reduced apparent C turnover time and increased ^{14}C

activity compared to the bulk soil (Table 3.1; Table 4.4). This can be attributed to the high amounts of particulate black carbon at the Halle site (Table 3.1).

Table 4.4: SOC loss by density fractionation given as percentage of total SOC, SOC contents, C/N ratio, $\delta^{13}C$, and apparent C turnover time determined by ^{13}C natural abundance (means and standard deviation, n = 4), ^{14}C activity, and ^{14}C age (means and 1-σ measurement uncertainty) of the soil after density fractionation for the Rotthalmünster and for the Halle soils.

	Soil depth	SOC loss	C_{org}	C/N	$\delta^{13}C$	Apparent C turnover	^{14}C activity	Mean ^{14}C age
	/cm	/%	/g kg^{-1}		/‰ V-PDB	/years	/pMC	/years BP
Rotthalmünster site								
Wheat	0–30	27.6 (10.6)	8.6 (0.8)	7.4 (0.1)	-26.5 (0.1)		n.d.	n.d.
Maize	0–30	18.6 (9.8)	10.6 (1.2)	7.9 (0.8)	-21.9 (0.5)	58 (+9/-8)	106.5 (0.3)	modern
Grass	0–10	8.7 (10.3)	24.5 (4.8)	9.6 (0.1)	-28.0 (0.1)		n.d.	n.d.
Forest	0–7	56.3 (8.3)	17.8 (3.8)	16.8 (0.7)	-26.0 (0.1)		n.d.	n.d.
Wheat	30–45	21.3 (7.3)	3.6 (0.2)	4.4 (0.1)	-25.5 (0.1)		n.d.	n.d.
Maize	30–45	23.2 (9.1)	5.2 (0.7)	5.9 (0.2)	-23.5 (0.2)	151 (+23/-18)	97.5 (0.3)	205 (22)
Halle site								
Rye	0–20	39.5 (5.9)	6.4 (0.7)	10.8 (0.3)	-25.7 (0.1)		n.d.	n.d.
Maize	0–20	38.4 (10.5)	6.5 (0.5)	10.9 (0.2)	-23.0 (0.2)	220* (+24/-20)	64.9* (0.3)	3479* (32)

n.d.: not determined
* The apparent C turnover time, ^{14}C activity and mean ^{14}C age in the Halle soil are influenced by large amounts of fossil C in this soil (Rethemeyer, 2004)

4.2.2 Chemical fractionations, Rotthalmünster soil, A horizon

Stable SOC contents after chemical fractionation ranged from 0.8–4.2 g kg^{-1} soil and comprised 5–27% of the initial mineral-associated SOC (Table 4.5; Figure 4.4). The amount of $Na_2S_2O_8$- and H_2O_2-resistant SOC did not vary with land

use, hence, was independent from the current management practice. In contrast, the SOC contents of the hydrolysis- and NaOCl-resistant fractions were significantly larger than those of H_2O_2- and $Na_2S_2O_8$-resistant fractions and differed significantly among land uses. Demineralisation of the NaOCl-resistant fraction (NaOCl+HF) approximately halved the SOC contents of the NaOCl-resistant fraction, indicating that dissolution of minerals released only about 50% of mineral-associated SOC (Table 4.5).

The proportion of stable SOC after treatment with H_2O_2, $Na_2S_2O_8$, or stepwise hydrolysis increased with decreasing contents of initial, mineral-associated SOC under the different land uses. However, no such relation existed after treatment with NaOCl and NaOCl+HF (Figure 4.4).

Mineral-associated SOC in the Ap horizon of the maize soil contained 33% maize-derived SOC (Figure 4.5). All chemical treatments selectively removed this younger, maize-derived SOC (Figure 4.6a). Hence, all residual SOC fractions had higher apparent C turnover times than the corresponding mineral-associated SOC, which increased in the following order: hydrolysis < NaOCl < NaOCl+HF < H_2O_2 ≈ $Na_2S_2O_8$ (Table 4.6). Since treatment with H_2O_2 and $Na_2S_2O_8$ left very small amounts of maize-derived SOC, we were unable to derive reliable apparent C turnover times. Therefore only estimated maximum apparent C turnover times are given (Table 4.6).

The mean ^{14}C age of the removed SOC fractions as calculated by mass balance corroborated the results obtained by ^{13}C analysis. All methods preferentially removed young material with a ^{14}C activity of > 100 pMC and left a residual SOC fraction comprising a lower ^{14}C activity (Table 4.6). The mean ^{14}C age of the residual SOC fractions increased in a slightly different order compared to the apparent C turnover times determined by ^{13}C natural abundance: the hydrolysis-resistant SOC fraction had a mean ^{14}C age that was about twice that of the NaOCl-resistant fraction (2780 compared to 1110 years, Table 4.6). In contrast, the ^{13}C data indicated that the apparent C turnover time was higher after NaOCl treatment (211 years) than hydrolysis (104 years, Table 4.6).

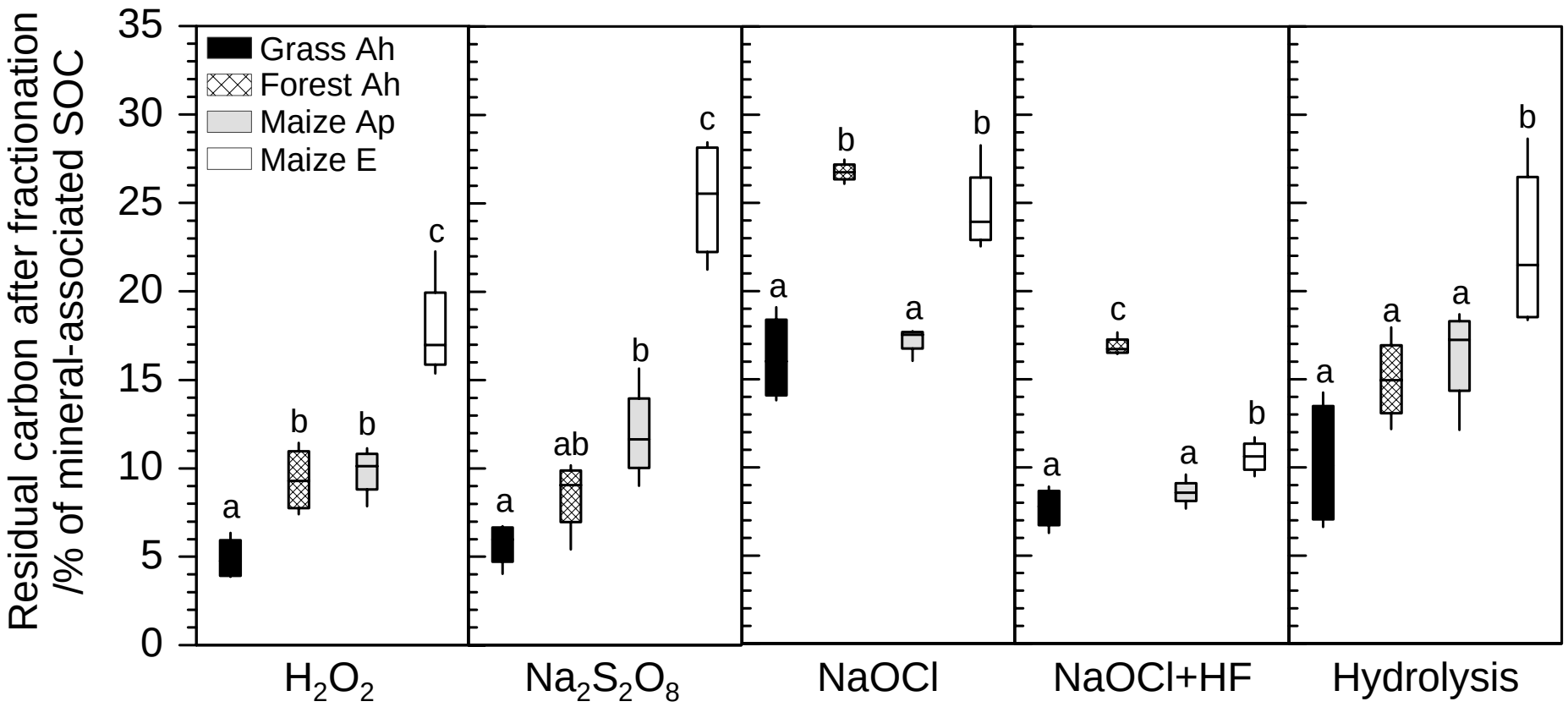

Figure 4.4: Box plots (n = 4) showing proportions of SOC resistant to chemical fractionation in the A horizon under continuous grass, forest and maize cultivation and E horizon under maize at the Rotthalmünster site; Letters from a–c indicate significant differences between land uses and soil depth for a single chemical treatment ($p < 0.05$).

Table 4.5: SOC contents and C/N ratio after the different chemical fractionations in the Rotthalmünster and Halle soils (means and standard deviation, n = 4). Letters from a-e (read from the left to the right) indicate significant differences between the chemical treatments for a single land use type.

	H_2O_2	$Na_2S_2O_8$	NaOCl	NaOCl +HF	Hydro-lysis	H_2O_2	$Na_2S_2O_8$	NaOCl	NaOCl +HF	Hydro-lysis
	C /g kg^{-1}					C/N				
Rotthalmünster site										
Wheat	1.1^{a}	1.5^{b}	1.7^{bc}	0.8^{a}	1.9^{c}	2.5^{a}	6.0^{b}	5.3^{b}	7.6^{c}	8.1^{c}
0–30 cm	(0.2)	(0.1)	(0.2)	(0.1)	(0.2)	(0.5)	(0.3)	(0.3)	(1.4)	(0.3)
Maize	1.0^{a}	1.3^{a}	1.7^{b}	0.8^{a}	1.7^{b}	2.2^{a}	4.4^{b}	5.4^{c}	7.0^{d}	11.0^{e}
0–30 cm	(0.0)	(0.2)	(0.1)	(0.1)	(0.1)	(0.1)	(0.6)	(0.3)	(0.6)	(0.4)
Grass	1.2^{a}	1.4^{a}	3.3^{c}	1.5^{a}	2.4^{b}	2.0^{a}	5.5^{b}	8.7^{c}	10.7^{c}	10.8^{c}
0–10 cm	(0.3)	(0.2)	(0.5)	(0.2)	(0.7)	(0.1)	(0.2)	(0.7)	(3.0)	(1.8)
Forest	1.6^{a}	1.4^{a}	4.2^{c}	2.6^{b}	2.7^{b}	3.3^{a}	6.5^{a}	13.9^{b}	21.2^{c}	13.8^{b}
0–7 cm	(0.3)	(0.1)	(0.4)	(0.2)	(1.0)	(0.1)	(0.8)	(1.0)	(4.0)	(3.8)
Wheat	0.9^{b}	1.3^{c}	1.0^{b}	0.4^{a}	1.0^{b}	1.6^{a}	5.2^{c}	3.3^{b}	3.8^{b}	6.8^{d}
30–45 cm	(0.1)	(0.2)	(0.1)	(0.03)	(0.1)	(0.0)	(1.6)	(0.3)	(0.3)	(1.1)
Maize	0.9^{b}	1.3^{c}	1.2^{c}	0.5^{a}	1.2^{c}	2.3^{a}	5.5^{c}	3.8^{b}	4.3^{b}	7.5^{d}
30–45 cm	(0.0)	(0.2)	(0.1)	(0.0)	(0.1)	(0.3)	(0.2)	(0.3)	(0.4)	(1.3)
Halle site										
H-Rye	1.3^{a}	1.3^{a}	2.0^{b}	1.2^{a}	2.2^{b}	5.7^{a}	6.3^{a}	8.2^{a}	8.5^{a}	18.8^{b}
0–20 cm	(0.3)	(0.2)	(0.3)	(0.1)	(0.7)	(1.2)	(0.5)	(1.3)	(2.5)	(3.1)
H-Maize	1.4^{a}	1.5^{a}	2.3^{b}	1.4^{a}	1.9^{ab}	5.3^{a}	7.0^{ab}	9.7^{b}	9.7^{b}	19.1^{c}
0–20 cm	(0.6)	(0.2)	(0.0)	(0.1)	(0.3)	(2.2)	(1.2)	(0.9)	(2.4)	(2.5)

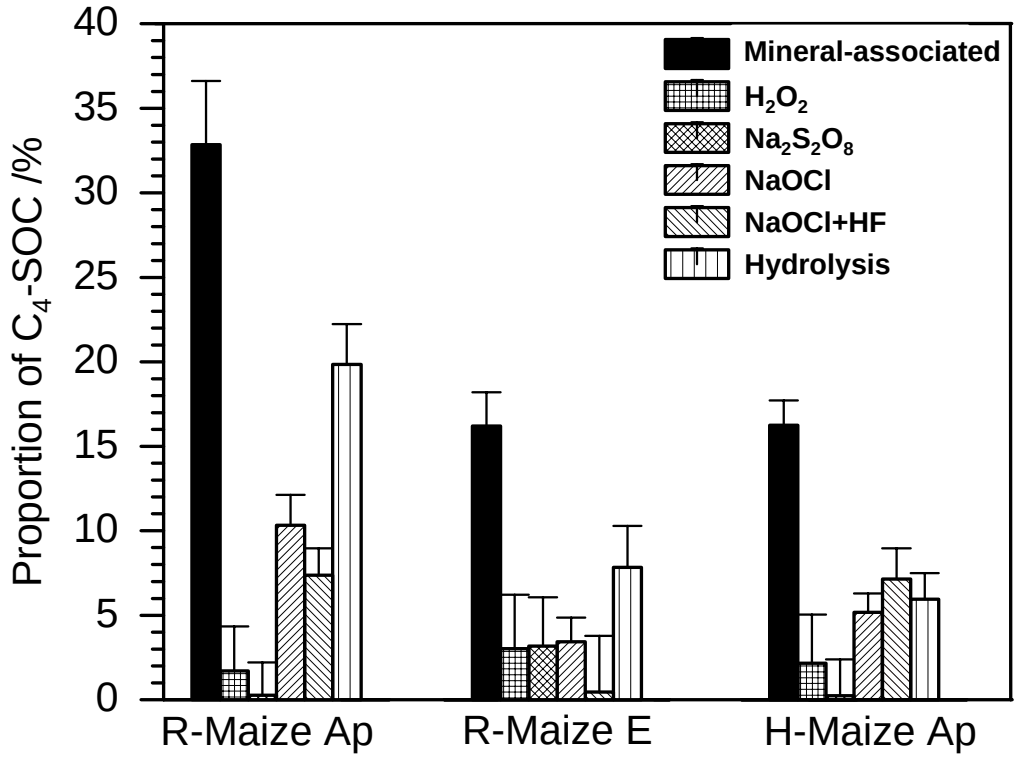

Figure 4.5: Proportion of C_4-derived SOC in the maize soils after density fractionation ('Mineral-associated'), and after subsequent chemical fractionation in the Ap and E horizon of the Rotthalmünster soil (R-Maize Ap and E) and the Ap horizon of the Halle soil (H-Maize Ap) (means and standard deviation, n = 4).

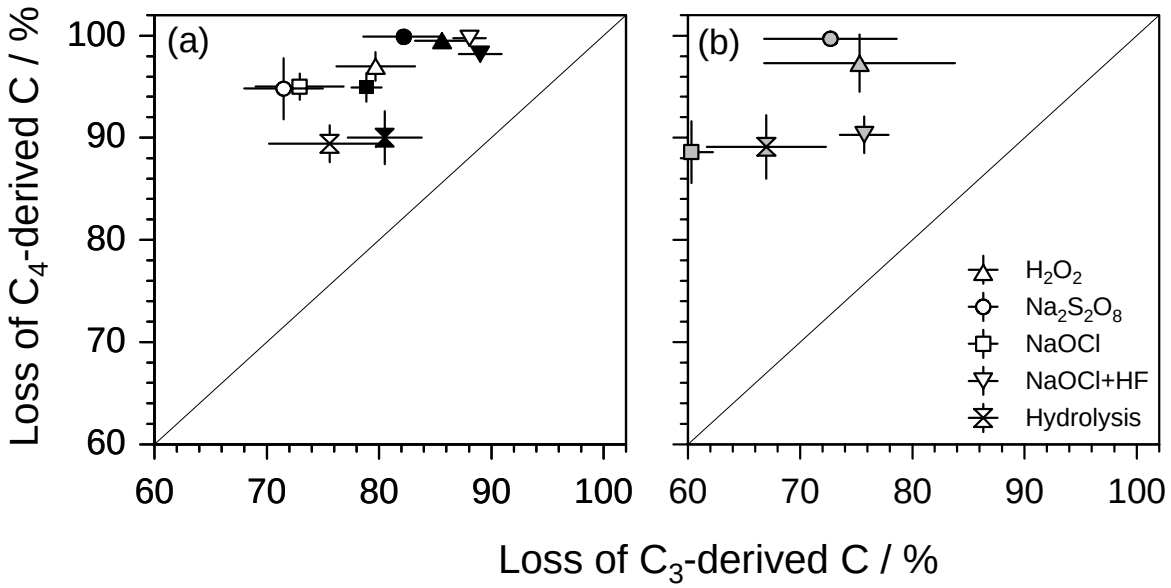

Figure 4.6: Loss of C_3-derived versus C_4-derived SOC in the maize soils after hydrolysis, oxidation with H_2O_2, $Na_2S_2O_8$, and NaOCl and after demineralization of the NaOCl treated samples with HF (NaOCl+HF) in (a) the Ap (white symbols) and E horizon (black symbols) of the Rotthalmünster soil and (b) the Ap horizon of the Halle soil (means and standard deviation, n = 4).

Table 4.6: Apparent C turnover time of the residual SOC after chemical fractionation determined by ^{13}C natural abundance (means and standard deviation, n = 4), ^{14}C activity, and ^{14}C age (means and 1-σ measurement uncertainty) for the Rotthalmünster ("R") and Halle ("H") soils under continuous maize cropping.

Site	H_2O_2	$Na_2S_2O_8$	NaOCl	NaOCl +HF	Hydro-lysis
			Apparent C turnover time /years		
R-Maize 0–30 cm	>> 560*	>> 560*	211 (+47/-33)	300 (+85/-55)	104 (+16/-13)
R-Maize 30–45 cm	>> 560*	>> 560*	754 (+541/-224)	>> 560*	324 (+152/-80)
H-Maize 0–20 cm	>> 950*	>> 950*	735 (+210/-135)	526 (+187/-111)	635 (+218/-134)
			^{14}C activity /pMC		
R-Maize 0–30 cm	35.8 (0.2)	28.1 (0.2)	87.1 (0.3)	75.3 (0.3)	70.8 (0.3)
R-Maize 30–45 cm	31.3 (0.2)	26.5 (0.2)	73.4 (0.3)	60.5 (0.3)	57.4 (0.3)
H-Maize 0–20 cm	18.6 (0.2)	20.9 (0.2)	49.5 (0.2)	43.3 (0.3)	33.6 (0.2)
			Mean ^{14}C age /years BP		
R-Maize 0–30 cm	8243 (47)	10203 (60)	1110 (25)	2276 (31)	2777 (28)
R-Maize 30–45 cm	9328 (56)	10659 (69)	2483 (32)	4035 (34)	4455 (38)
H-Maize 0–20 cm	13512 (82)	12583 (84)	5649 (37)	6733 (50)	8764 (57)

* corresponding to a proportion of maize-derived C of < 4.0 % of total SOC in the sample

4.2.3 Chemical fractionation, Rotthalmünster soil, E horizon

In the subsoil, 11–38% of the initial, mineral-associated SOC were resistant to the chemical treatments. Thus, the efficiency of all fractionation procedures in removing mineral-associated SOC was significantly lower in the E than the A horizon (Figure 4.4). This trend was most pronounced for the H_2O_2- and $Na_2S_2O_8$-resistant SOC fractions and least obvious for those left after treatment with NaOCl+HF. All chemical treatments preferentially removed organic materials with a modern isotope signature (data not shown). Except for hydrolysis, the C/N ratios of SOM decreased after all treatments (Table 4.5).

Similarly, the proportion of maize-derived SOC declined from 14% in mineral-associated SOC to 7% in hydrolysis-resistant SOC and to 2–3% in SOC resistant to oxidative treatments (Figure 4.5). No maize-derived SOC was detected in the (NaOCl+HF)-residuum. As with the A horizons, the relative loss of maize-derived SOC was always significantly higher than that of C_3-derived SOC for all tested methods (Figure 4.6a) and the increase in mean ^{14}C age followed the same order as seen for the A horizon: NaOCl < NaOCl+HF < hydrolysis < H_2O_2 < $Na_2S_2O_8$ (Table 4.6).

Among all treatments tested, again, consistently highest mean ^{14}C ages of residual SOC were found after treatment with H_2O_2 and $Na_2S_2O_8$. The mean ^{14}C ages of the $Na_2S_2O_8$-resistant SOC fractions was only slightly higher in the E than in the Ap horizon whereas slightly larger differences were observed between the horizons after the other chemical treatments (Table 4.6).

4.2.4 Chemical fractionations, Halle soil

The proportion of residual SOC differed significantly among the treatments and increased in the following order (% of initial, mineral-associated SOC): NaOCl+HF (15–19%) ≤ H_2O_2 (20–21%) ≤ $Na_2S_2O_8$ (21–23%) ≤ NaOCl (24–31%) ≤ hydrolysis (29–35%).

Again, C/N ratios decreased after all oxidative treatments but increased after hydrolysis (Table 4.5). The C/N ratio of the residual SOM was generally higher if the treatments with $Na_2S_2O_8$ and H_2O_2 were applied without preliminary density fractionation (9–11 versus 5–7; cp. Tables 4.5 & 8.3).

Mineral-associated SOC in the Halle soil contained 17% of maize-derived C. Losses of maize-derived SOC (89–100%) were significantly higher than losses of C_3-SOC (60–76%) for all fractionation procedures (Figure 4.6b). In accordance with the Rotthalmünster soil, treatment with H_2O_2 and $Na_2S_2O_8$ left a significantly lower proportion of maize-derived C (1.2 and 0.6% C_4-SOC, respectively) in the residual SOC fraction than the other treatments (5–7% C_4-SOC) (Figure 4.5). The apparent C turnover time increased in the following order: NaOCl+HF < hydrolysis < NaOCl < H_2O_2 ≈ $Na_2S_2O_8$ (Table 4.6). As with the Rotthalmünster soils, no reliable apparent C turnover times were derived for

the H_2O_2- and $Na_2S_2O_8$-resistant SOC fractions due to very low amounts of maize-derived C. These SOC fractions also had a considerably lower ^{14}C activity than the residual SOC fraction after any of the other treatments (similar to the horizons of the Rotthalmünster soil; Table 4.6). The mean ^{14}C age increased in the order: NaOCl < NaOCl+HF < hydrolysis < $Na_2S_2O_8 \approx H_2O_2$, which was only slightly different to the order observed for the Rotthalmünster soil (Table 4.6). The calculated mean ^{14}C age of SOC removed by chemical fractionation was again younger than that of the initial, mineral-associated SOC, confirming selective removal of younger SOC components. Treatment of the Halle maize and rye soils with H_2O_2 and $Na_2S_2O_8$ without preliminary density fractionation resulted in higher, though statistically not significantly different, contents of residual SOC (Table 8.3, Appendix). The mean ^{14}C age of H_2O_2- and $Na_2S_2O_8$-resistant SOC was even higher for samples without preliminary density fractionation (16600 and 16200 years for H_2O_2- and $Na_2S_2O_8$-resistant SOC, respectively).

4.3 Suitability of thermal oxidation for obtaining stable pools of soil organic matter

Weight losses ranged from 0.5 to 3.2% after thermal oxidation at 200°C and increased to between 2.5 and 7.6% after thermal oxidation at 600°C (Table 4.7). Comparing the agricultural soils, weight losses were steadily lower in the Halle than the Rotthalmünster soil. Between land uses, weight losses increased with increasing C contents in the order agricultural soil < forest ~ grassland soil (Table 4.7).

Soil organic carbon contents decreased significantly with increasing temperature for all investigated soils and land uses except for the temperature step 250°C (Table 4.8). Losses of SOC were between 10 and 20% lower in the Ap horizon of the Rotthalmünster than the Halle maize soil. After thermal oxidation at 400°C, only the continuous grassland of the Rotthalmünster soil and the Ap horizon of the Halle maize soil still contained small amounts of SOC (Table 4.8), which were lost after thermal oxidation at 500°C. Hence, degradation of SOC was completed at 400 and 500°C for the Rotthalmünster and Halle soils, respectively.

Table 4.7: Weight loss of the A horizon of the Halle maize soil ("H"), the A horizon of the Rotthalmünster grass, forest and maize soils ("R") as well as the E horizon of the Rotthalmünster maize soil after thermal oxidation at 200, 225, 250, 275, 300, 400, 500 or 600°C (means and standard error, n = 4). Letters from a–g (read from left to right) indicate significant differences between temperature steps for a single land use.

Soil	Depth	200°C	225°C	250°C	275°C	300°C	400°C	500°C	600°C
	/cm	Weight loss /% of sample							
R-Grass Ah	0–10	2.8^{a} (0.4)	4.2^{b} (0.5)	4.0^{b} (0.3)	5.1^{bc} (0.1)	4.6^{b} (0.5)	6.0^{cd} (0.3)	6.7^{de} (0.4)	7.6^{e} (0.4)
R-Forest Ah	0–7	3.2^{a} (0.1)	3.8^{a} (0.4)	3.2^{a} (0.4)	5.5^{b} (0.4)	4.2^{a} (0.3)	5.7^{b} (0.2)	6.2^{bc} (0.1)	6.8^{c} (0.2)
R-Maize Ap	0–30	1.2^{a} (0.2)	2.0^{b} (0.0)	2.0^{b} (0.1)	2.8^{d} (0.1)	2.3^{c} (0.1)	3.2^{e} (0.1)	3.9^{f} (0.0)	4.5^{g} (0.1)
R-Maize E	30–45	2.2^{ab} (0.2)	2.0^{ab} (0.4)	1.5^{a} (0.1)	2.4^{ab} (0.1)	1.9^{ab} (0.1)	2.7^{b} (0.2)	3.5^{c} (0.3)	4.3^{d} (0.3)
H-Maize Ap	0–20	0.5^{a} (0.1)	1.3^{bc} (0.1)	1.0^{b} (0.1)	1.0^{b} (0.0)	1.4^{c} (0.1)	1.7^{d} (0.0)	2.2^{e} (0.1)	2.5^{f} (0.0)

No significant differences in SOC resistant to thermal oxidation were observed between the Ap and E horizon of the Rotthalmünster soil except for oxidation at 200°C where a considerable larger proportion of stable C was observed in the upper soil horizon (Figure 4.7). Between land uses, the content of SOC resistant to thermal oxidation did not increase with decreasing contents of initial, mineral-associated SOC (Figure 4.7).

Total nitrogen was less affected by thermal oxidation than the SOC fraction: C/N ratios decreased significantly with increasing temperature for all soils and land uses again with the exception of the 250°C temperature step (Table 4.8). Furthermore, all samples still contained about 0.2 g N kg^{-1} soil after thermal oxidation at 600°C (Table 8.4, Appendix), pointing to a higher resistance of the N fraction to thermal oxidation.

Table 4.8: Soil organic carbon contents and C/N ratios of the A horizon of the Halle soil ("H") under maize cropping, and the A horizon under continuous grass, forest and maize cropping as well as the E horizon of the Rotthalmünster soil ("R") under maize cropping after density fractionation (mineral-associated) and after subsequent thermal oxidation at 200, 225, 250, 275, 300 or 400°C (means and standard error, n = 4). Letters from a–f (read from left to right) indicate significant differences between temperature steps for a single land use.

Soil	Depth	Mineral-associated	200°C	225°C	250°C	275°C	300°C	400°C
	/cm	C content /g kg^{-1} soil						
R-Grass Ah	0–10	24.5^{a} (2.4)	11.1^{b} (2.2)	8.2^{bc} (0.5)	8.2^{bc} (0.6)	4.8^{c} (0.8)	3.2^{c} (0.5)	1.1 (n.d.)
R-Forest Ah	0–7	17.8^{a} (1.9)	5.8^{b} (0.3)	4.3^{c} (0.5)	4.8^{c} (0.5)	2.5^{c} (0.1)	1.2^{c} (0.1)	0.0
R-Maize Ap	0–30	10.6^{a} (0.6)	7.0^{b} (0.4)	2.9^{c} (0.5)	4.1^{c} (0.3)	2.5^{d} (0.0)	1.9^{d} (0.1)	0.0
R-Maize E	30–45	5.2^{a} (0.4)	2.7^{b} (0.3)	1.8^{cd} (0.3)	2.0^{c} (0.2)	1.2^{de} (0.0)	1.0^{e} (0.1)	0.0
H-Maize Ap	0–20	6.5^{a} (0.3)	5.0^{b} (0.2)	3.2^{d} (0.1)	3.8^{c} (0.1)	3.1^{d} (0.1)	1.7^{e} (0.1)	0.8^{f} (0.0)
		C/N ratio						
R-Grass Ah	0–10	9.6^{a} (0.2)	6.9^{b} (0.6)	5.3^{cd} (0.3)	5.3^{cd} (0.3)	3.9^{de} (0.6)	3.5^{e} (0.4)	2.2 (n.d.)
R-Forest Ah	0–7	16.8^{a} (0.4)	8.3^{b} (1.2)	5.1^{c} (0.1)	5.7^{c} (0.3)	2.6^{d} (0.4)	2.6^{d} (0.1)	n.c.
R-Maize Ap	0–30	7.9^{a} (0.4)	6.6^{b} (0.1)	4.2^{c} (0.1)	4.6^{c} (0.1)	3.4^{d} (0.0)	3.3^{d} (0.1)	n.c.
R-Maize E	30–45	5.9^{a} (0.1)	3.7^{b} (0.1)	2.8^{c} (0.1)	3.0^{c} (0.1)	2.0^{d} (0.1)	2.0^{d} (0.1)	n.c.
H-Maize Ap	0–20	10.9^{a} (0.1)	9.5^{b} (0.2)	8.2^{c} (0.1)	8.3^{c} (0.2)	7.8^{c} (0.2)	5.5^{d} (0.2)	4.1^{e} (0.0)

n.c.: not calculated because no residual C was left upon thermal oxidation
n.d.: not determined, because only one sample contained a detectable amount of C

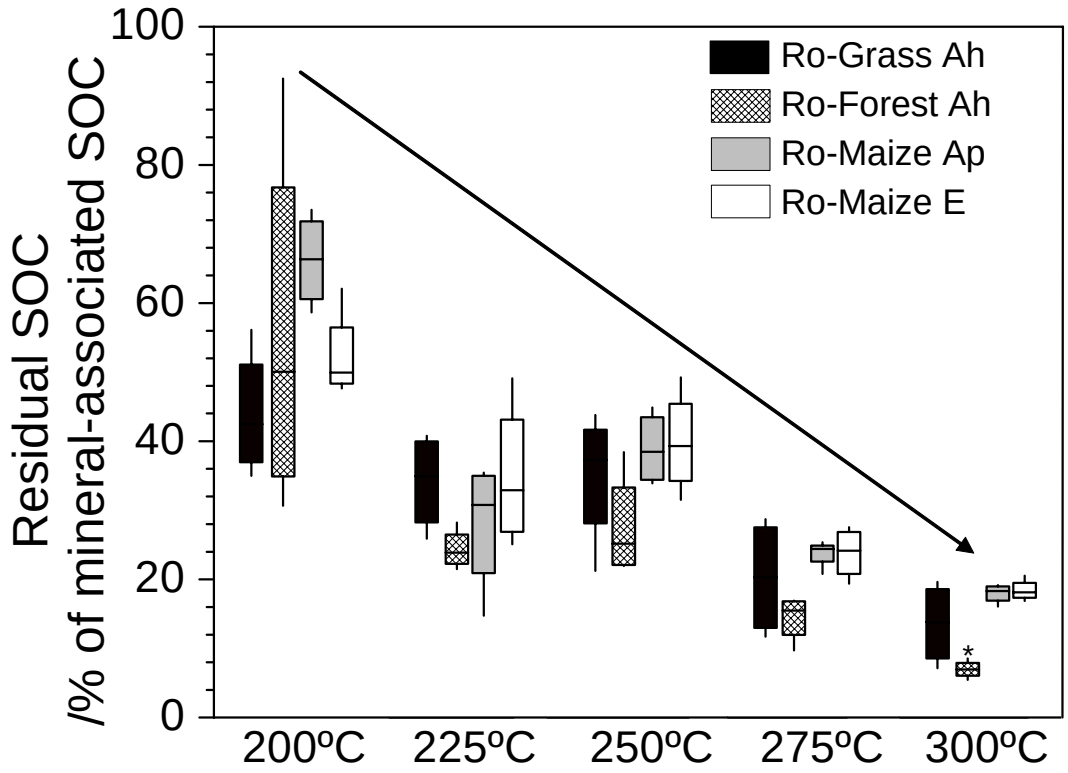

Figure 4.7: Proportions of SOC resistant to thermal oxidation at 200, 225, 250, 275 or 300°C in the Rotthalmünster soil under different land use regimes (Box Plots; n = 4). "*" indicates significant differences between land use regimes.

Table 4.9: $\delta^{13}C$ values of the initial, mineral-associated SOC and the SOC fractions obtained after thermal oxidation at 200, 225, 250, 275 or 300°C in the maize and corresponding reference soils of the Halle soil ("H") and the Rotthalmünster soil ("R") (means and standard deviation, n = 4); Letters from a–e (read from left to right) indicate significant changes in $\delta^{13}C$ between temperature steps.

Soil	Depth	Mineral-associated	200°C	225°C	250°C	275°C	300°C
	/cm	$\delta^{13}C$ /‰ V-PDB					
H-Rye Ap	0–20	-25.7^{a} (0.1)	-24.9^{b} (0.2)	-24.5^{c} (0.1)	-24.5^{c} (0.2)	-24.2^{d} (0.1)	-23.5^{e} (0.1)
H-Maize Ap	0–20	-23.0^{a} (0.2)	-22.4^{b} (0.1)	-23.0^{a} (0.1)	-23.0^{a} (0.2)	-23.0^{a} (0.1)	-22.9^{a} (0.1)
R-Wheat Ap	0–30	-26.5^{a} (0.1)	-25.6^{b} (0.2)	-25.0^{c} (0.0)	-25.1^{c} (0.1)	-25.0^{c} (0.1)	-24.6^{d} (0.1)
R-Maize Ap	0–30	-21.9^{a} (0.5)	-20.6^{b} (0.1)	-20.6^{b} (0.2)	-20.5^{b} (0.3)	-20.9^{b} (0.1)	-20.9^{b} (0.2)
R-Wheat E	30–45	-25.5^{a} (0.1)	-24.5^{b} (0.2)	-24.3^{bc} (0.4)	-24.0^{bc} (0.4)	-24.2^{bc} (0.2)	-23.8^{c} (0.2)
R-Maize E	30–45	-23.5^{a} (0.2)	-21.9^{c} (0.2)	-22.4^{b} (0.1)	-22.4^{b} (0.2)	-22.5^{b} (0.2)	-22.5^{b} (0.1)

Thermal oxidation caused a significant isotope fractionation (up to 2.2‰) as can be seen in the reference soils (Table 4.9). This fractionation completely masked the shift in $\delta^{13}C$ in the maize soils due to thermal oxidation. This underlines the importance of a reference site when working with ^{13}C natural abundance: ^{13}C discrimination by thermal oxidation is assumed to be similar in the maize and the reference soil and hence, the difference in $\delta^{13}C$ between the two sites is used for determination of proportions of young, maize-derived C and calculation of apparent C turnover times.

In the Halle soil, proportions of young, maize-derived SOC decreased strongly from 16% of initial mineral-associated SOC to 3% after thermal oxidation at 300°C and the apparent C turnover time increased by 940 years (Figure 4.8).

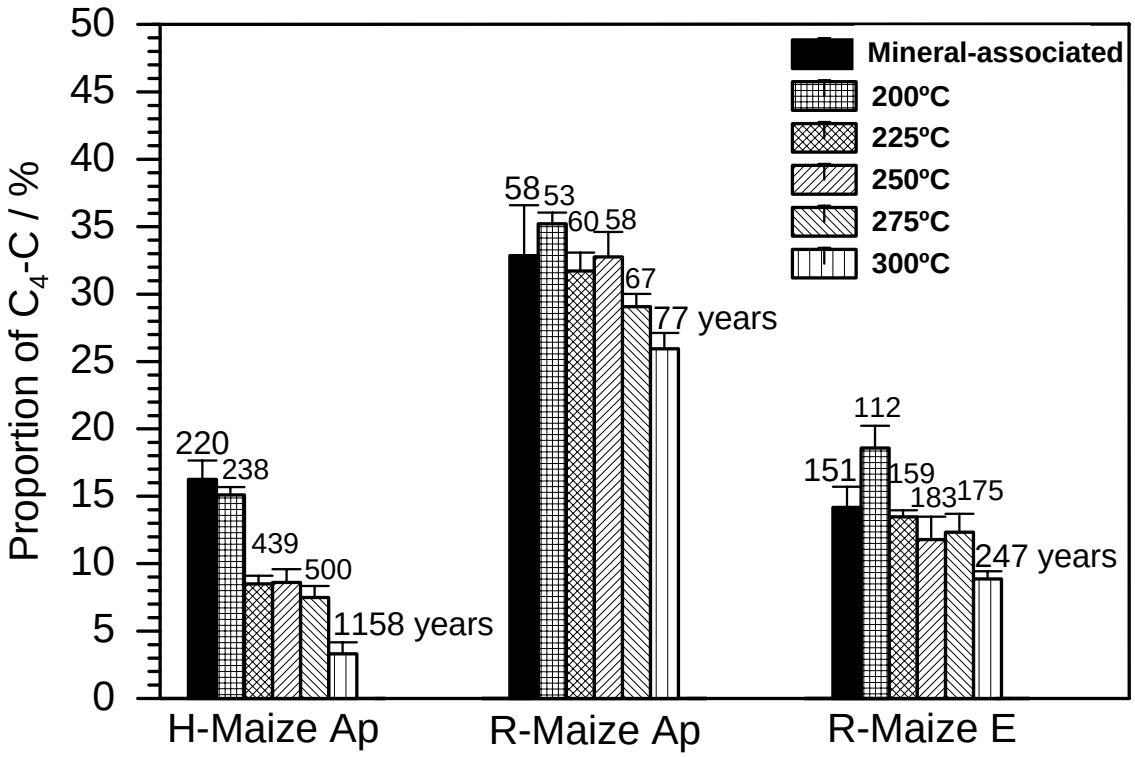

Figure 4.8: Proportions of maize-derived SOC (C_4-C) in the Ap horizon of the Halle maize soil and the Ap and E horizon of the Rotthalmünster maize soil for the mineral-associated SOC fraction and the fractions resistant to thermal oxidation at 200, 225, 250, 275 or 300°C (means and standard deviation). Numbers above the bars indicate the corresponding calculated apparent C turnover times.

In the Ap horizon of the Rotthalmünster soil, the proportion of maize-derived SOC decreased only slightly (from 33% of initial, mineral-associated SOC to 26% of SOC after thermal oxidation at 300°C) and the apparent C turnover time

increased by only 20 years (Figure 4.8). The same trend was observed in the E horizon where the proportion of maize-derived SOC decreased by only 5% (from 14% to 9% after thermal oxidation at 300°C). This led to an increase of 100 years in apparent C turnover time in the E horizon (Figure 4.8).

4.4 Effect of residue decomposability and the structure and activity of the microbial biomass on the formation of macroaggregates and C sequestration in aggregate fractions

4.4.1 Mineralization of the added residue

During the first two days of incubation, a large emission of CO_2 due to the rewetting of the soil was observed, which was excluded from the evaluation of CO_2 data. A second flush of CO_2 due to mineralization of the added maize residues was visible after day three of incubation and the maximum respiration of about 2.5 mg CO_2-C h^{-1} kg^{-1} soil was reached after 7 days of incubation for the leaf treatment, whereas the root treatment reached its maximum of 1.3 mg kg^{-1} two days later (Figure 4.9). The CO_2 production of the treatments with the added fungicide was considerably lower than those of the corresponding non-fungicide-treated soils (Figure 4.9).

After the maximum, the respiration decreased faster in the leaf than the root treatment. The cumulative CO_2-production differed significantly between treatments and decreased in the order: “Leaf” > “Root” > “Leaf Captan” > “Root Captan” > “Control” > “Control Captan” (Table 4.10). A high percentage (up to 90%) of the evolved CO_2-C derived from the added maize residue (Figure 4.9; Table 8.6, Appendix). During the first two weeks of incubation, the percentage of maize-derived C in the evolved CO_2 was higher in the leaf than the root treatment, but generally lower thereafter. With some exceptions, the Captan treated soils had a lower percentage of maize-derived C in the evolved CO_2 than their non-fungicide treated counterparts.

The greatest dynamics in soil respiration ended after 35 days of incubation. At this point, 25 and 30% of the added maize-C was mineralized in the root and leaf treatment, respectively, and 10 and 17% in their fungicide-treated counterparts. After 84 days of incubation only slightly more of the added maize-C was

mineralized ("Root Captan" (13%) < "Leaf Captan" (21%) < "Root" (30%) < "Leaf" (34%)) (Table 4.10).

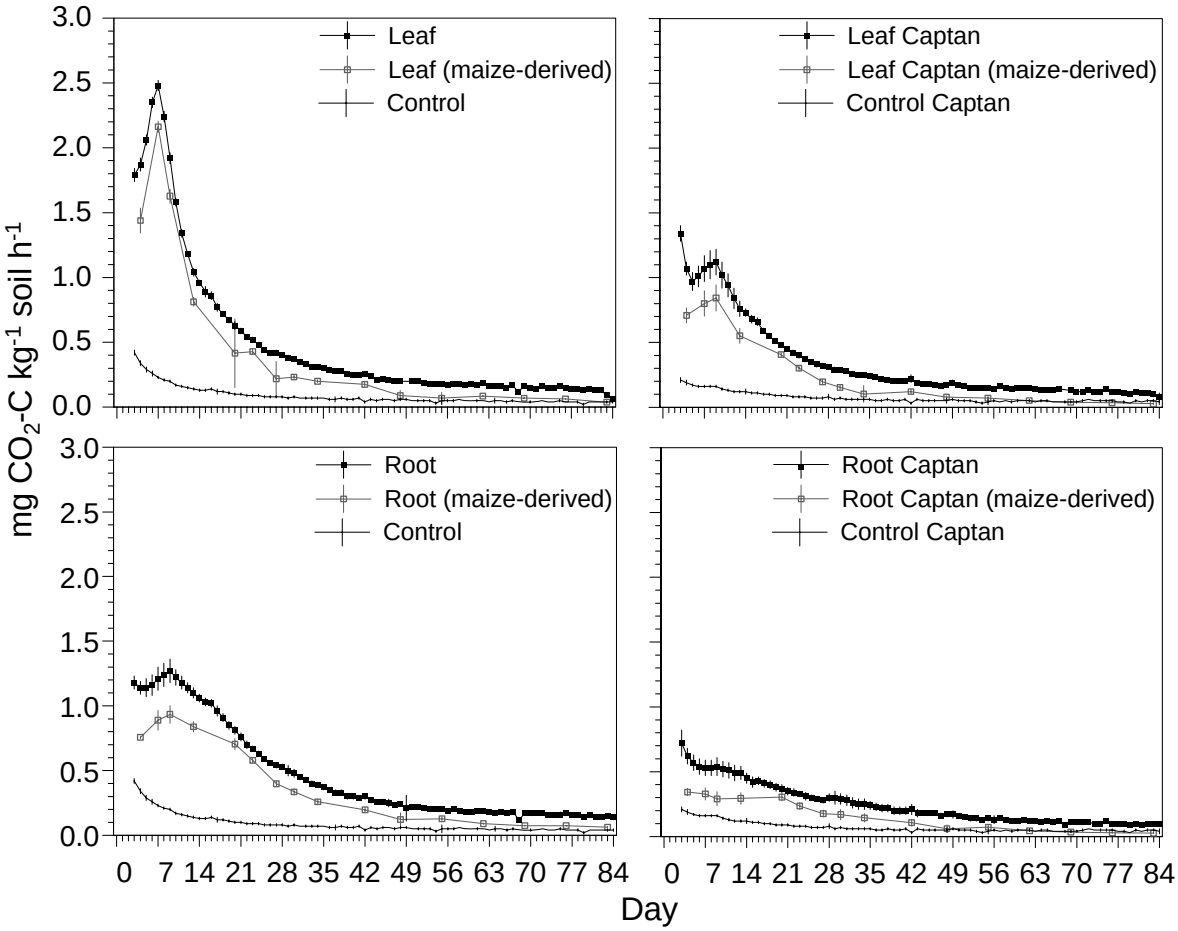

Figure 4.9: CO_2-C evolution rate and maize-derived CO_2-C for the applied treatments (Control, Leaf, Root, Control Captan, Leaf Captan and Root Captan) (means and standard deviation, n = 8 (day 0–56); n = 4 (day 56–84)).

Table 4.10: Cumulative CO_2-C emission in 84 days and 6 treatments (means and standard deviation, n = 4). Letters from a–e indicate significant differences between treatments.

Treatment	Cumulative CO_2-C production /mg kg^{-1} soil	Cumulative maize-derived CO_2-C production /mg kg^{-1} soil	Proportion of the added maize-C mineralized /%
Control	159.2 (3.3)^{a}		
Control	130.1 (10.9)^{a}		
Leaf	962.1 (19.5)^{e}	718.5 (85.0)^{d}	34.1 (4.0)^{c}
Leaf Captan	672.0 (17.6)^{c}	451.4 (8.5)^{b}	21.4 (0.4)^{b}
Root	876.5 (17.4)^{d}	630.5 (20.5)^{c}	29.9 (1.0)^{c}
Root Captan	456.2 (40.3)^{b}	264.9 (32.2)^{a}	12.6 (1.5)^{a}

4.4.2 Soil aggregation

The amount of water-stable macroaggregates (250–2000 µm) made up between 0.1 (Control treatment) and 8% (Leaf Captan) of the soil mass, while 50–69% of the soil mass was allocated to the microaggregate fraction (53–250 µm) and 31–50% were found in the non-aggregated fraction (0–53 µm) (Table 8.8, Appendix).

The maximum macroaggregation occurred later than maximum respiration in both the leaf and the root treatment, but with a higher retardation in the root than the leaf treatment; the maximum amount of macroaggregates was observed 17 and 7 days after the maximum respiration, respectively.

4.4.2.1 The influence of residue quality

Macroaggregation was significantly enhanced by the addition of maize residues. The amount of water-stable macroaggregates remained higher in the residue-treated soils than the control during the whole incubation experiment (Figure 4.10a–c).

Further, the formation of water-stable macroaggregates was significantly influenced by the type of the added residue: the amount of macroaggregates was significantly higher (except for day 56) in the leaf than the root treatment. Besides the amount of water-stable macroaggregates, also the dynamics in aggregation differed between treatments: in the leaf treatment, the amount of water-stable macroaggregates was highest at the time of the first sampling (day 14) and decreased thereafter. In the root treatment, the maximum amount of water-stable macroaggregates was reached at day 28, but was significantly lower than the maximum amount of water-stable macroaggregates of the leaf treatment (Figure 4.10b–c).

4.4.2.2 Aggregation after addition of fungicide

The addition of fungicide, i.e. the suppression of the fungal biomass markedly influenced soil aggregation dynamics: the formation of water-stable macro-aggregates in the leaf Captan treatment was significantly lower at day 14 of incubation and the maximum aggregation was reached 42 days later than in

the leaf treatment without fungicide (Figure 4.10b). In the root plus fungicide treatment ("Root Captan"), the amount of water-stable macroaggregates remained significantly lower than in the non-fungicide treated counterpart up to day 28 of incubation and the maximum aggregation was reached 28 days later than in the root treatment (Figure 4.10c). The maximum aggregation was slightly, but not significantly higher in the fungicide treatments than in their non-fungicide counterparts (Figure 4.10a–c).

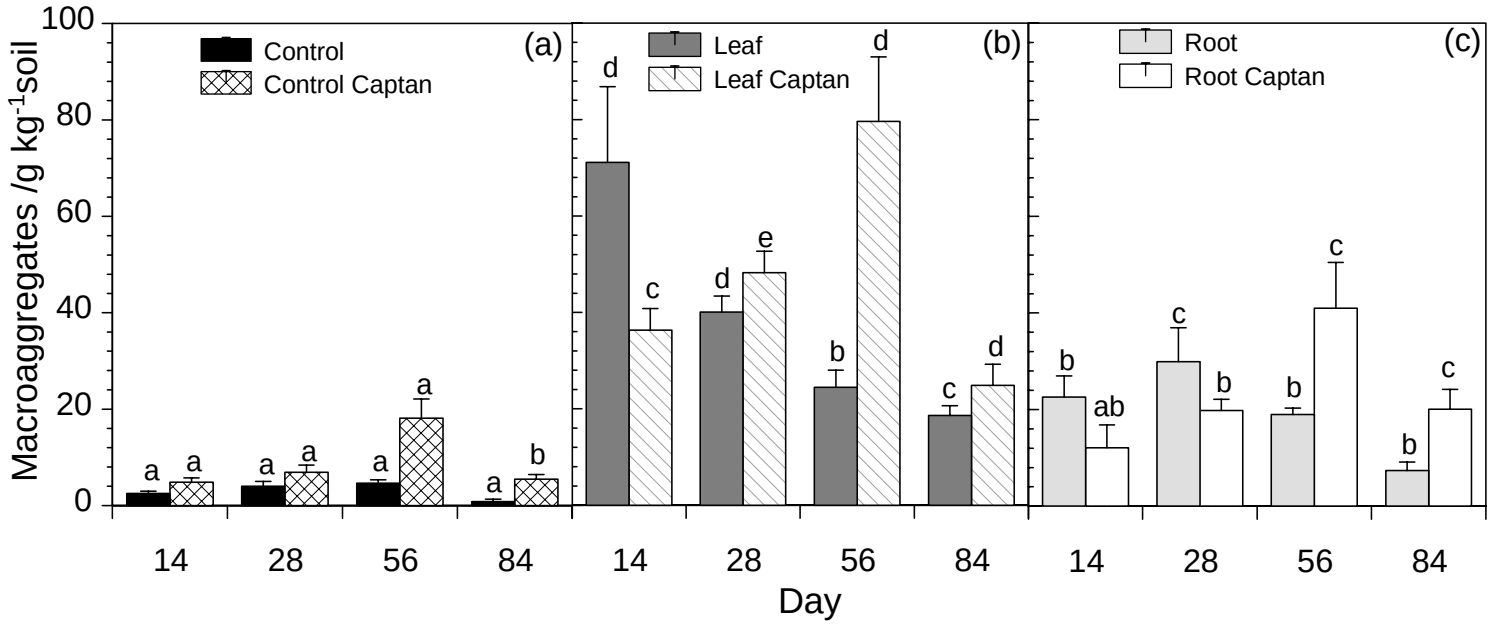

Figure 4.10: Amount of water-stable macroaggregates after 14, 28, 56 and 84 days of incubation for the control soil, soil with addition of leaf material and soil with addition of root material without ("Control", "Leaf", "Root") and with addition of fungicide ("Control Captan", "Leaf Captan", "Root Captan") (means and standard deviation, n = 4). Letters from a–e indicate significant differences between the six applied treatments for a single day.

4.4.2.3 The role of size, activity and structure of the microbial community for soil aggregation

The size of the microbial community, represented by the chloroform-labile C pool extracted with 0.05 M K_2SO_4, was significantly increased by the addition of maize residues and remained higher than in the control soil during the whole experiment (Table 4.11). Except for sampling day 56, the size of microbial biomass C pool differed significantly between the leaf and the root treatment. At day 14, it was significantly higher in the leaf than the root treatment, but lower (days 28 and 84) or equally high (day 56) thereafter.

In the leaf and root treatment without addition of fungicide, the incorporation of maize-derived C into the microbial biomass was highest (42%) at days 14 and 28, respectively, corresponding to the days of maximum aggregation (Table 4.11). Thereafter, the proportion of maize-derived C decreased to 34–36%. In the fungicide treated counterparts, two maxima in the maize-label of the microbial biomass C were detected; the first at the beginning of the experiment and the second at the date of maximum aggregation (root Captan) or thereafter (leaf Captan). For the microbial biomass N pool, the incorporation of maize-derived N ranged from 17–30% for the leaf and leaf Captan treatments and from 6–10% for the root and root Captan treatments with the highest maize-label at the beginning of the incubation and a continuous decrease thereafter (Table 4.11).

The amount of ergosterol, as an indicator for the fungal biomass, was also increased by the addition of maize residues and remained higher in the leaf and root treatment than in the control soil during the whole incubation (Table 4.11). While the ergosterol content was lower in the root than the leaf treatment at day 28, it was higher at all other sampling dates. The addition of fungicide did not reduce the ergosterol content in the leaf Captan treatment and in the control Captan treatment; the ergosterol content was only lower in the control Captan treatment at the beginning of the experiment. Only in the root Captan treatment, the addition of fungicide led to significantly lower ergosterol content compared to the root treatment during the whole incubation (Table 4.11).

The size of the microbial biomass C pool was related to the amount of water-stable macroaggregates: an increase in the microbial biomass C pool was

accompanied by an increasing amount of water-stable macroaggregates for both the non-fungicide and fungicide treated samples and for all sampling dates (Figure 4.11a). The same relation was observed between the mean respiration as an indicator of the activity of the microbial biomass and the amount of water-stable aggregates (Figure 4.11b).

Table 4.11: Microbial parameters (Microbial biomass C and N pool extracted with 0.05 M K_2SO_4, proportion deriving from the added maize residue in the microbial biomass C and N pools, and ergosterol content after 0, 14, 28, 56, and 84 days of incubation (means and standard deviation, n = 4). Letters from a–f indicate significant differences between the six applied treatments for a single day.

Day	Control	Control Captan	Leaf	Leaf Captan	Root	Root Captan
			Microbial biomass C /mg kg^{-1} soil			
0	93 (5)^{a}	89 (3)^{a}	n.d	n.d.	n.d.	n.d.
14	102 (2)^{b}	83 (1)^{a}	198 (11)^{e}	137 (3)^{c}	169 (21)^{d}	117 (126)^{b}
28	90 (4)^{b}	72 (2)^{a}	142 (6)^{e}	133 (2)^{d}	156 (3)^{f}	117 (1)^{c}
56	96 (3)^{b}	76 (4)^{a}	142 (2)^{e}	114 (5)^{d}	140 (7)^{e}	107 (5)^{c}
84	110 (5)^{b}	85 (4)^{a}	142 (58)^{d}	122 (8)^{c}	151 (48)^{e}	109 (7)^{b}
			Microbial biomass N /mg kg^{-1} soil			
0	5 (1)^{a}	8 (3)^{a}	n.d	n.d.	n.d.	n.d.
14	8 (3)^{a}	6 (1)^{a}	22 (2)^{c}	20 (1)^{c}	17 (1)^{b}	16 (2)^{b}
28	10 (3)^{a}	8 (2)^{a}	15 (1)^{b}	18 (2)^{bc}	18 (1)^{c}	9 (1)^{a}
56	11 (3)^{a}	12 (2)^{ab}	16 (2)^{bc}	16 (2)^{bc}	18 (2)^{c}	11 (2)^{a}
84	11 (3)^{ab}	6 (3)^{a}	17 (2)^{ab}	11 (1)^{ab}	19 (2)^{b}	12 (3)^{ab}
			Maize-derived C in microbial biomass C /%			
14			42 (1)^{b}	39 (4)^{b}	38 (1)^{b}	28 (5)^{a}
28			41 (2)^{c}	30 (5)^{b}	42 (3)^{c}	23 (3)^{a}
56			36 (2)^{b}	28 (2)^{a}	34 (0)^{b}	32 (4)^{b}
84			37 (1)^{b}	37 (6)^{b}	36 (3)^{b}	27 (4)^{a}
			Maize-derived N microbial biomass N /%			
14			28 (0)^{c}	30 (1)^{d}	8 (0)^{a}	10 (0)^{b}
28			30 (1)^{c}	26 (4)^{b}	8 (15)^{a}	9 (18)^{a}
56			24 (2)^{b}	25 (3)^{b}	8 (0)^{a}	8 (1)^{a}
84			24 (1)^{b}	17 (8)^{b}	7 (0)^{a}	6 (1)^{a}
			Ergosterol /mg kg^{-1} soil			
0	0.5 (0.1)^{b}	0.4 (0.1)^{a}	n.d	n.d.	n.d.	n.d.
14	0.4 (0.1)^{a}	0.4 (0.0)^{a}	0.7 (0.1)^{a}	1.0 (0.4)^{b}	1.2 (0.2)^{b}	0.5 (0.1)^{a}
28	0.5 (0.1)^{a}	1.0 (0.1)^{b}	1.1 (0.3)^{bc}	1.3 (0.2)^{c}	0.9 (0.2)^{b}	0.6 (0.1)^{a}
56	0.5 (0.1)^{a}	0.6 (0.1)^{a}	0.6 (0.1)^{a}	0.5 (0.0)^{a}	0.8 (0.1)^{b}	0.6 (0.0)^{a}
84	0.4 (0.1)^{ab}	0.2 (0.0)^{a}	0.3 (0.1)^{ab}	0.3 (0.1)^{a}	0.7 (0.0)^{c}	0.4 (0.1)^{b}

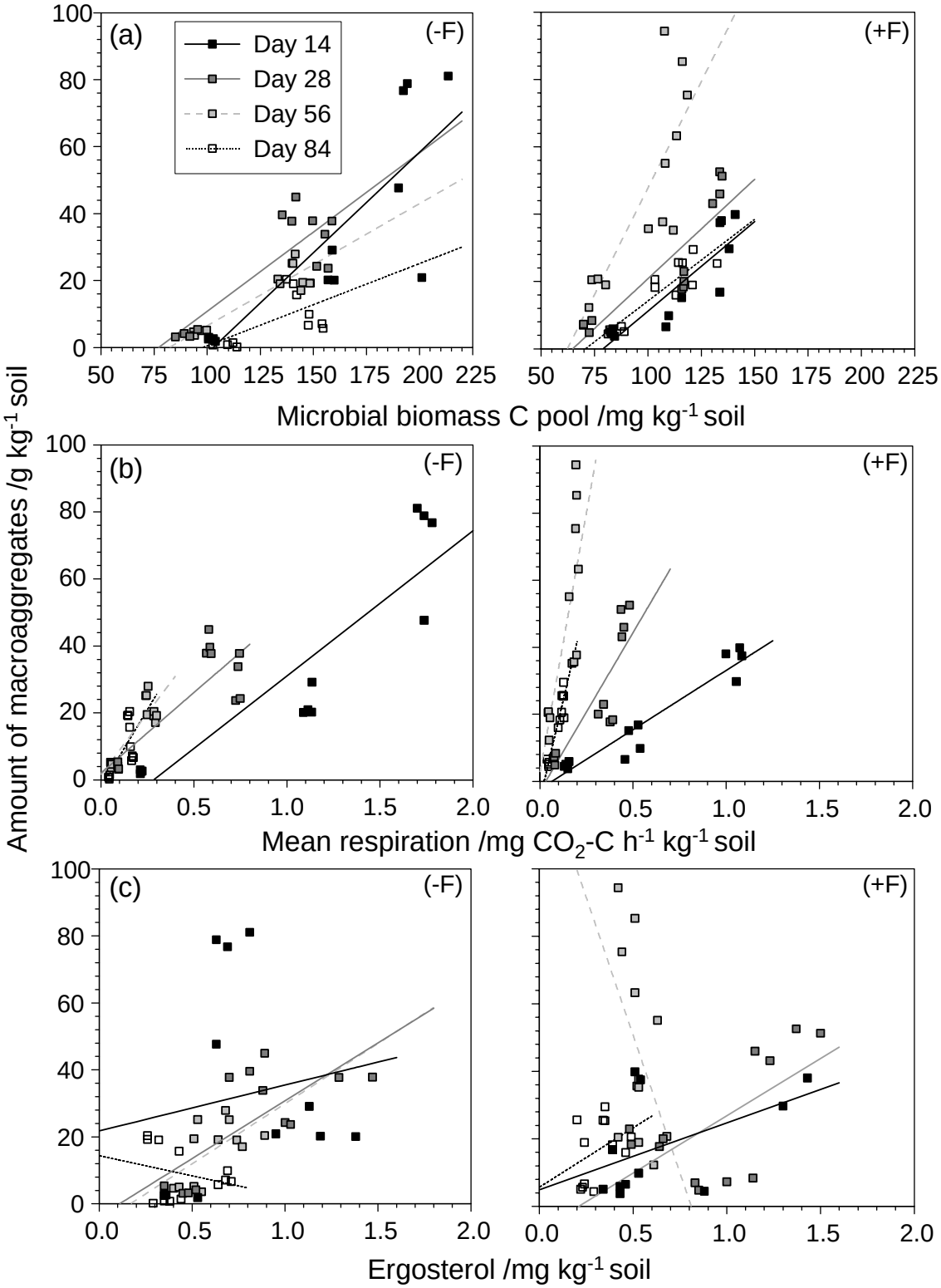

Figure 4.11: Relationship between the amount of water-stable macro-aggregates (250–2000 µm) formed after 14, 28, 56, and 84 days of incubation and (a) the mean respiration, (b) the size of the chloroform-labile C pool extracted using 0.05 M K_2SO_4, and (c) the ergosterol content and for the treatments without (-F) and with (+F) fungicide.

For the non-fungicide treated samples, high ergosterol contents were accompanied by a higher amount of water-stable macroaggregates for sampling days 28 and 56 (Figure 4.11c), but this was not the case at days 14 and 84. For the fungicide treated samples, this trend was observed except for day 56 where the maximum amount of macroaggregates was reached. Thus, this maximum aggregation was not accompanied by a high content of ergosterol.

4.4.3 The role of water-stable aggregates for C and N stabilization

The water-stable macroaggregates contained between 27 and 73 g C kg^{-1} sand-free aggregates, which was considerably more C than found in the micro-aggregate (15–18 g C kg^{-1}) and non-aggregated soil fractions (9–12 g kg^{-1})) (Table 8.8, Appendix). This accounted for an up to twice as large C concentration in macroaggregates compared to microaggregates and an around three times higher C concentration than in the non-aggregated fraction (Figure 4.12). The C concentration per kg sandfree aggregates decreased with increasing amount of water-stable macroaggregates in the order: “Control” > “Root” > “Leaf” (Table 8.8, Appendix).

Due to the low amount of water-stable macroaggregates (less than 10% of the soil mass), their contribution to the C storage was lower compared to micro-aggregates or the non-aggregated fraction when considering the amount of C stored in this fraction per kg soil. In this case, the fraction of water-stable microaggregates (50–68% of the soil) played the most important role in C storage: while water-stable macroaggregates contained between 0.1 and 1.9 g C kg^{-1} soil, water-stable microaggregates stored between 6 and 9 g C kg^{-1} and the non-aggregated soil comprised 3–5 g C kg^{-1}soil (Table 8.8, Appendix).

The formation of water-stable macroaggregates was accompanied by a considerable incorporation of maize-derived C (up to 60%). A much lower proportion of maize-derived C was detected in the microaggregate fraction (10–15%) and the non-aggregated soil (5–10%) (Figure 4.12).

The quantities of ^{13}C and ^{15}N associated with each aggregate size fraction can be expressed as percentage of residual maize-C and -N, i.e. the portion of added ^{13}C and ^{15}N still available in the soil. In the leaf treatment, no free POM was

observed at all four sampling dates. After two weeks of incubation, 46% of the residual maize-C was stored in water-stable macroaggregates, 41% in water-stable microaggregates and 11% in the non-aggregated soil fraction. At the next sampling date, a higher percentage of residual maize-C was stabilized in the microaggregate fraction (61%) compared to the macroaggregate fraction (31%) and the importance of the macroaggregate fraction for storing the maize-C further decreased to 19% of residual maize-C at day 84, while the importance of

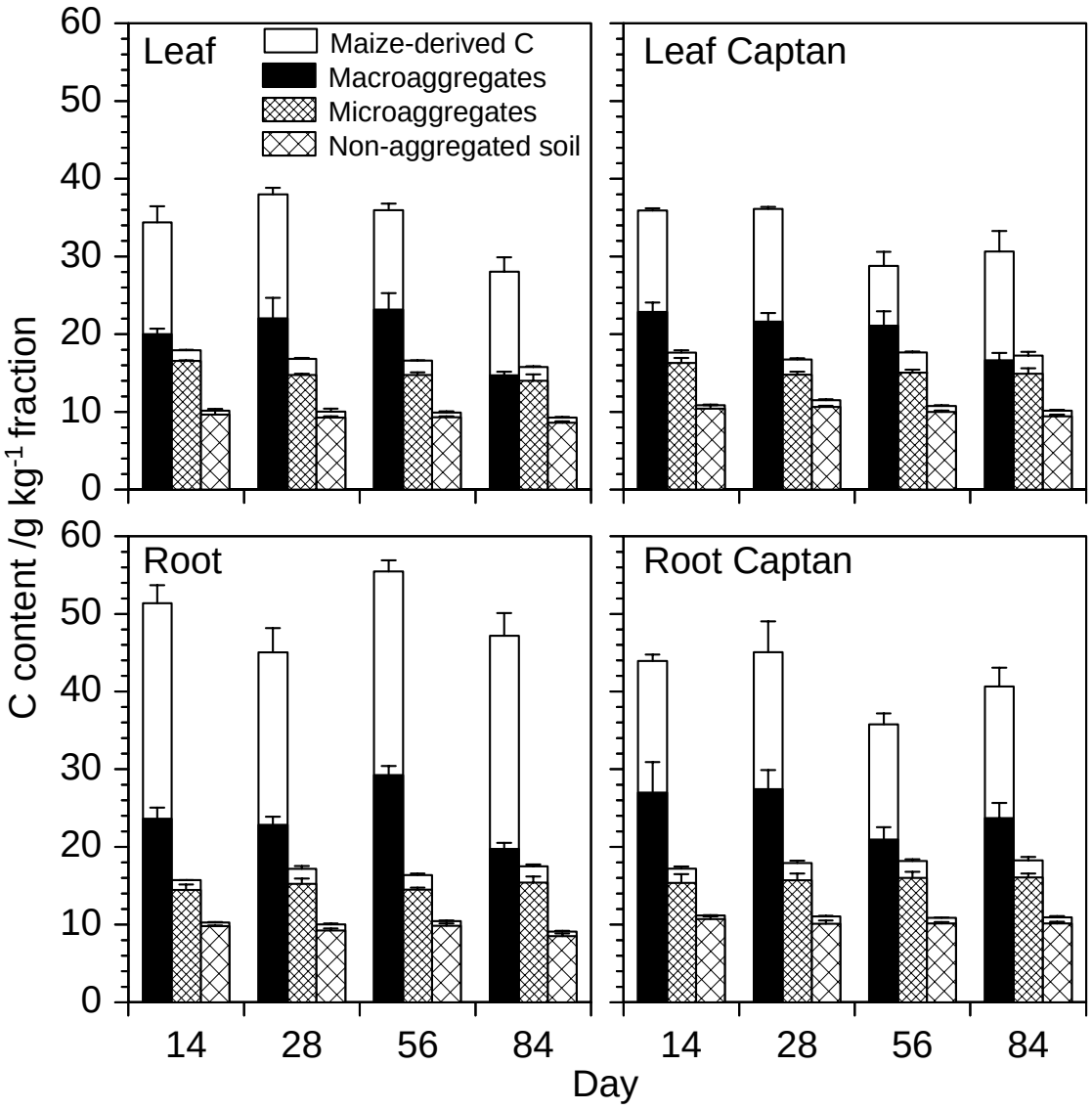

Figure 4.12: Carbon content and content of maize-derived C of water-stable macroaggregates (250–2000 µm), microaggregates (53–250 µm) and the non-aggregated soil (< 53 µm) of the leaf treatment without fungicide ("Leaf"), with application of fungicide "Leaf Captan"), and the root treatment without fungicide ("Root") and with application of fungicide ("Root Captan") after 14, 28, 56 and 84 days of incubation (means and standard deviation, n = 4).

the microaggregate and the non-aggregated soil fraction further increased – up to 62 and 20% at day 84, respectively.

In the other three treatments with addition of maize residues, a considerable part of the residual maize-C remained as free POM in the soil during the first two weeks of incubation (13%, 24% and 30% in the leaf Captan, root and root Captan treatments, respectively) and was removed by flotation in water during aggregate fractionation. In these treatments, the maximum portion of the residual maize-C was stored in the microaggregate fraction during the whole experiment.

As with the C concentrations, the N concentration was higher in water-stable macroaggregates than in the microaggregate or the non-aggregated fractions (Figure 4.13; Table 8.8, Appendix), but differences between fractions were less pronounced than observed for C concentrations due to narrowing C/N ratios with increasing decomposition in the order water-stable macroaggregates > water-stable microaggregates > non-aggregated fraction (Table 8.8, Appendix).

Similar to the C fraction, the formation of water-stable macroaggregates implied the incorporation of considerable amounts of maize-derived N (Figure 4.13). The proportion of maize-derived N in water-stable macroaggregates decreased in the order “Leaf” (15–19%) > “Leaf Captan” (9–16%) > “Root” (8–12%) > “Root Captan” (5–6%). In the leaf and leaf Captan treatment, the proportion of maize-derived N in the microaggregate fraction decreased to 4–5% in the micro-aggregate fraction and to 3% in the non-aggregated fraction. The micro-aggregates of the root and root Captan treatments contained 1–2% maize-N and a proportion of about 1% maize-derived N was found in the non-aggregated fraction.

Considering the low amount of water-stable macroaggregates, they contributed less to the storage of the residual maize-derived N than the microaggregate fraction at all sampling dates and for all four residue treatments.

The ^{15}N recovery in aggregate size fractions varied between 91 and 99% for the leaf and leaf Captan treatments (C/N 27.4) and ranged from 83–102% for the root treatment and from 66–74% for the root Captan treatment (C/N 86.4). The

lower ^{15}N recovery rates, especially in the root Captan treatment, can partly be explained by the removal of free maize-residues during aggregate fractionation.

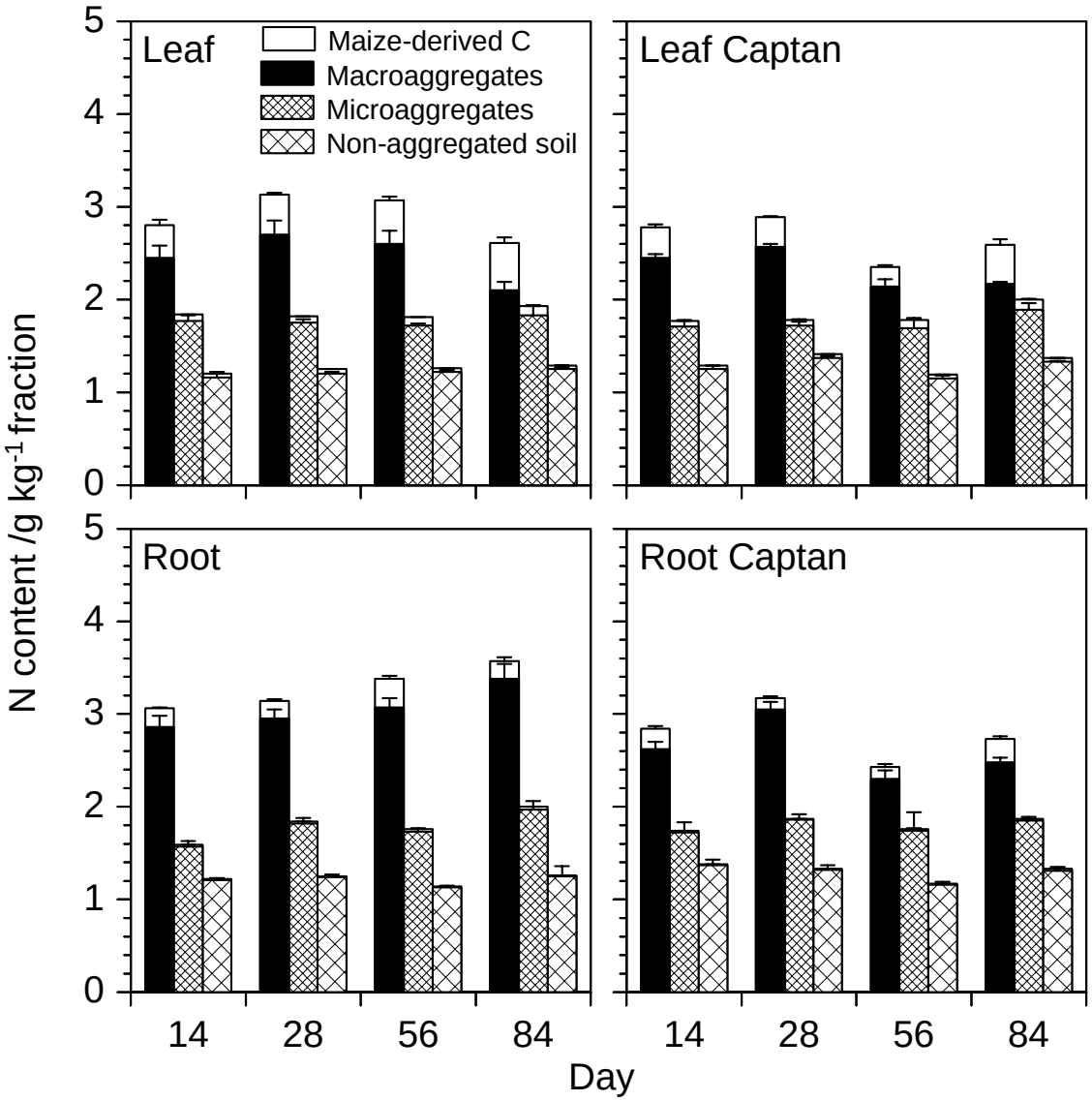

Figure 4.13: Nitrogen content and content of maize-derived N of water-stable macroaggregates (250–2000 µm), microaggregates (53–250 µm) and the non-aggregated soil (< 53 µm) of the leaf treatment without fungicide ("Leaf"), with application of fungicide "Leaf Captan"), and the root treatment without fungicide ("Root") and with application of fungicide ("Root Captan") after 14, 28, 56 and 84 days of incubation (means and standard deviation, n = 4).

5 Discussion

5.1 Investigations of organic matter composition in litter, aggregate and density fractions

5.1.1 Compositional changes in organic matter composition during decomposition

The chemical composition of the L horizon of the investigated spruce site was similar to that of forest litter from other sites with spruce, beech or ash, where the litter layers consisted of 16–23% alkyl-C, 52–56% O-alkyl-C, 15–23% aromatic C, and 5–10% carboxyl C (Kögel *et al.*, 1988; Kögel-Knabner *et al.*, 1988). Differences between ^{13}C CPMAS NMR spectra of the spruce needles and the litter horizon can be explained by the admixture of woody materials such as twigs and cones in the litter which increase the aryl-C content, and by the beginning decomposition of polysaccharides.

The changes in alkyl-C and O-alkyl-C contents when progressing from fresh needles to organic materials in the Ah horizon were consistent with results of Kögel *et al.* (1988) and Zech *et al.* (1992), who analyzed the structural composition of forest litter during the decomposition/humification process using ^{13}C NMR spectroscopy. The increasing alkyl-C/O-alkyl-C ratio indicated an increasing extent of decomposition as seen in previous studies, corroborating its usefulness as sensitive indicator of the degree of decomposition (Baldock *et al.*, 1997; Kölbl & Kögel-Knabner, 2004). Additionally, in a study on arable soils of different texture, the aryl-C/O-alkyl-C ratio was proposed as an indicator for the stage of decomposition of POM (Kölbl & Kögel-Knabner, 2004). For the maize site, the aromaticity and the aryl-C/O-alkyl-C ratio, increased in the order maize litter < $fPOM_{<1.6}$ < $oPOM_{1.6\text{-}2.0}$ < $Mineral_{>2.0}$, corroborating this suggestion. For the spruce site, the aryl-C/O-alkyl-C ratio was approximately constant and for the grassland site, it increased considerably from the $fPOM_{<1.6}$ to the $oPOM_{1.6\text{-}2.0}$ fraction, but remained about the same in the $Mineral_{>2.0}$ fraction. Thus, this ratio was no useful indicator for the degree of SOM decomposition at the spruce or the grassland site. These findings suggest that the application of this indicator may be restricted to arable soils with easily decomposable plant litter.

Despite similar C/N ratios in the POM fractions, which suggested a similar degree of decomposition, the compositional changes in density fractions and the resulting increase in the alkyl-C/O-alkyl-C ratio indicates an increasing degree of decomposition in the order $fPOM_{<1.6}$ < $oPOM_{1.6\text{-}2.0}$ < $oPOM_{<1.6}$. The chemical changes the POM underwent during decomposition were characterized by a loss of easily decomposable carbohydrates and a concomitant selective preservation of the more recalcitrant alkyl-C. These results are in agreement with those of Golchin *et al.* (1994a), who found that the extent of decomposition of the organic materials in the density fractions increased in the order $fPOM_{<1.6}$ < $oPOM_{1.8\text{-}2.0}$ < $oPOM_{1.6\text{-}1.8}$ in five Australian virgin soils. They are also in accordance with the results of Zech *et al.* (1992), who reported an increase in the alkyl-C/O-alkyl-C ratio with soil depth for seven forest soils covering different tree species and humus forms.

The composition of the $fPOM_{<1.6}$ fraction at the spruce site was similar to that of the litter layer, indicating that the $fPOM_{<1.6}$ fraction consisted mainly of relatively fresh plant materials. The more advanced extent of decomposition in the $oPOM_{<1.6}$ fraction compared to the $oPOM_{1.6\text{-}2.0}$ fraction suggests that the degree of association of occluded POM with mineral particles decreased with increasing decomposition. Our results support the model of aggregate formation and stabilization proposed by Golchin *et al.* (1994a). According to this concept, young occluded POM rich in carbohydrates and susceptible to microorganisms is closely associated with mineral particles (indicated by $oPOM_{1.6\text{-}2.0}$) and the formation of stable aggregates. As decomposition proceeds within aggregates, the more labile portions of the oPOM cores are consumed and the association with mineral particles decreases (indicated by $oPOM_{<1.6}$), resulting in less stable aggregates. However, we did not find an accumulation of aryl-C structures in the oPOM fractions as reported by Golchin *et al.* (1997).

The considerably higher proportion of O-alkyl-C and smaller content of alkyl-C in the heavy mineral-associated SOM compared to the oPOM fraction was in accordance with findings from Baldock *et al.* (1997) and Golchin *et al.* (1997) and can be attributed to selective stabilization of microbial metabolites which were enriched in carbohydrate C by adsorption on mineral surfaces. This conclusion is supported by the observation that microbially-synthesized

carbohydrates accumulate in SOM of the clay fraction (Guggenberger *et al.*, 1994).

The missing influence of the aggregate size on the alkyl-C/O-alkyl-C ratio or the aryl-C/O-alkyl-C ratio observed in the spruce and the grassland site suggests that the degree of SOM decomposition was similar in all aggregate size fractions. This conclusion is supported by a similar C/N ratio in all aggregate fractions (John *et al.*, 2005). In agricultural soils, Tisdall & Oades (1982) and Six *et al.* (2000), found an increase in SOC concentration and a decrease in SOM decomposition, indicated by wider C/N ratios, with increasing aggregate size. They explained this by the presence of young organic matter as a binding agent between microaggregates. This effect was not observed in the Ah horizon of the spruce soil.

5.1.2 Effect of land use on the composition of soil organic C

The composition of SOM is influenced by the composition of plant litter inputs, mesofaunal, and microbial products, and by the degree of degradation and stabilization of these primary and secondary SOM sources (Swift *et al.*, 1979). Despite differences in vegetation at the investigated experimental sites (spruce, grassland, maize), the composition of all plant materials was dominated by O-alkyl-C structures which made up 64%–79% of the total signal intensity of the ^{13}C CPMAS NMR spectra. Maize litter had the highest proportions of O-alkyl-C and the lowest contents of alkyl-C, aryl-C and carbonyl-C compared with the grass and spruce litter. Probably caused by different lignin contents, the aromaticity of the litter material increased in the order maize residues < grass residues < spruce residues. The aliphatic signal at 32 ppm was largest for spruce needles and indicated the highest contents of lipids and cutins in this plant material. These results reveal that forest litter is enriched in aromatic, phenolic and alkyl-C, which is in agreement with results of Gregorich *et al.* (1996) and Kögel-Knabner (2002).

When analyzing the effect of land use and vegetation on the chemical composition of SOM pools, one has to take account of the effect of litter placement. In the acid forest soil, litter decomposition occurred already in the humus layer; whereas in agricultural soils, fresh plant residues decomposed

directly in the mineral soil. The decomposition and humification processes in the humus layer resulted in increasing proportions of alkyl-C and decreasing contents of O-alkyl and aryl-C thus, some of the SOM entering the mineral soil of the spruce site might already be depleted in plant-derived polysaccharides.

In all soils, the $fPOM_{<1.6}$ fraction had a considerably lower alkyl-C/O-alkyl-C ratio than the oPOM fractions, indicating that oPOM was at a more advanced stage of plant litter decomposition. There was a clear influence of litter composition on the composition of the POM fractions: the POM fractions of the forest soil had higher alkyl-C contents and smaller O-alkyl-C contents than the grassland and the maize soil. The litter composition was also reflected in the aromatic C content of the $fPOM_{<1.6}$ fraction which was highest in the forest soil. In contrast to that, the proportion of aromatic C in the oPOM was higher in the grassland and maize soils than in the forest soil. These results suggest that the accumulation of aromatic C in the oPOM fraction was favored by a high microbial activity and a high content of easily available carbohydrates in the organic matter inputs. Both factors can promote the selective preservation and relative accumulation of recalcitrant aromatic C compounds. A higher proportion of aryl-C in the oPOM than in the fPOM fraction was also found by Kölbl & Kögel-Knabner (2004) in agricultural soils.

The mineral-associated SOM fraction, which had the lowest C/N ratio at all sites, showed a lower (forest, maize) or equal (grassland) alkyl-C/O-alkyl-C ratio compared to the oPOM fraction, which agrees with the finding that microbial products accumulate in the clay fraction (Guggenberger *et al.*, 1995). The aromaticity of the mineral-associated SOM was much greater in the maize soil (27%) than in the other soils (14–16%). This probably resulted from cultivation, which significantly increased SOC mineralization and reduced the SOC concentration in the mineral-associated fraction by about 50%. Gregorich *et al.* (1996) analyzed the transformation of plant residues into SOM in a maize field and in a nearby mixed hardwood forest. They also found a higher aromaticity of SOM in the arable soil (31%) than in the forest soil (23%), although the content of aromatic C in the plant litter was higher in the forest than in the field. Guggenberger *et al.* (1995) reported that aromatic C accumulated particularly in silt associated SOM. The accumulation of aromatic C in the $oPOM_{1.6\text{-}2.0}$ and the

$Mineral_{>2.0}$ fraction of the maize soil was accompanied by an increasing carbonyl-C content, in particular of the peak at 175 ppm, which originates from carboxyl and amide groups. These results indicate a more advanced degradation of mineral-associated SOM in the maize soil and thus a growing contribution of microbially derived C to the total SOC. The mineral-associated SOM fraction of the forest soil had the highest proportion of alkyl-C (32%), which could be the result of the selective preservation of recalcitrant alkyl-C structures from the spruce litter.

There was no difference in the chemical composition of SOM associated with different aggregate size fractions at a specific site. However, SOM in aggregates of the forest soil had a greater proportion of alkyl-C and a smaller proportion of O-alkyl-C than that within aggregates of the grassland soil, resulting in consistently higher alkyl-C/O-alkyl-C ratios of SOM in the forest soil aggregates. The presented data indicate that this difference was caused primarily by the litter quality and the accumulation of less decomposed POM in the forest soil, which can also be seen in the much lower C/N ratio of aggregates in the grassland (9.0–10.9) compared to the forest soil (19.6–21.2) (John *et al.*, 2005).

5.1.3 Structure and stability of SOM fractions

John *et al.* (2005), who separated the density fractions from the maize soil, determined the stability of the SOC in these fractions on the basis of $\delta^{13}C$ values and it was found that the apparent C turnover time increased in the order $fPOM_{<1.6}$ (22 years) < $oPOM_{1.6\text{-}2.0}$ (49 years) < $Mineral_{>2.0}$ (63 years). However, it was not clear whether the greater stability of the oPOM compared to the fPOM fraction was solely due to physical protection within aggregates or if chemical recalcitrance contributed to the slower turnover of the oPOM. The decrease of O-alkyl-C in $oPOM_{1.6\text{-}2.0}$ and the increases of alkyl-C (by 34%), aryl-C (by 26%), and carbonyl-C (by 51%) with respect to $fPOM_{<1.6}$ suggest that the greater stability of the POM occluded in aggregates depended largely on the lower content of carbohydrates and the relative accumulation of more recalcitrant C compounds. These results support the findings of Golchin *et al.* (1997), who also compared the ^{13}C NMR spectra of POM fractions with the isotopic composition of SOC and observed that the content of O-alkyl-C was inversely related to the

stability, whereas the content of aromatic C was directly related to the SOC stability. The mineral-associated SOM showed a greater stability than the occluded $POM_{1.6\text{-}2.0}$ even though the alkyl-C/O-alkyl-C ratio decreased from 0.41 to 0.36. Thus, organic matter attached to mineral particles appeared to be more resistant to mineralization.

5.2 Suitability of chemical fractionation methods for obtaining stable pools of soil organic matter

5.2.1 Treatment with H_2O_2

The content of H_2O_2-resistant SOC was not significantly different between land uses in the Rotthalmünster soils (0.9–1.6 g kg^{-1}), despite large differences in initial, mineral-associated SOC contents (3.6–24.5 g kg^{-1}). The proportion of H_2O_2-resistant SOC was highest in C-depleted subsoils and lowest in soils with high C saturation of minerals. A similar trend was observed by Eusterhues *et al.* (2005) who found that in soil profiles of sandy texture under temperate forest, the H_2O_2-resistant SOC fraction increased with soil depth. This likely arises from a more intimate association of SOM with mineral surfaces at lower SOC loadings, which resulted in a larger protection of SOC against chemical attack (Kaiser & Guggenberger, 2003). In addition, the results presented in this study agree with the view that the proportion of stable SOM to total SOC increases for soils with similar texture and mineral assemblage as total C is depleted through reduced inputs, e.g. by cultivation of native soils, because the more labile components are consumed first (Paul *et al.*, 2006; Plante *et al.*, 2006).

However, the results of this study are contrary to findings of Plante *et al.* (2004), who investigated SOC contents in untreated and H_2O_2-treated clay fractions of a humic acid loam with different land managements. They found that H_2O_2-resistant SOC was a constant proportion of initial C contents. This may be a result of the different size fractions studied, because Plante *et al.* (2004) investigated the clay fraction only, whereas in this study bulk soil after density fractionation was used. Still, it is unclear to which extent the findings of this study may be generalized.

The low C/N ratios of the H_2O_2-resistant fractions in this study indicated a high stability of some N compounds against treatment with H_2O_2, which accords with

findings of other studies (Cheshire *et al.*, 2000; Leifeld & Kögel-Knabner, 2001; Plante *et al.*, 2004). The protection of N was suggested to result from interaction with mineral surfaces, protection within microaggregates, or fixation of NH_4^+ to minerals. The higher C/N ratios in the H_2O_2-resistant fraction of the Halle compared with the Rotthalmünster soil may result from the larger contribution of H_2O_2-resistant black carbon in the Halle soil (Schmidt *et al.*, 1999) or from a larger amount of NH_4^+ fixed to clay minerals in the Rotthalmünster soil (16–17% clay content compared to 7–9% in the Halle soil).

Treatment with H_2O_2 removed a significantly higher percentage of young, maize-derived SOC compared to C_3-derived SOC. This indicates that the main proportion of maize-C that entered the soil over the last three to five decades was held in weaker mineral-organic associations likely due to the occupation of 'high-affinity' sites by pre-existing SOM. These weaker associations probably offered less protection against chemical attack. This may also explain the reduced efficiency of H_2O_2 to remove mineral-associated SOC in horizons with lower SOC content. Similarly, Kaiser & Guggenberger (2007) showed that SOC most strongly attached to goethite at small organic carbon loadings (0.9 mg m^{-2}) was barely removable by NaOCl compared with organic carbon contained in weaker, more bulky agglomerations at larger organic carbon loadings (1.9 mg m^{-2}).

The strong decrease in ^{14}C activity, corresponding to an increase in mean ^{14}C age by 8000–10000 years upon treatment with H_2O_2, confirms a preferential removal of younger organic constituents. Mean ^{14}C ages in the present study were considerably higher than those reported by Eusterhues *et al.* (2005) who found that the mean ^{14}C age of the H_2O_2-resistant fraction in two forest soils ranged from modern to 5680 years. The lower mean ^{14}C ages in the latter study may, at least partly, be attributed to the presence of H_2O_2-resistant aliphatic components derived from fresh particulate organic matter (Eusterhues *et al.*, 2005; v. Lützow *et al.*, 2006a), which had not been removed prior to the treatment. The remarkably high mean ^{14}C age of H_2O_2-resistant SOC of about 13000 years in the loess-derived silty loam of Rotthalmünster suggests that some of this old, stable SOC was already present in the loess deposits.

Overall, the presented findings indicate that the H_2O_2 treatment is suitable to separate a stable SOC fraction, because it (i) preferentially removed younger SOC and (ii) the residuum exhibited a high stability as revealed by both, ^{13}C and ^{14}C analysis.

5.2.2 Treatment with $Na_2S_2O_8$

The $Na_2S_2O_8$-resistant SOC fraction was neither a constant portion of the initial, mineral-associated SOC nor dependent on land use. Similar to the H_2O_2 treatment, the amount of residual SOC increased with decreasing contents of mineral-associated SOC. This is in agreement with results of Eusterhues *et al.* (2003), who reported an increasing proportion of $Na_2S_2O_8$-resistant SOC with increasing soil depth in two forest soils (0.7–2.3% in topsoils; 29–84% in subsoils).

$Na_2S_2O_8$-treated samples had noticeably higher C/N ratios than the H_2O_2-resistant fraction. These differences in C/N ratios suggest that treatments cause preferential removal of certain SOM components with the N fraction being more susceptible to treatment with $Na_2S_2O_8$ than H_2O_2. Beside differences in the reactivity of oxidants, this may additionally result from the varying reaction conditions: While the pH was kept between 7 and 8.5 during treatment with $Na_2S_2O_8$, the suspension was not buffered during H_2O_2 treatment. The neutral to alkaline ($Na_2S_2O_8$) versus acidic (H_2O_2) conditions may cause selective losses of certain SOM fractions.

The removal efficiency of $Na_2S_2O_8$ for maize-derived SOC was similar to that of H_2O_2 and the selective removal of more recently incorporated SOC by $Na_2S_2O_8$ was additionally confirmed by the ^{14}C data. Mean ^{14}C ages were in the same order of magnitude as those observed for the H_2O_2-resistant fraction, but consistently lower. This indicates that the selective removal of young SOC components by $Na_2S_2O_8$ appears to be more efficient than by H_2O_2. Our findings are thus in line with Eusterhues *et al.* (2003) who found a strong negative correlation between the percentage of $Na_2S_2O_8$-resistant SOC and ^{14}C activity in the bulk soil, indicating that chemically resistant SOM is enriched in old SOC.

I think that the $NaHCO_3$ buffer did not significantly influence the age determination, because (i) the mean ^{14}C ages of residual SOC fractions after the carbonate-buffered $Na_2S_2O_8$ were of the same order of magnitude as those after the non-buffered H_2O_2 treatment and (ii) there was no general shift to more positive $\delta^{13}C$ values of $Na_2S_2O_8$-resistant fractions of the reference sites (wheat/rye), which would be the case if traces of the buffer ($\delta^{13}C$: -5.3‰ V-PDB) were left in the residue. The shift in $\delta^{13}C$ of SOM from the reference sites was below ±1‰ V-PDB for all tested chemical fractionation protocols without a general trend of more positive or negative $\delta^{13}C$ values in the residual C fractions.

Consequently, the presented findings suggest that treatment with $Na_2S_2O_8$ was able to separate a stable SOC fraction, corroborating findings of Cuypers *et al.* (2002), who observed that changes in SOM composition due to persulphate treatment were similar to compositional changes upon humification.

5.2.3 Treatment with NaOCl

Losses of mineral-associated SOC upon treatment with NaOCl (69–84%) agree well with findings of other studies (Kleber *et al.*, 2005; Mikutta *et al.*, 2005a). The larger amount of NaOCl-resistant SOC compared to SOC fractions resistant to the other oxidative treatments likely resulted from the lower reaction temperature and/or from a lesser ability of NaOCl to remove mineral-associated SOM. In contrast to hydrolysis and treatment with H_2O_2 or $Na_2S_2O_8$, the proportion of NaOCl-resistant SOC in the Rotthalmünster soil did not increase with decreasing amounts of initial, mineral-associated SOC. It was significantly higher in the forest soil than in the continuous grassland and the maize soil. The considerably larger amount of NaOCl-resistant SOC in the forest soil can partly be explained by differences in the composition of SOM (cp. chapter 4.1.1 and 5.1.2): the decomposition and transformation processes in the organic layer of the forest soil resulted in a higher proportion of alkyl-C that enters the mineral soil. Aliphatic constituents of SOM are supposed to be less reactive upon oxidation and thus to enrich during oxidative treatments (Mikutta *et al.*, 2005b).

The efficiency to remove young, maize-derived SOC was lower for NaOCl than for H_2O_2 or $Na_2S_2O_8$, but still, the NaOCl-resistant SOC had a two to four fold higher apparent C turnover time than the initial, mineral-associated SOC. The

mean ^{14}C age corroborated this finding: the NaOCl-residue was about 1000–2000 years older than the mineral-associated SOC fraction. This is in accordance with findings of Kleber *et al.* (2005) who reported an increasing mean ^{14}C age of several hundreds to 1600 years in 12 acid subsoil samples upon NaOCl treatment.

Therefore, the results of this work suggest that NaOCl is capable of isolating a stable SOC fraction, but compared with treatment with H_2O_2 and $Na_2S_2O_8$, younger SOM associated with the mineral matrix is not attacked by this treatment. A larger efficiency to isolate stable SOC, however, can be expected at higher reaction temperature.

5.2.4 Demineralization of NaOCl treated soils (NaOCl+HF)

Demineralization with HF released 38–57% of NaOCl-resistant SOC, which agrees with findings of an investigation of 12 acid subsoil horizons of tropical and temperate forest subsoils (Mikutta *et al.,* 2006). The presented data indicate that about half of the NaOCl-resistant SOC attached to minerals in our soils was insoluble in HF. Since no minerals except for quartz and muscovite in 12 acid subsoil horizons survived treatment with HF (Mikutta *et al.,* 2006), this may predominantly be ascribed to the highly aliphatic nature of the residuum (Mikutta *et al.,* 2006).

Demineralization induced further losses of both C_3- and C_4-derived SOC. In the Rotthalmünster soil, the HF treatment further enriched older SOM components as evidenced by lower proportions of maize-derived SOC and a mean ^{14}C age that was about twice that of the NaOCl-resistant C fraction. Similarly, Mikutta *et al.* (2006) reported older mean ^{14}C ages in 10 out of the 12 investigated acid subsoil samples.

In contrast, in the Halle soil, demineralization was less effective in removing young SOC; a larger loss of C_3-SOC (39% of NaOCl-resistant SOC) than of C_4-SOC (5% of NaOCl-resistant SOC) was observed. The ^{14}C activity increased by 6 pMC whereas the apparent C turnover time calculated from the ^{13}C data decreased. Consequently, the ^{13}C data reveal a preferential loss of older C_3-derived SOC compared to the NaOCl treatment without HF, while the ^{14}C data

indicates the loss of SOC with higher ^{14}C activity (~59 pMC) compared to that of the NaOCl-residuum (49.5 pMC). However, the low ^{14}C activity of the removed SOC also reveals a considerable loss of old carbon.

In consequence, treatment with NaOCl+HF isolates a SOC pool, which may represent either (bio)chemically resistant materials attached to mineral surfaces or the chemically least reactive 'backbone' of mineral-associated SOM. Based on ^{13}C and ^{14}C data, this fraction can be considered as stable SOM, but, as noted for the treatment with NaOCl, it depended on initial SOC contents and was considerably younger than the SOC fractions isolated by $Na_2S_2O_8$ or H_2O_2.

5.2.5 Stepwise hydrolysis

The SOC fraction resistant to stepwise hydrolysis comprised 10–35% of the initial, mineral-associated SOC. These proportions were larger than those reported in the < 10-µm fraction of arable and forest soils (5–6% of initial SOC; Augris *et al.*, 1998; Poirier *et al.* 2000, 2003), but comparable to quantities found in the topsoil of a sandy Spodosol in south-west France under pine forest (34% of initial SOC; Quénéa *et al.*, 2005a,b) and maize cropping (20% of initial SOC; Quénéa *et al.*, 2006).

The investigation of different land uses and soil depths in the Rotthalmünster soil revealed an increase of hydrolysis-resistant SOC with decreasing contents of mineral-associated SOC in the order: continuous grassland (10%) < forest (15%) < agricultural top soil (16–22%) < agricultural subsoil (22–27%), which is in line with the notion that the proportion of resistant SOM will increase upon SOM depletion (Plante *et al.*, 2004; Paul *et al.*, 2006). However, the results of the present study are contrary to findings by Plante *et al.* (2006), who observed no relation between the proportion of hydrolysis-resistant SOC with C depletion in silt- and clay-sized fractions of soils under native vegetation, no-till and conventional till, but a strong correlation of C concentrations in the hydrolysis-resistant fraction with initial C contents. This was partly explained by a contribution of physical protection within microaggregates, while the strength of protection of SOC by microaggregates may have been reduced in this study due to the more vigorous hydrolysis procedure applied (multiple step versus single step hydrolysis).

The smaller proportion of hydrolysis-resistant SOC in the Rotthalmünster than the Halle soil can be explained by a smaller amount of black carbon, which is, besides aliphatic components, melanoidins, and tannins, a major component of the hydrolysis-resistant SOM fraction (Poirier *et al.*, 2000; 2002; 2003; Quénéa *et al.*, 2005a,b).

The higher C/N ratios of the hydrolysis-resistant SOM fraction compared to the residual fractions obtained by the other chemical fractionation methods were expected, since proteinaceous compounds are preferentially hydrolyzed (Paul *et al.*, 2006).

Hydrolysis preferentially removed young, maize-derived SOC, which accords with findings by Poirier *et al.* (2005a). The selective removal of younger SOC was also confirmed by the ^{14}C data. The hydrolysis-resistant SOC fraction had a mean ^{14}C age that was 2800–5300 years older than the initial, mineral-associated SOC; this is considerably older than the resistant SOC fraction in a silty loam investigated by Poirier *et al.* (2005a) (120–340 years) but consistent with other studies (Leavitt *et al.*, 1996; Paul *et al.*, 1997, 2001, 2006).

The proportion of maize-derived SOC in the non-hydrolyzable fraction was higher than in the residual fraction of any other method tested. This resulted in a comparably shorter apparent C turnover time whereas the mean ^{14}C age was higher than those of the NaOCl- and (NaOCl+HF)-resistant fractions. Hydrolysis is supposed to leave black carbon unaffected, which may explain the higher ^{14}C age, but it also left some young but chemically resistant maize-derived materials, leading to a shorter apparent C turnover time calculated on the basis of ^{13}C natural abundance. This result demonstrates that differences in the apparent stability of SOM arise from the different methodologies applied (^{13}C versus ^{14}C). Consequently, for assessing the stability of treatment residuals, the natural ^{13}C abundance method should always be combined with radiocarbon dating because both approaches complement each other.

I conclude that despite being older, the hydrolysis-resistant SOC fraction does not adequately represent a stable SOC pool, since freshly synthesized materials may resist hydrolysis whereas older SOM components may be selectively hydrolyzed. The presented results thus corroborate previous findings that there is

no close parallel between the resistance to hydrolysis and the ability to resist degradation in the soil (Balesdent, 1996; Poirier *et al.*, 2003).

5.2.6 Comparison of isolated stable SOC fractions with the IOM pool of the Rothamsted Carbon model

For the agricultural Rotthalmünster soils, the H_2O_2- and $Na_2S_2O_8$-resistant SOC fractions were about twice as large as the size of the IOM pool calculated by the Rothamsted Carbon Model and isotope data (0.7 g kg^{-1}; Ludwig *et al.*, 2005). For the Halle soil with its large amounts of fossil C, the obtained stable SOC fractions (1.2–2.3 g kg^{-1}) were considerably smaller than the calculated IOM pool (6.7 g kg^{-1}; Ludwig *et al.*, 2003). Treatment with H_2O_2 and $Na_2S_2O_8$ without preliminary density fractionation resulted in residual SOC fractions (3.2 and 3.5 g kg^{-1} for the maize soil) which were closer to the IOM pool, but deviations were still considerable. Thus, neither H_2O_2- nor $Na_2S_2O_8$-resistant SOC appears to match the size of the IOM pool as obtained by using stable isotopes and modeling for the two sites (Ludwig *et al.*, 2003; 2005). The other chemical fractionation methods recovered even larger amounts of residual SOC in the Rotthalmünster soil while in the Halle soil the size of the residual SOC fraction was comparable with those after H_2O_2 and $Na_2S_2O_8$ treatment. Therefore, none of the tested methods were capable of isolating a stable SOC pool that may be used in the Rothamsted Carbon model.

5.3 Assessing the suitability of thermal oxidation for isolating stable SOM pools

The magnitude of the weight losses observed in the present study are in line with findings of several other studies on various soil types and SOM fractions (Leinweber & Schulten, 1992; Leinweber *et al.*, 1992; Lopez-Chapel *et al.*, 2005; Plante *et al.*, 2005; Kuzyakov *et al.*, 2006). The increasing weight losses from the agricultural soil to the grassland and forest soils were expected, because weight losses occur mainly due to degradation of SOM. Correlations between SOM content and weight loss due to thermal oxidation were observed in several other studies (Plante *et al.*, 2005; Siewert, 2001).

The complete SOM degradation at temperatures between 400 and 500°C is also in accordance with observations in other SOM studies (Leinweber & Schulten,

1992; Plante *et al.*, 2005; Kuzyakov *et al.*, 2006). Weight losses above these temperatures mainly reflect mineral lattice changes with loss of O, S, P and H (Kuzyakov *et al.*, 2006). The complete oxidation of SOC points out that the black carbon of the Halle soil was not thermally inert, which is in line with findings by Derenne & Largeau (2001) and Schmidt *et al.* (2001) who found that determination of black carbon by benzenepolycarboxylic acids or photooxidation led to much higher values for black carbon than thermal or chemical oxidations, pointing either to an overestimation of black carbon by these methods or to a partial destruction of black carbon by oxidative treatments.

In the Rotthalmünster soil, the proportion of SOC resistant to thermal oxidation did not increase with C depletion. According to Plante *et al.* (2005) this would be implied for a stable C fraction comparing soils with similar texture and mineralogy as the more labile fractions are decomposed first. In contrast to results from the present study, Plante *et al.* (2005) observed a shift in SOM composition from a higher contribution of thermally labile OM in a forest soil to a higher contribution of thermally resistant fractions in a long-term bare fallow in a study on the clay fraction of a cultivation sequence on a silty soil. Thus, it remains unclear to which extent the results of this work may be generalized.

The different behavior of total N compared to the SOC fraction was also observed during chemical fractionation (cp. chapter 4.2). Further, Kuzyakov *et al.* (2006) reported a different behavior of total N to thermal analysis compared with the SOC fraction. It can be assumed that the different behavior of the N fraction partly results from the presence of NH_4^+ fixed to clay minerals, which may resist thermal oxidation as was found for chemical oxidative treatments (cp. chapter 5.2.1).

For obtaining a SOC fraction of considerably higher stability, a strong preferential loss of young, C_4-SOC is implied. This was observed for the Halle soil, but the preferential loss of young C_4-SOC in the Halle soil likely resulted from high amounts of black carbon in this soil (~2 g kg^{-1} soil; Brodowski, 2005). Part of the black carbon was lost by density fractionation, but according to findings by Brodowski (2005) who reported 65% of the black carbon to be contained in the density fraction < 2 g cm^{-3} considerable amounts of black carbon

were left in the sample. This is also in accordance with a still very high ^{14}C age (~3500 years) in the Halle maize soil after density fractionation as reported in chapter 4.2.1. Hence, the Halle soil contained a fairly great pool of thermally stable C_3-SOC and the proportion of C lost from the C_4-SOC was much higher than the respective losses from the C_3-SOC pool. No such trend could be observed in the non-contaminated Rotthalmünster soil. Thus, in the soil with no "fossil carbon contamination", young C_4-SOC was hardly lost selectively. The resulting low increase in apparent C turnover in this soil corroborated the findings of Kuzyakov *et al.* (2006) who investigated a silty loam 10.5 years after C_3-/C_4-vegetation change and stated that the proportion of C with short-term turnover was nearly similar in thermally labile and stable fractions.

The incapability of thermal oxidation to leave residual organic matter of high stability can partly be explained by its reaction pathway: thermal stability is mostly compound-specific: In the low temperature range, aliphatic components and carboxyls are degraded and at higher temperatures, more refractory compounds like aromatic C are lost – independently of their age. Further, lignin has a high thermal stability (Leinweber *et al.*, 1992), but was found to be readily degraded in soil (Poirier *et al.*, 2003). Thus, the reaction pathway in some way resembles that of hydrolysis, which was previously found to be incapable of isolating a functionally stable SOM pool (chapter 5.2.5).

5.4 Effect of residue decomposability and the structure and activity of the microbial biomass on the formation of macroaggregates and C sequestration in aggregate fractions

5.4.1 Mineralization of the added residue

The significantly enhanced soil respiration after addition of maize residues reflected the easily available nature of the residues as C source, which was confirmed by the high contribution of maize-derived C to the CO_2-production of up to 90%. The lower soil respiration in the root treatment and the later maximum compared to the leaf treatment derived from the higher amount of easily available C in the leaf treatment due to its much narrower C/N ratio and to a higher content of persistent compounds, such as waxes or lignin, in the coarse roots compared to the leaves. Increasing proportions of soluble (and therefore

easily available) C and N with decreasing C/N ratios of the residue have been reported by Reinertsen *et al.* (1984).

The further decrease in soil respiration and mineralization of the added residue after the addition of fungicide shows that the fungicide successfully suppressed part of the microbial biomass. This was confirmed by lower amounts of chloroform-labile C extractable by 0.05 M K_2SO_4 throughout the incubation, but not by a generally lower ergosterol concentration. Captan was used previously in incubation studies and found to drastically reduce the fungal biomass as detected by microscopy and substrate induced respiration for up to 44 days (Denef *et al.*, 2001; Bossuyt *et al.*, 2001; Frey *et al.*, 2003). Therefore, and because of the significant decrease in the amount of the chloroform-labile C pool observed in the incubation experiment, it is unlikely that the fungicide did not work. This questions ergosterol as a generally suitable biochemical marker for the quantification of fungal biomass. While ergosterol was used as valuable indicator for the fungal biomass in the field or in laboratory studies with addition of substrates (West *et al.*, 1987; Joergensen, 2000; Klamer & Bååth, 2004; Malosso *et al.*, 2004), Zhao *et al.* (2005) found (i) that ergosterol was hardly mineralized when added to soil as substrate and (ii) no response or just a slight decline in the ergosterol concentration after fumigation of the soil as well as after addition of several pesticides (including Captan) despite a considerable decrease in the fungal biomass as determined under the microscope and by the substrate induced respiration method according to Beare *et al.* (1991). Consequently, despite providing a good index of fungal growth, ergosterol seems to be inappropriate as indicator for the fungal biomass in periods of rapid decline in the fungal biomass. Therefore, I agree with the view of Klamer & Bååth (2004) and Zhao *et al.* (2005) that relying on a single biomarker bears uncertainties if the objective is to monitor soil microbial communities. These can be minimized by complementing measures of one biomarker by the use of another, e.g. phospholipid fatty acids (PLFA), which were found to be rapidly degraded following cell death (Zelles *et al.*, 1992).

5.4.2 Soil aggregation

The differences in the availability of C and N deriving from the added maize residues resulted in significant differences in the amounts of water-stable aggregates and significantly different aggregation dynamics between the control, leaf and root treatment. The treatment with residues of lower decomposability (root) developed a lower amount of macroaggregates and showed a later maximum aggregation in the incubation experiment. The influence of residue decomposability on soil aggregation was highlighted earlier by some authors. For example, Bossuyt *et al.* (2001) incubated a silt loam with addition of plant residues of different degradability (C/N ratios of 19.7 and 108) and observed a significantly smaller amount of water-stable macroaggregates in the treatment with addition of residues of lower decomposability after two weeks of incubation. Further, Martens (2000) found the most rapid increase in soil aggregation in treatments with residues of low phenolic acid content, but the highest aggregation after 84 days was observed in treatments with residues of high phenolic acid contents during 84 days of incubation of a silty-clay loam.

The good relation of the amount of water-stable aggregates to the size of the microbial biomass pool and its activity emphasizes the important role of the microbial community in soil aggregation. The decomposition of the added maize-residues resulted in the production of microbial products that serve as binding agents for aggregate formation (Tisdall & Oades, 1982; Golchin *et al.*, 1997). These need to be produced in sufficient amounts before significant aggregate formation can occur. This may explain the observed retardation in maximum aggregation compared to the maximum respiration. The more distinct retardation in the root compared to the leaf treatment can be explained by the lower availability of C and N from maize residues in the root compared to the leaf treatment, resulting in a lower microbial activity and a longer time until sufficient synthesis products for significant macroaggregation were released in the root treatment.

The reduced CO_2 release and delayed macroaggregate formation for up to 42 days after the elimination of the fungal biomass in the leaf and root treatments with addition of Captan points out the influence of the fungal biomass on the

initial mineralization of fresh plant residues and it shows the vital impact of soil fungi on the formation of water-stable macroaggregates. This is in accordance with findings by Bossuyt *et al.* (2001), who observed reduced macroaggregation after the suppression of the fungal biomass, but not after the suppression of the bacterial biomass, and points out the importance of the fungal biomass in soil macroaggregation. Similarly, Denef *et al.* (2001) and Frey *et al.* (2003) reported a significant influence of the presence of the fungal biomass on the formation of water-stable macroaggregates.

Further, the presented results confirm previous findings that changes in aggregation can happen fast (Angers *et al.*, 1997; Golchin *et al.*, 1997; De Gryze *et al.*, 2005) – especially the formation of macroaggregates is a very rapid process: In the leaf treatment, maximum macroaggregation was reached two weeks after the start of incubation, which is in accordance with findings by Martens (2000) and Bossuyt *et al.* (2001), who observed the maximum amount of water-stable macroaggregates after 9 and 12 days of incubation of soil with addition of plant residues, respectively.

However, disaggregation was observed early in the incubation experiment: when the most easily available C was consumed and soil respiration and thus microbial activity started to decrease, the disintegration of newly formed macroaggregates commenced.

5.4.3 The role of water-stable aggregates for C and N stabilization

The results of the C and N analysis showed a trend of decreasing C concentrations and decreasing C/N ratios with decreasing aggregate size, which supports the concept of aggregate hierarchy postulated by Tisdall & Oades (1982) and strengthened by findings of several subsequent studies (e.g. Jastrow *et al.*, 1996; Puget *et al.*, 2000; John *et al.*, 2005; Yamashita *et al.*, 2006). The high C concentrations of the few water-stable macroaggregates in the control soil likely result from abiotic aggregation processes, i.e. the flocculation of clay particles, which were found to contain the highest amount of C per kg fraction among particle-size fractions in soil (Puget *et al.*, 2000; Ludwig *et al.*, 2005). Biotic macroaggregation, being the main aggregating process in the leaf and root

treatment, includes the entanglement of bigger, less C enriched soil particles, e.g. of the silt-sized fraction.

Besides a high C concentration in water-stable macroaggregates, a high proportion of maize-derived C within water-stable macroaggregates (36–61% of total C) was observed. The proportion of maize-derived C incorporated into water-stable macroaggregates during the incubation experiment is comparable to the 38–50% of maize-derived C observed in water-stable macroaggregates isolated from the field soil after 23 years of maize-cropping by John *et al.* (2005), showing that the maximum incorporation of recently deposited C is achieved instantly. This emphasizes the rapid dynamics of macroaggregation and the importance of soil macroaggregates in incorporating and fresh SOM (Angers *et al.*, 1997; Gale *et al.*, 2000a; Puget *et al.*, 2000; John *et al.*, 2005). However, the results of this study suggest that there is no long-term protection of fresh SOM in macroaggregates since disaggregation of macroaggregates occurred after less than two months of incubation.

Significantly lower proportions of added maize-derived C in the microaggregate and non-aggregated fractions were not surprising, since it is well known that the C turnover is much slower in the latter two fractions (Besnard *et al.*, 1996; Puget *et al.*, 2000; John *et al.*, 2005; Yamashita *et al.*, 2006). Still, these aggregate size fractions seem to incorporate young C much faster than possibly assumed: Despite the relatively short incubation time of three months, a considerable part of the added maize-C was found in the microaggregate (8–12%) and non-aggregated fraction (5–8%), corresponding to one fifth and one third of the maize-C incorporated in these fractions after 23 years of maize-cropping in the field (John *et al.*, 2005). According to findings of Yamashita *et al.* (2006) that the major part of the C in is in association with the mineral matrix it can be assumed that most of the maize-derived C was located in the mineral-associated SOM fraction and only part of it was stored as particulate organic matter occluded in microaggregates.

Further, due to the much higher contribution of microaggregates to the soil mass compared to macroaggregates, the microaggregate fraction was found to be the most important fraction for the storage of the added maize residues during the

investigated time period for both the C and N fraction. This accords with findings from Angers *et al.* (1997), who incubated a silty soil with addition of $^{13}C^{15}N$-labeled wheat straw for a 18 months period and found less than 20% of water-stable macroaggregates during the whole incubation period and a rapid increase in ^{13}C in the 50–250 µm fraction with proceeding incubation up to 50% of residual ^{13}C and ^{15}N at day 574.

The quick incorporation of fresh plant residues, but also the rapid destabilization of water-stable macroaggregates as no more easily substrate was added to the soil highlights the sensitivity of soil macroaggregates to cultivation changes as reported in several studies (Chaney & Swift, 1984; Oades, 1984; Besnard *et al.*, 1996). These rapid dynamics of macroaggregation questions the function of soil macroaggregates for stabilizing SOM. Only in a system with continuous addition of fresh, easily decomposable residues in the form of decomposing plant materials and/or root exudates, a high level of macroaggregation can be maintained, but these turn over rapidly. Undoubtedly, a well aggregated soil is essential, because the soil structure influences all soil functions (Kononova, 1961; Stevenson & Elliott, 1989; Doran & Parkin, 1994; Piccolo, 1996), but a stabilization of SOC in the long-term cannot be granted by soil macroaggregates. At the aggregate level, the medium-term stabilization seems to happen in the microaggregate fraction, while the long-term stabilization presumably derives from the non-aggregated fraction, i.e. the mineral-associated SOM.

6 Concluding remarks

The overall objective of the present thesis was to evaluate present techniques for SOM fractionation and to get a better understanding of the factors controlling SOM sequestration and stabilization. For modeling long-term changes in SOM, e.g. following land-use changes or variations due to global warming, there is a special need of fractionation procedures that are capable of isolating functional SOM fractions with respect to SOM storage and stability. One of the difficulties in developing such fractionation procedures is the marked heterogeneity of the soil environment, the resulting high complexity of SOM and the various stabilization mechanisms present in soils and soil horizons with different texture and mineralogy (often several mechanisms operating simultaneously).

Therefore, SOM fractions of two soils with different texture and under different land use isolated by several fractionation procedures were investigated in terms of their importance as C and N storage, their molecular structure, age/turnover, and the factors controlling their stability.

In this final chapter, the most relevant conclusions of the presented experiments are drawn.

Investigations of organic matter composition in litter, aggregate and density fractions

Results from the ^{13}C NMR analysis show that the applied density fractionation produced SOM pools which differed in their chemical composition. These changes, especially the differences in the alkyl-C/O-alkyl-C ratio, suggest that the density fractions represented different stages of SOM decomposition. Therefore, density fractionation is an appropriate method for tracing different degrees of decomposition (entailing different degrees of protection – free, occluded and mineral-associated) and offers a way of isolating functional SOM pools. However, in biologically active soils, the mineral-associated SOM generally makes up the major part of SOM and can be assumed as a still very heterogeneous pool that can be further divided into more homogeneous pools, e.g. by chemical fractionation (see below).

Additionally, the ^{13}C CPMAS NMR spectra showed that changes in land use and management do not only influence the total SOC and N stocks in soils and SOM fractions, but also change the composition of SOM in soil density fractions. Thus, land use effects on SOM structure were caused by the different contribution of specific SOM fractions to the total SOM storage, but they were also based on differences in the chemistry of specific SOM fractions.

The composition of SOM was influenced by litter quality, the intensity of litter decomposition and the related production and accumulation of microbially-derived substances. Soil organic matter of the acid forest soil was characterized by large amounts of POM with a high content of spruce litter-derived alkyl-C. In the biologically more active grassland and maize soil, litter-derived POM was decomposed more rapidly and SOC stocks were dominated by mineral-associated SOM. The mineral-associated SOM contained greater proportions of aryl-C and carbonyl-C and is considered to enclose more microbially-derived organic substances. Mineralization of POM and mineral-associated SOM was fastest in the maize soil. The rapid turnover was associated with an accumulation of aromatic and carbonyl-C structures in the mineral-bound SOM. Soil organic matter in the separated aggregates did not differ between aggregate size classes, most likely due to a major contribution of mineral-associated SOM to soil aggregates, which did not differ in the degree of decomposition. The spectra of aggregate size fractions resembled those of the mineral-associated SOM fraction isolated by density fractionation and thus showed differences between land uses.

Consequently, land use can alter the structure and stability of SOM fractions. Therefore, the effect of land use on SOM storage should not only be assessed in terms of total C stocks, but also with respect to changes of SOC structure, stability and function.

Suitability of chemical fractionation methods for obtaining stable pools of soil organic C

According to both, ^{13}C and ^{14}C data, all investigated chemical fractionation methods preferentially removed younger SOC and hence resulted in a considerably older, by definition more stable SOC fraction compared to the initial, mineral-associated SOC. The highest apparent C turnover times and mean

^{14}C ages were obtained in the residual C fractions after treatment with H_2O_2 and $Na_2S_2O_8$. The highest proportion of young, maize-derived C was left upon stepwise hydrolysis.

Stepwise hydrolysis aims to isolate a SOM fraction composed of recalcitrant biomacromolecules, which are supposed to accumulate upon reduction of C inputs due to consumption of easier available SOM. The nature of the hydrolytic treatment also explains the low increase in apparent C turnover time and mean ^{14}C age – hydrolysis removes old non-recalcitrant C, which may be protected by the mineral phase and preserves recalcitrant biomacromolecules (e.g. aliphatics, cutans, suberans), independent of their age. Thus, stepwise hydrolysis isolates a recalcitrant SOM pool – which could be termed functional, but as stated above, not all the biomacromolecules that survive hydrolysis were found to survive degradation in the soil. Therefore, I would not define this pool as functional. Further, due to the omnipotence of the microbial biomass, the hydrolysis-resistant pool likely represents no passive SOM fraction.

The H_2O_2- and $Na_2S_2O_8$-resistant SOM presumably survived these treatments due to a very close association with the mineral phase and/or metal ions, which protected this SOM fraction from oxidation. Consequently, treatment with H_2O_2 and $Na_2S_2O_8$ were most efficient in obtaining a functionally stable SOC fraction. The very high mean ^{14}C age of H_2O_2- and $Na_2S_2O_8$-resistant SOC (about 13000 years) in the loess derived silty loam suggests that some of this old, stable SOC was not formed in situ, but was already present in the loess deposits.

However, none of the tested chemical fractionation procedures accurately represented the IOM pool as calculated for the investigated sites using the Rothamsted Carbon Model and isotope data. However, despite this mismatch in size with the IOM pool, the SOC fractions isolated by the H_2O_2 and $Na_2S_2O_8$ treatments did not differ in size between land uses, which is in line with the view that stable SOM is independent of the current land use and therefore agrees with the concept of an IOM pool.

<u>Assessing the suitability of thermal oxidation for isolating stable SOM pools</u>

Thermal oxidation with increasing temperature was assumed to be capable of isolating SOM fractions of increasing stability, but according to the results

obtained in this study, this is not the case. Isolation of stable SOM would imply a preferential loss of young, maize-derived C, but this was not generally observed: In the Rotthalmünster soil, young, maize-derived SOC was not preferentially removed. Thus, the apparent C turnover times of the residual C fractions increased just slightly. Only in the fossil C contaminated Halle soil, thermal oxidation isolated SOM fractions with high apparent C turnover times due to the relative accumulation of thermally stable C_3-C. Because of its fairly great pool of thermally stable fossil C_3-SOC, the Halle soil has to be considered as an exception.

Effect of residue decomposability and the structure and activity of the microbial biomass on the formation of macroaggregates and C sequestration in aggregate fractions

The $^{13}C^{15}N$ label of the maize residues were very helpful in tracing the partitioning of maize-C and -N during litter decomposition into several soil fractions. It was shown that the decomposability of the added residue significantly influenced the size of the microbial biomass pool, its activity and thereby the amount of water-stable aggregates and aggregation dynamics. Further, the fungal biomass was found to be of vital importance for the process of macroaggregation.

The rapid dynamics of macroaggregate formation and disintegration led to the conclusion that despite being important for a soil in terms of providing good soil structure, soil macroaggregates cannot grant a long-term stabilization of SOM. At the aggregate level, the medium-term stabilization seems to appear in the microaggregate fraction, while the long-term stabilization most likely derives from the non-aggregated fraction, i.e. the mineral-associated SOM.

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8 Appendix

Table 8.1: Carbon content, C/N ratio, $\delta^{13}C$, ^{15}N and ergosterol content of the soil and plant materials used for the incubation experiment (means and standard deviation, n=4).

	Soil < 250 µm	Leaf material	Root material
C /g kg^{-1} DW	12.6 (0.1)	421 (0.7)	422 (1.8)
C/N	8.9 (0.1)	27.4 (0.7)	86.4 (1.6)
$\delta^{13}C$ /‰ V-PDB	-26.5 (0.1)	-12.7 (0.2)	-11.9 (0.2)
^{15}N /atom%	0.37 (0.0)	7.4 (0.4)	7.9 (0.5)
Ergosterol /mg kg^{-1} DW	0.41 (0.0)	8.2 (1.7)	5.5 (1.9)

Table 8.2: Properties of the leaf and root material determined by the Weender analysis and the Van Soest method.

	Leaf material	Root material
	/% of dry matter	
Ash	11.4	18.4
Crude protein	15.5	5.6
Crude fibre	26.3	29.9
Crude fat	1.4	1.4
Cellulose	26.5	10.0
Hemicellulose	28.0	29.5
Lignin	6.2	32.0

Table 8.3: C content, C loss, C/N ratio, $\delta^{13}C$, proportion of maize-derived C, apparent C turnover time and mean ^{14}C age after chemical fractionation using H_2O_2 and $Na_2S_2O_8$ without preliminary density fractionation maize and rye soils of the Halle site ("H") (means and standard deviation, n = 4).

	H_2O_2		$Na_2S_2O_8$	
	H-Maize	H-Rye	H-Maize	H-Rye
C /g kg^{-1} soil	3.2 (1.5)	2.4 (0.8)	3.5 (2.2)	1.7 (0.2)
C loss /% of SOC	77.1 (10.1)	84.1 (3.1)	75.3 (14.0)	88.6 (2.8)
C/N	11.4 (4.7)	9.0 (2.9)	n.d.	n.d.
$\delta^{13}C$ /‰ V-PDB	-23.6 (0.7)	-25.0 (0.6)	-23.5 (0.2)	-23.7 (0.5)
Maize-derived C /%	6.1 (4.6)		1.0 (3.2)	
Apparent C turnover time	n.d.		n.d.	
Mean ^{14}C age /years BP	16647		16200	

n.d.: not determined

Table 8.4: Nitrogen contents of the A horizon of the Halle soil ("H") under maize cropping, the A horizon of the Rotthalmünster soil ("R") under continuous grass, forest and maize cropping as well as the E horizon of the Rotthalmünster soil under maize cropping after density fractionation (mineral-associated) and after subsequent thermal oxidation at 200, 225, 250, 275, 300 and 400°C (means and standard deviation, n = 4). Letters from a–g (read from left to right) indicate significant differences between temperature steps for a single land use.

Soil	Depth	Mineral-associated	200°C	225°C	250°C	275°C	300°C	400°C	500°C	600°C
	/cm	N content /g kg^{-1} soil								
R-Grass Ah	0-10	2.55^{e} (0.5)	1.55^{cd} (0.3)	1.55^{cd} (0.1)	1.54^{d} (0.2)	1.18^{bc} (0.2)	0.90^{b} (0.1)	0.44^{a} (0.1)	0.26^{a} (0.0)	0.18^{a} (0.0)
R-Forest Ah	0-7	1.06^{b} (0.2)	0.91^{b} (0.1)	0.83^{b} (0.1)	0.75^{b} (0.1)	0.82^{b} (0.1)	0.47^{a} (0.0)	0.34^{a} (0.0)	0.21^{a} (0.0)	0.19^{a} (0.0)
R-Maize Ap	0-30	1.34^{g} (0.1)	1.05^{f} (0.1)	0.77^{d} (0.1)	0.88^{e} (0.1)	0.74^{d} (0.0)	0.58^{c} (0.1)	0.34^{b} (0.0)	0.22^{a} (0.0)	0.19^{a} (0.0)
R-Maize E	30-45	0.88^{e} (0.1)	0.70^{d} (0.1)	0.63^{d} (0.2)	0.65^{d} (0.0)	0.60^{cd} (0.0)	0.48^{bc} (0.0)	0.37^{b} (0.0)	0.24^{a} (0.0)	0.19^{a} (0.0)
H-Maize Ap	0-20	0.60^{g} (0.1)	0.52^{f} (0.1)	0.39^{d} (0.2)	0.46^{e} (0.0)	0.39^{d} (0.0)	0.30^{c} (0.0)	0.20^{b} (0.0)	0.17^{b} (0.0)	0.10^{a} (0.0)

Table 8.5: Changes in C and N content, C/N ratio, proportion of maize-derived C and N and $\delta^{13}C$ and ^{15}N values of the bulk soil after 0, 14, 28, 56 and 84 days of incubation (means and standard deviation, n = 4). Letters from a–e indicate significant differences between treatments for a single day.

Day	Control	Control Captan	Leaf	Leaf Captan	Root	Root Captan
			C content /g kg^{-1}			
0	12.4 (0.1)^{a}	13.5 (0.2)^{b}	n.d.	n.d.	n.d.	n.d.
14	12.3 (0.1)^{ab}	13.3 (0.1)^{b}	13.6 (0.2)^{acd}	14.9 (0.4)^{d}	12.3 (0.1)^{ab}	13.3 (0.1)^{b}
28	12.5 (0.2)^{a}	13.8 (0.3)^{bc}	13.6 (0.2)^{b}	15.1 (0.2)^{d}	12.5 (0.2)^{a}	13.8 (0.3)^{bc}
56	12.1 (0.1)^{a}	14.0 (0.2)^{c}	13.4 (0.2)^{b}	14.7 (0.4)^{d}	12.1 (0.1)^{a}	14.0 (0.2)^{c}
84	11.8 (0.1)^{a}	13.1 (0.2)^{c}	12.7 (0.1)^{b}	14.1 (0.2)^{d}	11.8 (0.1)^{a}	13.1 (0.2)^{c}
			N content /g kg^{-1}			
0	1.5 (0.0)^{a}	1.6 (0.0)^{b}	n.d.	n.d.	n.d.	n.d.
14	1.5 (0.0)^{a}	1.5 (0.2)^{a}	1.5 (0.0)^{ab}	1.7 (0.0)^{b}	1.5 (0.0)^{a}	1.5 (0.2)^{a}
28	1.3 (0.0)^{a}	1.5 (0.0)^{b}	1.4 (0.1)^{b}	1.6 (0.0)^{d}	1.3 (0.0)^{a}	1.5 (0.0)^{b}
56	1.4 (0.0)^{a}	1.5 (0.0)^{c}	1.4 (0.0)^{ab}	1.5 (0.0)^{c}	1.4 (0.0)^{a}	1.5 (0.0)^{c}
84	1.4 (0.0)^{a}	1.6 (0.0)^{c}	1.5 (0.0)^{b}	1.6 (0.0)^{c}	1.4 (0.0)^{a}	1.6 (0.0)^{c}
			C/N ratio			
0	8.5 (0.1)^{a}	8.5 (0.1)^{a}	n.d.	n.d.	n.d.	n.d.
14	8.3 (0.1)^{a}	8.4 (0.1)^{a}	8.9 (0.1)^{b}	9.0 (0.0)^{b}	8.3 (0.1)^{a}	8.4 (0.1)^{a}
28	9.5 (0.1)^{a}	9.4 (0.1)^{a}	9.4 (0.6)^{a}	9.2 (0.1)^{a}	9.5 (0.1)^{a}	9.4 (0.1)^{a}
56	8.6 (0.3)^{a}	9.3 (0.1)^{b}	9.4 (0.0)^{b}	9.7 (0.1)^{c}	8.6 (0.3)^{a}	9.3 (0.1)^{b}
84	8.2 (0.1)^{a}	8.2 (0.1)^{a}	8.4 (0.1)^{b}	8.7 (0.1)^{c}	8.2 (0.1)^{a}	8.2 (0.1)^{a}
			Proportion of maize-derived C /% of SOC			
0			n.d.	n.d.	n.d.	n.d.
14			10.1 (1.2)^{a}	10.9 (1.4)^{a}	11.4 (3.0)^{a}	10.4 (1.1)^{a}
28			11.3 (1.3)^{a}	10.0 (1.3)^{a}	13.0 (1.4)^{a}	12.5 (2.8)^{a}
56			10.6 (1.3)^{a}	10.4 (2.3)^{a}	11.4 (3.0)^{a}	10.2 (2.2)^{a}
84			8.3 (0.6)^{a}	10.9 (1.8)^{b}	9.5 (0.9)^{a}	9.5 (0.9)^{a}
			Proportion of maize-derived N /% of N_t			
0			n.d.	n.d.	n.d.	n.d.
14			6.3 (0.2)^{c}	5.1 (0.2)^{b}	1.9 (0.2)^{a}	1.7 (0.2)^{a}
28			6.1 (0.2)^{c}	5.4 (0.3)^{b}	2.0 (0.2)^{a}	2.0 (0.4)^{a}
56			5.9 (0.2)^{c}	5.4 (0.2)^{b}	1.9 (0.2)^{a}	2.1 (0.8)^{a}
84			6.3 (0.2)^{c}	5.6 (0.4)^{b}	2.1 (0.1)^{a}	1.9 (0.1)^{a}
			$\delta^{13}C$ /‰ V-PDB			
0	-26.3 (0.1)^{a}	-26.1 (0.1)^{b}	n.d.	n.d.	n.d.	n.d.
14	-26.3 (0.0)^{a}	-26.1 (0.1)^{a}	-24.9 (0.2)^{b}	-24.6 (0.3)^{b}	-26.3 (0.0)^{a}	-26.1 (0.1)^{a}
28	-26.4 (0.1)^{a}	-26.1 (0.1)^{a}	-24.8 (0.2)^{b}	-24.7 (0.2)^{b}	-26.4 (0.1)^{a}	-26.1 (0.1)^{a}
56	-26.4 (0.0)^{a}	-26.2 (0.2)^{a}	-24.9 (0.2)^{b}	-24.7 (0.3)^{b}	-26.4 (0.0)^{a}	-26.2 (0.2)^{a}
84	-26.3 (0.1)^{a}	-26.3 (0.0)^{a}	-25.1 (0.1)^{b}	-24.8 (0.1)^{d}	-26.3 (0.1)^{a}	-26.3 (0.0)^{a}
			^{15}N /atom%			
0	0.37 (0.0)^{a}	0.37 (0.0)^{a}	n.d.	n.d.	n.d.	n.d.
14	0.37 (0.0)^{a}	0.37 (0.0)^{a}	0.81 (0.0)^{d}	0.73 (0.0)^{c}	0.37 (0.0)^{a}	0.37 (0.0)^{a}
28	0.37 (0.0)^{a}	0.37 (0.0)^{a}	0.79 (0.0)^{d}	0.75 (0.0)^{c}	0.37 (0.0)^{a}	0.37 (0.0)^{a}
56	0.37 (0.0)^{a}	0.37 (0.0)^{a}	0.78 (0.0)^{c}	0.74 (0.0)^{c}	0.37 (0.0)^{a}	0.37 (0.0)^{a}
84	0.37 (0.0)^{a}	0.37 (0.0)^{a}	0.81 (0.0)^{d}	0.76 (0.0)^{c}	0.37 (0.0)^{a}	0.37 (0.0)^{a}

Table 8.6: Percentages of maize-derived C in the evolved CO_2-C at different days of soil incubation (means and standard deviation, n = 4).

Day	Leaf	Leaf Captan	Root	Root Captan
	Proportion of maize-derived C in the evolved CO_2 /%			
0	62 (3)	75 (1)	46 (2)	63 (1)
1	57 (1)	58 (2)	40 (2)	52 (3)
2	79 (2)	69 (1)	69 (0)	56 (1)
4	87 (0)	77 (0)	73 (1)	60 (5)
7	85 (1)	78 (1)	73 (1)	51 (4)
9	79 (1)	74 (1)	76 (2)	59 (5)
13	74 (4)	84 (2)	85 (2)	83 (3)
16	83 (2)	75 (3)	87 (2)	71 (3)
20	72 (1)	59 (1)	74 (3)	62 (7)
23	63 (3)	55 (1)	70 (1)	56 (5)
27	67 (4)	53 (2)	66 (6)	56 (8)
30	74 (5)	55 (3)	66 (2)	49 (5)
34	52 (7)	45 (3)	57 (1)	38 (7)
42	74 (5)	55 (3)	66 (2)	49 (5)
48	52 (7)	45 (3)	57 (1)	38 (7)
55	58 (1)	52 (5)	64 (6)	47 (7)
62	45 (2)	33 (1)	46 (1)	36 (3)
69	44 (1)	30 (1)	45 (1)	32 (2)
76	43 (1)	28 (1)	44 (2)	29 (2)
84	41 (1)	27 (1)	42 (2)	27 (2)

Table 8.7: $\delta^{13}C$ and ^{15}N of the chloroform-labile C pool extracted with 0.05 M K_2SO_4 after 0, 14, 28, 56 and 84 days of incubation (means and standard deviation, n = 4). Letters from a–d indicate significant differences between treatments for a single day.

Day	Control	Control Captan	Leaf	Leaf Captan	Root	Root Captan
			$\delta^{13}C$ of extracted C /‰ V-PDB			
0	-24.2 (1.2)^{a}	-23.6 (0.5)^{a}	n.d.	n.d.	n.d.	n.d.
14	-24.3 (0.2)^{a}	-24.7 (1.6)^{a}	-18.5 (0.1)^{c}	-19.3 (0.5)^{c}	-18.8 (0.1)^{c}	-20.5 (0.7)^{b}
28	-24.7 (0.3)^{a}	-23.4 (1.3)^{b}	-19.1 (0.3)^{cd}	-19.3 (0.7)^{cd}	-18.6 (0.4)^{d}	-20.1 (0.5)^{c}
56	-24.6 (0.1)^{a}	-24.3 (0.1)^{a}	-19.7 (0.2)^{c}	-20.4 (0.2)^{b}	-19.7 (0.0)^{c}	-19.6 (0.5)^{c}
84	-25.2 (0.1)^{a}	-25.2 (0.1)^{a}	-20.2 (0.2)^{c}	-20.1 (0.8)^{c}	-20.0 (0.5)^{c}	-21.2 (0.6)^{b}
			^{15}N of extracted N /atom%			
0	0.38 (0.02)^{a}	0.51 (0.17)^{a}	n.d.	n.d.	n.d.	n.d.
14	0.45 (0.19)^{a}	0.32 (0.16)^{a}	2.39 (0.02)^{c}	2.39 (0.09)^{c}	1.07 (0.01)^{b}	1.08 (0.03)^{b}
28	0.42 (0.03)^{a}	0.43 (0.05)^{a}	2.51 (0.09)^{d}	2.27 (0.29)^{c}	1.01 (0.04)^{b}	1.09 (0.06)^{b}
56	0.39 (0.04)^{a}	0.40 (0.02)^{a}	2.10 (0.12)^{c}	2.17 (0.23)^{c}	0.96 (0.02)^{b}	1.00 (0.10)^{b}
84	0.41 (0.05)^{a}	0.88 (0.83)^{a}	2.13 (0.06)^{b}	1.67 (0.59)^{b}	0.94 (0.03)^{a}	0.90 (0.04)^{a}

Table 8.8: Changes in yield of water-stable aggregates, soil organic carbon (SOC) of water-stable aggregates based on one kg of soil, SOC in sand-free aggregates, C/N ratio and isotopic label of aggregates (^{13}C, ^{15}N) during the incubation procedure. Letters from a–f indicate significant differences between treatments for a single day (means and standard deviation, n = 4).

Water-stable macroaggregates (250–2000 µm)						
Day	**Control**	**Control Captan**	**Leaf**	**Leaf Captan**	**Root**	**Root Captan**
			Yield /%			
14	0.25 (0.0)^{a}	0.48 (0.1)^{a}	7.11 (1.6)^{d}	3.63 (0.4)^{c}	2.26 (0.4)^{b}	1.21 (0.5)^{ab}
28	0.40 (0.1)^{a}	0.69 (0.1)^{a}	4.00 (0.3)^{d}	4.83 (0.4)^{e}	2.99 (0.7)^{c}	1.98 (0.2)^{b}
56	0.46 (0.1)^{a}	1.81 (0.4)^{b}	2.44 (0.4)^{b}	7.96 (1.3)^{d}	1.89 (0.1)^{b}	4.10 (1.0)^{c}
84	0.08 (0.1)^{a}	0.55 (0.1)^{b}	1.86 (0.2)^{c}	2.49 (0.4)^{d}	0.74 (0.2)^{b}	2.01 (0.4)^{c}
			SOC /g kg^{-1} soil			
14	0.14 (0.0)^{a}	0.20 (0.0)^{a}	1.91 (0.4)^{c}	1.02 (0.1)^{b}	0.91 (0.1)^{b}	0.41 (0.1)^{a}
28	0.22 (0.1)^{a}	0.30 (0.1)^{a}	1.19 (0.1)^{cd}	1.33 (0.1)^{d}	1.07 (0.2)^{c}	0.68 (0.1)^{b}
56	0.22 (0.0)^{a}	0.44 (0.1)^{b}	0.69 (0.1)^{c}	1.69 (0.2)^{e}	0.77 (0.1)^{c}	1.07 (0.2)^{d}
84	0.05 (0.0)^{a}	0.12 (0.0)^{a}	0.47 (0.0)^{c}	0.68 (0.1)^{d}	0.29 (0.0)^{b}	0.71 (0.1)^{d}
			SOC /g kg^{-1} fraction*			
14	70.1 (6.2)^{d}	53.1 (5.1)^{c}	34.4 (3.0)^{a}	35.9 (1.6)^{a}	51.4 (3.0)^{c}	43.9 (5.1)^{b}
28	71.1 (4.8)^{d}	54.1 (3.5)^{c}	38.0 (2.2)^{a}	35.2 (2.1)^{a}	45.0 (2.7)^{b}	45.0 (4.1)^{b}
56	59.3 (4.5)^{c}	31.7 (4.2)^{ab}	35.9 (2.9)^{b}	28.8 (2.1)^{a}	55.5 (2.0)^{c}	35.8 (1.6)^{b}
84	72.8 (7.9)^{c}	26.9 (2.2)^{a}	28.1 (2.0)^{a}	30.6 (2.7)^{a}	47.2 (7.6)^{b}	40.6 (6.6)^{b}
			C/N			
14	14.7 (0.2)^{b}	13.0 (0.4)^{a}	12.3 (0.3)^{a}	12.9 (0.4)^{a}	16.8 (0.5)^{c}	15.5 (1.5)^{b}
28	14.5 (0.4)^{b}	13.7 (0.2)^{b}	12.1 (0.2)^{a}	12.3 (0.4)^{a}	14.3 (0.6)^{b}	14.2 (0.9)^{b}
56	13.3 (0.2)^{d}	10.8 (0.4)^{a}	11.7 (0.4)^{b}	12.2 (0.4)^{c}	16.4 (0.2)^{f}	14.7 (0.4)^{e}
84	12.2 (1.1)^{bc}	9.3 (0.2)^{a}	10.7 (0.3)^{b}	11.8 (0.8)^{bc}	13.3 (1.0)^{d}	14.8 (1.2)^{e}
			$\delta^{13}C$ /‰ V-PDB			
14	-27.4 (0.2)^{a}	-27.1 (0.2)^{a}	-21.9 (0.4)^{a}	-22.2 (0.1)^{a}	-19.6 (0.2)^{a}	-21.9 (0.7)^{a}
28	-27.7 (0.1)^{a}	-27.6 (0.1)^{a}	-21.8 (0.5)^{a}	-22.1 (0.1)^{a}	-20.6 (0.6)^{a}	-21.9 (0.9)^{a}
56	-27.5 (0.1)^{a}	-26.9 (0.2)^{b}	-22.6 (0.2)^{d}	-23.2 (0.7)^{c}	-20.6 (0.2)^{e}	-20.9 (0.5)^{e}
84	-27.5 (0.1)^{a}	-27.0 (0.1)^{a}	-21.0 (0.5)^{a}	-20.8 (0.7)^{a}	-18.6 (0.3)^{a}	-21.6 (0.6)^{a}
			^{15}N /atom%			
14	0.37 (0.0)^{a}	0.37 (0.0)^{a}	1.47 (0.1)^{b}	1.43 (0.1)^{b}	1.00 (0.0)^{ab}	0.82 (0.1)^{a}
28	0.37 (0.0)^{a}	0.37 (0.0)^{a}	1.60 (0.1)^{e}	1.37 (0.0)^{d}	0.95 (0.1)^{c}	0.72 (0.0)^{b}
56	0.37 (0.0)^{a}	0.37 (0.0)^{a}	1.45 (0.1)^{d}	0.99 (0.0)^{c}	1.05 (0.0)^{c}	0.75 (0.1)^{b}
84	0.37 (0.0)^{a}	0.37 (0.0)^{a}	1.74 (0.1)^{c}	1.51 (0.1)^{c}	1.29 (0.1)^{b}	0.76 (0.1)^{ab}

* sandfree aggregates

Table 8.8 (continued)

Water-stable microaggregates (53–250 µm)						
Day	**Control**	**Control Captan**	**Leaf**	**Leaf Captan**	**Root**	**Root Captan**
			Yield /%			
0	69.1 (2.3)^{a}	67.7 (1.2)^{a}	n.d.	n.d.	n.d.	n.d.
14	58.9 (4.0)^{ab}	57.0 (2.5)^{a}	59.2 (3.1)^{ab}	63.0 (1.6)^{b}	60.5 (1.4)^{ab}	58.0 (2.1)^{ab}
28	54.1 (0.9)^{a}	55.1 (1.0)^{ab}	60.1 (1.7)^{c}	57.0 (1.7)^{bc}	57.2 (0.8)^{abc}	57.7 (2.8)^{bc}
56	58.1 (1.4)^{a}	60.9 (2.3)^{a}	66.7 (1.9)^{b}	60.3 (1.4)^{a}	64.6 (1.5)^{b}	59.8 (1.5)^{a}
84	50.3 (2.7)^{a}	55.4 (2.7)^{b}	62.5 (2.2)^{c}	55.6 (2.9)^{b}	57.0 (1.7)^{b}	61.2 (2.1)^{c}
			SOC g kg^{-1} soil			
0	9.4 (0.2)^{a}	9.4 (0.2)^{a}	n.d.	n.d.	n.d.	n.d.
14	8.0 (0.5)^{a}	8.2 (0.5)^{a}	8.2 (0.5)^{a}	9.3 (0.5)^{b}	8.3 (0.2)^{a}	8.4 (0.2)^{a}
28	7.1 (0.2)^{a}	7.9 (0.4)^{b}	9.0 (1.4)^{b}	7.9 (0.3)^{b}	8.0 (0.1)^{b}	8.4 (0.2)^{b}
56	7.7 (0.2)^{a}	8.2 (0.3)^{a}	9.2 (0.3)^{b}	9.0 (0.7)^{b}	9.0 (0.3)^{b}	8.9 (0.2)^{b}
84	6.2 (0.4)^{a}	6.6 (0.4)^{a}	7.8 (0.2)^{b}	7.6 (0.2)^{b}	8.0 (0.3)^{b}	9.1 (0.3)^{c}
			SOC /g kg^{-1} fraction*			
0	15.3 (0.7)^{a}	15.8 (0.2)^{a}	n.d.	n.d.	n.d.	n.d.
14	15.8 (0.3)^{a}	17.3 (0.7)^{abc}	17.9 (0.3)^{c}	17.6 (1.6)^{abc}	15.7 (0.7)^{ab}	17.2 (1.3)^{bc}
28	16.1 (0.9)^{a}	17.3 (0.6)^{ab}	18.0 (2.1)^{ab}	16.7 (0.3)^{ab}	17.2 (0.4)^{ab}	17.9 (1.0)^{b}
56	16.1 (0.8)	16.4 (1.2)	16.6 (0.3)	17.7 (1.0)	16.3 (0.4)	18.2 (1.0)
84	14.8 (0.3)^{a}	14.6 (0.6)^{a}	15.8 (0.9)^{a}	17.2 (1.0)^{b}	17.5 (0.9)^{b}	18.3 (0.5)^{b}
			C/N			
0	9.4 (0.1)^{a}	9.4 (0.1)^{a}	n.d.	n.d.	n.d.	n.d.
14	9.8 (0.1)^{a}	9.8 (0.1)^{a}	9.8 (0.2)^{a}	9.9 (0.4)^{a}	9.9 (0.1)^{a}	9.9 (0.3)^{a}
28	9.4 (0.6)^{a}	9.9 (0.2)^{a}	9.3 (0.0)^{a}	9.4 (0.1)^{a}	9.3 (0.2)^{a}	9.6 (0.2)^{a}
56	9.3 (0.1)^{a}	9.0 (0.1)^{a}	9.2 (0.1)^{a}	10.0 (1.0)^{ab}	9.3 (0.1)^{a}	10.4 (0.8)^{b}
84	8.2 (0.1)^{a}	8.0 (0.0)^{a}	8.2 (0.0)^{ab}	8.6 (0.2)^{bc}	8.8 (0.5)^{c}	9.8 (0.2)^{d}
			δ^{13}C /‰ V-PDB			
0	-26.5 (0.1)^{a}	-26.4 (0.0)^{a}	n.d.	n.d.	n.d.	n.d.
14	-26.5 (0.1)^{a}	-26.5 (0.0)^{a}	-25.4 (0.1)^{b}	-25.0 (0.1)^{c}	-25.3 (0.1)^{b}	-24.9 (0.2)^{c}
28	-26.8 (0.1)^{a}	-26.9 (0.0)^{a}	-25.1 (0.3)^{b}	-25.3 (0.2)^{b}	-25.2 (0.3)^{b}	-25.1 (0.2)^{b}
56	-26.7 (0.1)^{a}	-26.5 (0.1)^{a}	-25.2 (0.1)^{b}	-24.8 (0.0)^{c}	-25.1 (0.1)^{b}	-24.8 (0.1)^{c}
84	-26.7 (0.1)^{a}	-26.6 (0.1)^{a}	-25.2 (0.1)^{b}	-24.8 (0.3)^{c}	-25.0 (0.1)^{bc}	-24.8 (0.3)^{c}
			^{15}N /atom%			
0	0.37 (0.0)^{a}	0.37 (0.0)^{a}	n.d.	n.d.	n.d.	n.d.
14	0.37 (0.0)^{a}	0.37 (0.0)^{a}	0.67 (0.0)^{b}	0.72 (0.0)^{c}	0.47 (0.0)^{b}	0.47 (0.0)^{b}
28	0.37 (0.0)^{a}	0.37 (0.0)^{a}	0.71 (0.0)^{d}	0.67 (0.0)^{c}	0.47 (0.0)^{b}	0.46 (0.0)^{b}
56	0.37 (0.0)^{a}	0.37 (0.0)^{a}	0.72 (0.0)^{c}	0.74 (0.0)^{c}	0.50 (0.0)^{b}	0.49 (0.0)^{b}
84	0.37 (0.0)^{a}	0.37 (0.0)^{a}	0.71 (0.0)^{d}	0.73 (0.0)^{d}	0.50 (0.0)^{c}	0.47 (0.0)^{b}

* sandfree aggregates

Table 8.8 (continued)

Non-aggregated fraction (0–53 µm)						
Day	**Control**	**Control Captan**	**Leaf**	**Leaf Captan**	**Root**	**Root Captan**
			Yield /%			
0	30.9 (2.3)^{a}	32.3 (1.2)^{a}	n.d.	n.d.	n.d.	n.d.
14	40.8 (4.0)^{bc}	42.6 (2.4)^{c}	33.7 (3.1)^{a}	33.3 (1.1)^{a}	37.3 (1.8)^{ab}	40.8 (2.1)^{bc}
28	45.5 (0.8)^{c}	44.2 (1.1)^{c}	35.9 (1.4)^{a}	38.1 (1.6)^{ab}	39.8 (1.0)^{b}	40.3 (2.7)^{b}
56	41.5 (1.5)^{c}	37.3 (2.3)^{b}	30.9 (1.8)^{a}	31.8 (1.1)^{a}	33.5 (1.5)^{a}	36.1 (1.7)^{b}
84	49.6 (2.7)^{c}	44.1 (2.6)^{b}	35.6 (2.3)^{a}	41.9 (2.5)^{b}	42.2 (1.6)^{b}	36.8 (2.4)^{a}
			SOC /g kg^{-1} soil			
0	3.2 (0.3)^{a}	3.7 (0.2)^{b}	n.d.	n.d.	n.d.	n.d.
14	4.3 (0.3)^{b}	4.7 (0.2)^{b}	3.4 (0.4)^{a}	3.6 (0.1)^{a}	3.8 (0.2)^{a}	4.5 (0.2)^{b}
28	4.6 (0.1)^{c}	5.1 (0.1)^{d}	3.6 (0.1)^{a}	4.4 (0.2)^{c}	4.0 (0.1)^{b}	4.4 (0.3)^{c}
56	4.1 (0.1)^{c}	4.0 (0.3)^{c}	3.1 (0.2)^{a}	3.4 (0.1)^{b}	3.5 (0.2)^{b}	3.9 (0.2)^{c}
84	4.7 (0.3)^{c}	4.3 (0.2)^{b}	3.3 (0.3)^{a}	4.2 (0.3)^{bc}	3.8 (0.1)^{b}	4.0 (0.3)^{b}
			SOC /g kg^{-1} fraction			
0	10.5 (0.2)^{a}	11.6 (0.5)^{b}	n.d.	n.d.	n.d.	n.d.
14	10.4 (0.4)^{a}	11.0 (0.3)^{a}	10.1 (0.7)^{a}	10.9 (0.5)^{a}	10.2 (0.1)^{a}	11.1 (0.4)^{a}
28	10.1 (0.2)^{a}	11.5 (0.4)^{bc}	10.0 (0.1)^{a}	11.5 (0.2)^{c}	10.0 (0.2)^{a}	11.0 (0.5)^{b}
56	10.0 (0.2)^{a}	10.8 (0.2)^{b}	9.9 (0.1)^{a}	10.8 (0.3)^{b}	10.5 (0.2)^{b}	10.8 (0.3)^{b}
84	9.4 (0.2)^{ab}	9.7 (0.4)^{c}	9.3 (0.1)^{ab}	10.1 (0.2)^{d}	9.1 (0.4)^{a}	10.9 (0.3)^{e}
			C/N			
0	8.3 (0.0)^{a}	8.3 (0.1)^{a}	n.d.	n.d.	n.d.	n.d.
14	8.5 (0.1)^{b}	8.4 (0.1)^{b}	8.4 (0.2)^{b}	8.4 (0.2)^{a}	8.4 (0.1)^{b}	8.1 (0.2)^{a}
28	8.2 (0.4)^{a}	8.4 (0.1)^{a}	8.1 (0.1)^{a}	8.1 (0.1)^{a}	8.0 (0.1)^{a}	8.3 (0.0)^{a}
56	8.4 (0.7)^{a}	8.0 (0.2)^{a}	7.9 (0.0)^{a}	9.0 (0.1)^{b}	9.2 (0.1)^{b}	9.3 (0.1)^{b}
84	7.2 (0.0)^{a}	7.1 (0.0)^{a}	7.2 (0.0)^{a}	7.4 (0.1)^{a}	7.3 (0.9)^{a}	8.2 (0.1)^{b}
			δ^{13}C /‰ V-PDB			
0	-26.0 (0.1)^{a}	-25.8 (0.1)^{a}	n.d.	n.d.	n.d.	n.d.
14	-26.0 (0.0)^{a}	-25.9 (0.0)^{b}	-25.3 (0.1)^{c}	-25.3 (0.1)^{c}	-25.3 (0.1)^{c}	-25.3 (0.1)^{c}
28	-26.4 (0.0)^{a}	-26.3 (0.1)^{a}	-25.3 (0.1)^{b}	-25.2 (0.1)^{b}	-25.2 (0.2)^{b}	-25.0 (0.1)^{c}
56	-26.1 (0.0)^{a}	-26.1 (0.0)^{a}	-25.3 (0.1)^{bc}	-25.1 (0.1)^{c}	-25.3 (0.1)^{b}	-25.3 (0.1)^{b}
84	-26.2 (0.2)^{a}	-26.2 (0.0)^{a}	-25.3 (0.2)^{b}	-25.2 (0.2)^{b}	-25.3 (0.2)^{b}	-25.1 (0.2)^{b}
			^{15}N /atom%			
0	0.37 (0.0)^{a}	0.37 (0.0)^{a}	n.d.	n.d.	n.d.	n.d.
14	0.37 (0.0)^{a}	0.37 (0.0)^{a}	0.61 (0.0)^{c}	0.60 (0.0)^{c}	0.44 (0.0)^{b}	0.44 (0.0)^{b}
28	0.37 (0.0)^{a}	0.37 (0.0)^{a}	0.61 (0.0)^{c}	0.60 (0.0)^{c}	0.44 (0.0)^{b}	0.44 (0.0)^{b}
56	0.37 (0.0)^{a}	0.37 (0.0)^{a}	0.60 (0.0)^{c}	0.61 (0.0)^{c}	0.45 (0.0)^{b}	0.44 (0.0)^{b}
84	0.37 (0.0)^{a}	0.37 (0.0)^{a}	0.60 (0.0)^{c}	0.60 (0.0)^{c}	0.45 (0.0)^{b}	0.45 (0.0)^{b}