

Review Article

How relevant is recalcitrance for the stabilization of organic matter in soils?[§]

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Abstract

Traditionally, the selective preservation of certain recalcitrant organic compounds and the formation of recalcitrant humic substances have been regarded as an important mechanism for soil organic matter (SOM) stabilization. Based on a critical overview of available methods and on results from a cooperative research program, this paper evaluates how relevant recalcitrance is for the long-term stabilization of SOM or its fractions. Methodologically, recalcitrance is difficult to assess, since the persistence of certain SOM fractions or specific compounds may also be caused by other stabilization mechanisms, such as physical protection or chemical interactions with mineral surfaces. If only free particulate SOM obtained from density fractionation is considered, it rarely reaches ages exceeding 50 y. Older light particles have often been identified as charred plant residues or as fossil C. The degradability of the readily bioavailable dissolved or water-extractable OM fraction is often negatively correlated with its content in aromatic compounds, which therefore has been associated with recalcitrance. But in subsoils, dissolved organic matter aromaticity and biodegradability both are very low, indicating that other factors or compounds limit its degradation. Among the investigated specific compounds, lignin, lipids, and their derivatives have mean turnover times faster or similar as that of bulk SOM. Only a small fraction of the lignin inputs seems to persist in soils and is mainly found in the fine textural size fraction (<20 µm), indicating physico-chemical stabilization. Compound-specific analysis of ¹³C : ¹²C ratios of SOM pyrolysis products in soils with C3-C4 crop changes revealed no compounds with mean residence times of > 40–50 y, unless fossil C was present in substantial amounts, as at a site exposed to lignite inputs in the past. Here, turnover of pyrolysis products seemed to be much longer, even for those attributed to carbohydrates or proteins. Apparently, fossil C from lignite coal is also utilized by soil organisms, which is further evidenced by low ¹⁴C concentrations in microbial phospholipid fatty acids from this site. Also, black C from charred plant materials was susceptible to microbial degradation in a short-term (60 d) and a long-term (2 y) incubation



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experiment. This degradation was enhanced, when glucose was supplied as an easily available microbial substrate. Similarly, SOM mineralization in many soils generally increased after addition of carbohydrates, amino acids, or simple organic acids, thus indicating that stability may also be caused by substrate limitations. It is concluded that the presented results do not provide much evidence that the selective preservation of recalcitrant primary biogenic compounds is a major SOM-stabilization mechanism. Old SOM fractions with slow turnover rates were generally only found in association with soil minerals. The only not mineral-associated SOM components that may be persistent in soils appear to be black and fossil C.

Key words: review / stabilization mechanism / ^{14}C age / $^{13}\text{C} : ^{12}\text{C}$ ratio / black carbon / DOM / lignin / lipids / SOM fractions / molecular turnover

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1 Introduction

Soil organic matter (SOM) constitutes approx. 2/3 of the global terrestrial C pool and is estimated to be in the order of approx. 1,500 Gt C (Batjes, 1996). Annually, about 75 Gt C are added to this pool through inputs of dead biomass and root deposits, but a similar amount is released as CO_2 so that the overall balance is close to equilibrium (Schlesinger and Andrews, 2000). However, depending on environmental conditions and land use, soils may act as sources of or sinks for C. Therefore, understanding of the mechanisms that control stabilization and release of C is important for the prediction of the effects of global climate change and for the development of management strategies to increase C sequestration of soils.

Various mechanisms of SOM stabilization are being discussed (Sollins et al., 1996; Gleixner et al., 2001; Krull et al., 2003; von Lützow et al., 2006). Physical protection of SOM through occlusion within aggregates or small pores and chemical protection through interaction with mineral surfaces or with other organic molecules are considered as important mechanisms to reduce the bioavailability and accessibility of organic matter (OM) for soil microorganisms and soil enzymes (Sollins et al., 1996; Kelleher and Simpson, 2006; von Lützow et al., 2006; Bachmann et al., 2008, this issue, pp. 14–26). The other main mechanism of SOM stabilization is assumed to be the selective preservation of certain recalcitrant organic compounds, due to their molecular-level characteristics such as elemental composition, presence of functional groups, and molecular conformation that restrict their decomposition. Sollins et al. (1996) and von Lützow et al. (2006) differentiate between primary recalcitrance of plant litter and rhizodeposits as a function of their indigenous molecular characteristics and secondary recalcitrance of microbial products, humic polymers, and charred materials (*i.e.*, black C). Krull et al. (2003) assume that physical-protection mechanisms can only retard the decomposition processes of biochemically labile materials while more recalcitrant materials are hardly affected by physical protection because they decompose slowly anyway. Finally, Gleixner et al. (2001) indicated that the presence of individual molecules in SOM is not necessarily due to stabilization but may also be caused by recycling of C. This mechanism suggests that soil microorganisms build their carbohydrates, proteins, and lipids preferentially from plant biomass consisting of the same compounds (Flessa et al., 2008, this issue, pp. 36–51). The chemical structure of these

newly synthesized compounds is identical to their precursors leading to an apparent biological stabilization. Only isotopic tracers can distinguish between recalcitrance and recycling.

Based on a short literature overview and recent results from a cooperative long-term research program (Kögel-Knabner et al., 2008a, this issue, pp. 5–13), this paper evaluates whether recalcitrance of biogenic compounds is indeed contributing to long-term SOM stabilization. Since SOM consists of a mixture of various materials and compounds representing a broad range of turnover rates, stability is a relative term within this continuum. In this paper, specific compounds or SOM fractions are considered to be stable, if their turnover or mean residence times are well above that of bulk SOM. Generally, this will then be in the range of centuries.

2 Background

Among the plant compounds considered to be resistant to microbial and enzymatic breakdown, lignin has been regarded as an important compound, because of its polymeric and disordered structure, which can only be degraded co-metabolically (Haider and Martin, 1975; Hedges et al., 1985). Early studies of litter decomposition showed that lignin content was inversely related to mass loss (Williams and Gray, 1974). However, several recent studies have shown, that a selective preservation of lignin appears to be only relevant during the early stages of litter decomposition and that later-on lignin degradation occurs at the same or even higher rate as the overall litter decomposition (Gleixner et al., 1999; Kerem et al., 1999; Jensen et al., 2005; Prescott, 2005; Kalbitz et al., 2006; Sollins et al., 2006). Recent studies using ^{13}C -CPMAS-NMR and pyrolysis techniques have confirmed that lignin is altered relatively quickly and does not appear to be stabilized in the long-term in any soil fraction (Baldock and Nelson, 2000; Gleixner et al., 2002; Kiem and Kögel-Knabner, 2003).

Aliphatic plant components like lipids derived from cutans or suberans in plants have also been considered to be recalcitrant in soils (Baldock et al., 1997; Stimler et al., 2006). But selective preservation of aliphatic compounds during SOM decomposition and their accumulation in old SOM fractions may also be due to other stabilization mechanisms, such as

surface interactions with minerals (Kögel-Knabner et al., 2008b, this issue, pp. 61–82) or hydrophobicity (Bachmann et al., 2008, this issue, pp. 14–26).

There is some evidence that microbially and faunally derived compounds such as murein, chitin, certain lipids, and so-called melanins accumulate in soils (Guggenberger et al., 1994; Marseille et al., 1999; Kiem and Kögel-Knabner, 2003; Knicker, 2004). Even carbohydrates and certain peptides produced by soil microorganisms seem to be more resistant to microbial degradation, since they make up a substantial part of the stable subsoil DOC (Guggenberger et al., 1994) and can persist in soils for several decades (Gleixner et al., 1999). Rillig et al. (2007) point out that protein misfolding resulting in amyloid aggregates and so-called fibrils can greatly reduce their biochemical degradation. They also identified a number of microbially produced proteins such as hydrophobins and glomalin that appear to be stable in soils, although data on degradation or turnover rates of these compounds are sparse.

Ekschmitt et al. (2005) question the concept of recalcitrance of biologically produced compounds on the basis of theoretical considerations. They argue that soil organisms have evolved techniques to overcome biochemical resistance of their food sources, so that for all natural substances corresponding decomposing enzymes or enzyme complexes exist that are produced by organisms as well. On the other hand, Fox et al. (2006) have shown that soil fauna can modify SOM compounds to become more recalcitrant. Constraints on decomposition rates are therefore largely attributed to the biology of the decomposing soil organisms (Ekschmitt et al., 2008, this issue, pp. 27–35).

For a long time, the formation of recalcitrant humic substances has been considered as the major pathway for SOM stabilization (Stevenson, 1994). The proposed formation pathways for such compounds generally include spontaneous reactions between small reactive metabolites or oxidative cross-linking within biomacromolecules, resulting in new condensation products or restructured compounds (Hedges, 1988; Guggenberger, 2005). Since these neoformations should be highly variable in size and structure, it is unlikely that specific enzymatic tools for their degradation could have evolved, thus making them recalcitrant to microbial breakdown.

Piccolo et al. (1996) as well as Sutton and Sposito (2005) even propose that covalent bonding or cross-linking are not prerequisites for the protection of biomolecules against enzymatic attacks, since already subtle chemical or conformational changes or the inclusion of smaller molecules within larger organic structures can cause profound changes in enzyme accessibility or function. Such supramolecular associations may form through relatively weak intermolecular interactions, such as hydrophobic interactions or H bonds (Sutton and Sposito, 2005). This concept is supported by recent investigations of Kelleher and Simpson (2006) who were able to assign nearly all of the NMR signals in traditional fractions of humic substances to intact or degrading biopolymers and therefore conclude that humic substances are not

chemically distinct, but a complex mixture of microbial and plant biopolymers.

In recent years, black C derived from the incomplete combustion of fossil fuels, and biomass has received much attention for its potential role in the stable C pool of soils (Gleixner et al., 2001; Swift, 2001; González-Pérez et al., 2004; Skjemstad et al., 2004). While individual charcoal particles found in soils have ^{14}C ages of up to several thousand years (Gavin, 2003; Sanborn et al., 2006), other studies have determined mean residence times of black C of only a few decades (Bird et al., 1999). These differences may largely be caused by different charring temperatures since Baldock and Smernick (2002) showed that the degradability of charred wood decreased with increasing heating temperature. Black C produced from vegetation fires will therefore consist of a continuum from highly to poorly thermally altered materials with a large range of recalcitrance. However, until now the factors controlling black-C degradation are largely unknown but knowledge of them is required for fate evaluation.

3 Methods to determine recalcitrance

There are two basic methodological approaches for the determination of recalcitrance of SOM, its fractions, or specific compounds. The most straightforward approach is to determine degradability with decomposition or incubation studies in the field or in the laboratory. High degradability then indicates low recalcitrance, but low degradability can be due to either high recalcitrance or other stabilization mechanism like physical protection or due to other limiting factors, such as nutrient availability, aeration, or toxicity from contaminants. Another approach is to determine the mean residence time of SOM, its fractions, or specific compounds in soils, either by direct analysis of ^{14}C or indirectly by the shift of ^{13}C natural abundance after a change of vegetation from C3 to C4 plants or *vice versa* (Balesdent and Mariotti, 1996). Short mean residence times of SOM fractions indicate low persistence which translates into high turnover rates if the system is at steady state (Trumbore, 2000). But as in the other approach, long mean residence times are not necessarily due to recalcitrance but may also be caused by other stabilization mechanisms. Within the Priority Program 1090 (SPP1090), soil samples were commonly collected at several sites from long-term field experiments (Kögel-Knabner et al., 2008a, this issue, pp. 5–13). All data reported here were obtained from analyses of these samples, unless stated otherwise. In the studies that are reported here, the following methodologies were used.

3.1 Decomposition and incubation experiments

For a long time, the dynamics of litter decomposition have been studied in the field or the laboratory, by exposing litter material enclosed in mesh containers on the soil surface or in the soil (Jenny et al., 1949; Williams and Gray, 1974; Agren and Bosatta, 1996; Kalbitz et al., 2006). Using this approach, mass loss, elemental composition, specific compounds, or isotopic signature have been monitored at specific sampling dates over periods of up to 3 years. Most of these studies

show that N-rich and water-soluble compounds as well as celluloses are rapidly degraded (Williams and Gray, 1974; Nelson et al., 1994; Kerem et al., 1999; Marschner and Noble, 2000; Webster et al., 2000; Jensen et al., 2005), while lignin and other polyphenols tend to accumulate during the initial stages of litter decomposition (Williams and Gray, 1974; Azam et al., 1985; Kalbitz et al., 2006). Since plant compounds are not uniformly labeled with ^{13}C , this parameter has also been used as a tool to identify compositional changes during litter decomposition (Schweizer et al., 1999). While the accumulation of the ^{13}C -depleted lignin should decrease the $\delta^{13}\text{C}$ value of the residual litter, this trend is counteracted by a preferential release of $^{12}\text{CO}_2$ from microbial-fractionation processes (Conteh et al., 1997). If the litter material or specific compounds are labeled with ^{14}C or have a different natural ^{13}C abundance than the soil, their decomposition in soils can also be monitored by determining the isotopic composition of the evolved CO_2 (Mary et al., 1992; Schweizer et al., 1999; Flieβbach et al., 2000; Hamer and Marschner, 2002, 2005a). However, considerable amounts of breakdown products may be immobilized in the microbial biomass (Flieβbach et al., 2000; Hamer and Marschner, 2005b) so that decomposition rates will be underestimated if based only on CO_2 release (Gaudinski et al., 2000).

The biodegradability of water-soluble or dissolved organic matter (DOM) is considered a crucial factor in SOM dynamics because most microbial transformation processes involve the soluble phase (Metting, 1993; Marschner and Kalbitz, 2003). Various incubation methods to determine the biodegradable DOM fraction are used, ranging from simple batch-solution assays, where the release of CO_2 or the loss of DOC are monitored (Qualls and Haines, 1992; Marschner and Bredow, 2002; Kalbitz et al., 2003b) to flow-through bed reactors with aged microbial biofilms (Yano et al., 1998). In a comparative study, McDowell et al. (2006) have shown that current short-term incubation methods produce largely comparable results for the most labile DOM fraction, while the determination of decomposition rate constants for the more refractory components requires incubation periods of at least 6 weeks.

Among the data presented in this paper, the decomposition of charred plant materials (black C) was determined in short- and long-term incubation experiments in quartz sand or soil, monitoring either CO_2 evolution (Hamer et al., 2004) or analyzing specific compounds characteristic of black C (Brodowski et al., 2005b). Similarly, lignin degradation and SOM mineralization from various soils and in soil size fractions were determined in 3–4-week laboratory incubations with CO_2 -efflux monitoring (Hamer and Marschner, 2002, 2005a; Ohm et al., 2007). The degradation of ^{14}C -labeled catechol in soil was monitored over 3–4 weeks (Hamer and Marschner, 2005a) and over a 4 y period (Ji, unpublished). The DOC degradation studies were carried out in solution batch assays over a period of 90 d with intermittent quantification of CO_2 release and characterization of the residue with simple spectroscopic methods (UV, fluorescence), $^1\text{H-NMR}$, FTIR, ^{13}C analysis, and pyrolysis–field ionization MS (Kalbitz et al., 2003b).

3.2 Carbon-14 analysis of SOM

The CO_2 assimilated by plants contains trace amounts of the radioactive isotope ^{14}C , which is continuously produced in the lower stratosphere by collision of low-energy cosmic-ray neutrons with N atoms. Since C cycling between the atmosphere and the living biosphere is relatively rapid, plants reflect the ^{14}C concentration of the atmosphere. When C exchange between an organism and the atmosphere is stopped by death, the radiocarbon concentration begins to decrease through radioactive decay with a half-life of $5,730 \pm 40$ y (Godwin, 1962). If left undisturbed, the ^{14}C concentration of OM decreases continuously and can be translated into an age.

Because soils are open systems which continuously receive organic C as plant residues and loose gaseous and dissolved C via mineralization and leaching, respectively, radiocarbon ages of SOM do not represent the time of soil formation but reflect the “apparent mean ^{14}C age”, of the mixture of different organic components in SOM (Wang et al., 1996). Thus, a heterogeneous mixture of organic components in SOM is reflected by a wide range in ^{14}C ages from recent, including post-1954 (“bomb”) material with absurd ^{14}C ages of up to more than 20,000 y (Scharpenseel and Becker-Heidmann, 1992; Trumbore and Zheng, 1996).

For the study of soil C dynamics, ^{14}C data give information on two different time scales: (1) Under steady-state conditions, the ^{14}C age reflects the mean residence time of C in bulk soil or of soil fractions provided the C input has a constant ^{14}C content. In modern soils, this interpretation is problematical because of the presence of bomb- ^{14}C . (2) Bomb- ^{14}C , derived from atmospheric testing of nuclear weapons, mainly in the late 1950s until the early 1960s, can be used as a tracer for C exchanges that occurred on decadal time scales (Trumbore and Zheng, 1996). However, this approach requires the availability of archived soil samples collected before the release of bomb- ^{14}C into the atmosphere, to correct the measured bomb- ^{14}C contributions for the effect of aging of SOM.

Carbon-14 analyses have been applied to numerous physical and chemical soil fractions to determine C-turnover times and identify different protection mechanisms (Trumbore and Zheng, 1996). Since even functionally defined SOM fractions are still composed of a large variety of organic compounds differing in ^{14}C age and thus represent the weighted average of individual compounds, a relatively new method is the analysis of ^{14}C in individual organic compounds ideally of known origin. Compound-specific radiocarbon analysis of soils and sediments became practicable with the development of preparative capillary gas-chromatography (Eglinton et al., 1996) and more recently of high-performance liquid chromatography (Smittenberg et al., 2006) that allows the isolation of individual molecules from complex mixtures and the reduction of the necessary sample size for ^{14}C measured by accelerator mass spectrometry (AMS) to <100 μg of C (Eglinton et al., 1996). In soil science, radiocarbon analyses at the molecular level are still scarce.

In this paper, we present data from the ^{14}C analysis of bulk soil samples (Rethemeyer et al., 2004a, 2005), size and density fractions (Rethemeyer et al., 2005; Ohm et al., 2007), SOM fractions obtained with different extraction solutions (Dreves, unpublished), and of soil lipid fractions and individual microbial phospholipid fatty acids (PLFAs) isolated from two agricultural topsoils (Rethemeyer et al., 2004b, 2005; Kramer and Gleixner, 2006).

3.3 Analysis of $\delta^{13}\text{C}$ values after vegetation change

Natural ^{13}C labeling exploits the difference in ^{13}C content between C4 plants (e.g., maize) and C3 plants (e.g., wheat). This isotopic difference is found at both the bulk and molecular level and is maintained during decomposition of plant biomass (Balesdent and Mariotti, 1996). When C4 vegetation replaces C3 vegetation, the new, ^{13}C -rich, C4-derived C gradually replaces the old, decomposing C3-C in SOM. Thus, the proportion of the new, C4-derived OM can be used to estimate the residence time and pool size of individual SOM components, such as size or density fractions (Conteh et al., 1997; Jolivet et al., 2003; Dalal et al., 2005), and specific chemical compounds (Gleixner et al., 1999). As an alternative to natural labeling, isotopic label can be introduced by fumigating plants with labeled CO_2 , as in free-air CO_2 -enrichment experiments (Hagedorn et al., 2003; Wiesenberger and Schwark, 2006).

The determination of compound-specific isotope values is achieved by extraction of the compounds from the soil, purification of the extract, gas-chromatographic (GC) separation, on-line combustion, and separate isotope-ratio-monitoring mass spectrometry of the CO_2 formed from each compound (Schmidt and Gleixner, 1998; Wiesenberger et al., 2004; Kramer and Gleixner, 2006) or by direct thermal extraction of pyrolysis products from soil followed by GC separation of the compounds, combustion, and isotope-ratio determination (Gleixner et al., 1999). In this paper, data on lipids (Wiesenberger et al., 2004; Wiesenberger and Schwark, 2006) and lignin

(Heim and Schmidt, 2007, and unpublished data) and pyrolysis products (Gude et al. unpublished) are presented, along with data from size or density fractions (John et al., 2005).

4 Recalcitrance of SOM and SOM fractions

4.1 Total SOM

In most soils, the radiocarbon age of SOM increases with depth. This may indicate increased recalcitrance or protection of the SOM remaining in the subsoils (Rumpel et al., 2002; Rethemeyer et al., 2005). In the two forest soils from N Bavaria described by Kögel-Knabner et al. (2008a, this issue, pp. 5–13), the mean ^{14}C concentration decreased with increasing soil depth. In the Cambisol, ^{14}C concentrations >100 pMC in the upper 24 cm (A and Bw horizons) indicate the presence of bomb- ^{14}C , which means a contribution of SOM younger than 50 y. In the Podzol and deeper layers of the Cambisol, ^{14}C concentrations are below that of the standard atmosphere and apparent mean ^{14}C ages increasing with depth can be calculated (Tab. 1). In the Podzol, these mean ^{14}C ages are higher than in the Cambisol, bomb- ^{14}C vs. 1,570 y, which corresponds with much lower soil organic carbon (SOC) mineralization rates in the Podzol. As the apparent mean ages are the result of both real aging of the SOM and admixture of bomb- ^{14}C material, as evident in the A and Bw of the Cambisol, a consistent relationship between ^{14}C age and SOC mineralization rate within the Podzol profile is not necessarily expected. The comparison between the two profiles indicates that the apparently longer SOC residence times in the Podzol are at least partly real and due to the low SOM degradation.

The degradation of SOM can be accelerated by the addition of organic substrates or nutrients that are easily available for microorganisms (Kuzyakov et al., 2000; Neff et al., 2002). This phenomenon denoted as positive priming effect has also been observed in various agricultural and forest soils, ranging from +10% to +91% after the addition of ^{14}C -labeled fructose or alanine (Hamer and Marschner, 2005a). For the Bs horizon

Table 1: Radiocarbon ages and substrate-induced priming effects (PE) on mineralization rates of soil samples from two forest sites in N Bavaria (data compiled from Rumpel et al. [2002] and Hamer and Marschner [2005a]).

		depth [cm]	SOC [g kg ⁻¹]	^{14}C age [y BP]	SOC mineralization [mg C g ⁻¹ d ⁻¹] ^a	PE after fructose addition [%]	PE after alanine addition [%]
Podzol	Oa	3–0	340	n.d.	0.18	+17	+14
	EA	0–10	32	525	0.22	+30	+49
	Bs	12–30	54	745	0.07	+91	+85
	Bw	30–55	24	1570	0.12	n.s.	+63
	C	55–70	2	3840	n.d.	n.d.	n.d.
Cambisol	Oa	1–0	122	n.d.	0.84	+10	+37
	A	0–5	44	<50	0.74	n.s.	+22
	Bw	5–24	6	<50	0.62	n.s.	n.s.
	Bg	24–30	1	655	n.d.	n.d.	n.d.

^a determined in a 26 d laboratory incubation at 20°C and 60% WHC

of the Haplic Podzol, it has been shown that the combined addition of fructose and alanine enhanced SOC mineralization stronger than the single additions (Hamer and Marschner, 2005b). Therefore, the high positive priming effects of >200% observed in planted soils (Cheng et al., 2003) may also be the result of such synergistic effects from different substrates present in the root exudates. Highest positive priming effects were mainly observed in samples with OM of low biodegradability, e.g., in the mineral horizons of the Haplic Podzol (Hamer and Marschner, 2005a). These results show that the apparent high SOM stabilities in the Podzol are at least partly due to a low availability of easily degradable cosubstrates or nutrients and not due to recalcitrance.

4.2 Physical SOM fractions

Physical fractionations to study SOM turnover are frequently used, since OM associated with different size or density fractions is characterized by different composition and stability (Christensen, 2001; Swanston et al., 2002). While OM associated with soil aggregates or minerals may be stabilized by various other mechanisms such as occlusion, inaccessibility, or sorption, the stability of free or uncomplexed OM should be mainly controlled by its inherent molecular-level resistance to microbial breakdown, i.e., its recalcitrance (Christensen, 2001; Swanston et al., 2002). This fraction is generally obtained by density fractionation in heavy liquids (1.2–2.0 g mL⁻¹) with the yield being strongly dependent on the pretreatment of the sample (i.e., air-drying, ultrasonic dispersion) and the density of the liquid (Amelung and Zech, 1999; Schmidt et al., 1999; Wander, 2004; Kaiser and Guggenberger, 2007). Without prior density separation, the coarse silt-sized soil fraction (>20–63 µm) as well as the sand-sized fraction contain free or uncomplexed OM (Christensen, 2001), so that its stability should also be primarily a function of its recalcitrance.

In two agricultural soils from long-term field experiments at Rothalmünster and Halle, SOM turnover was studied using a ¹³C tracer provided by a crop change from C3 (wheat or rye) to C4 (maize) and by using ¹⁴C. The ¹⁴C analyses of density SOM fractions showed fairly uniform ¹⁴C concentrations approx. 5 pMC below the atmospheric level at the time of sample collection at Rothalmünster. This indicates the OM is

a mixture of contributions from the last 50 y and before 1954. Only the light occluded fraction contained OM with a ¹⁴C content <100 pMC, corresponding to OM largely formed before 1954 (Tab. 2). This is due to a higher portion of older OM, possibly charcoal (Baisden et al., 2002) or black C which may also actively contribute to the aggregation process at an adjacent site (Brodowski et al., 2006). The small differences in ¹⁴C concentration of the density fractions from the arable soil at Rothalmünster corresponds to results of Baisden et al. (2002) for grassland soils yielding negligible differences in ¹⁴C-based mean residence time of OM in density fractions. Sollins et al. (2006), on the other hand, found decreasing ¹⁴C concentrations with increasing density of OM fractions from a forest soil suggesting a higher stability of mineral-associated OM.

If OM turnover times in density fractions from the Rothalmünster soil are calculated via the results of natural ¹³C labeling, some differentiation between the fractions can be seen (John et al., 2005). Clearly, OM not associated with soil minerals has very short turnover times, indicating that most of the OM within these fractions is not recalcitrant. Organic matter associated with soil minerals as well as occluded light-fraction OM show significantly longer turnover times, suggesting protection by mineral association and aggregate shielding, respectively. At the Halle site, the ¹⁴C contents of SOM are unrealistically low corresponding to radiocarbon ages between 4,300 and 21,200 y (Tab. 2), caused by the large amounts of fossil C derived from nearby lignite mining and industrial activities (Kögel-Knabner et al., 2008a, this issue, pp. 5–13). Due to the continuous lignite input until approx. 1990 and the high recalcitrance of the material (Rumpel and Kögel-Knabner, 2002), the admixture of this fossil C with a ¹³C signature of C3 vegetation is thought to have resulted in considerably overestimated C-turnover times determined by natural ¹³C abundance at the Halle site (Rethemeyer et al., 2004b; Wiesenberger et al., 2004). If the fossil-C contribution to total SOC is estimated via ¹⁴C using mass-balance calculation and then excluded from SOM, the corrected turnover times of plant-derived OM show a similar distribution as the ¹³C-based data for the Rothalmünster soil with the slowest turnover of the occluded light OM fraction (John et al., 2005; Rethemeyer et al., 2008). The turnover time in this fraction is roughly 3–4 times longer than that of free light OM fraction at both sites. These corrected turnover

Table 2: Radiocarbon ages and turnover times calculated from natural ¹³C abundance in density fractions from topsoils of the maize plots in the long-term agricultural field experiments at Halle and Rothalmünster (data compiled from John et al. [2005], Rethemeyer et al. [2005], and Rethemeyer et al. [2008]).

SOM density fraction	Rothalmünster		Halle		¹⁴ C-corrected turnover time from ¹³ C data [y]
	¹⁴ C content [pMC]	turnover time from ¹³ C data [y]	¹⁴ C content [pMC]	turnover time from ¹³ C data [y]	
mineral >2.0 g cm ⁻³	103.5 ± 0.3	64	58.7 ± 0.2	308	125
free light <1.6 g cm ⁻³	102.9 ± 0.5	23	57.2 ± 0.4	148	52
mineral occluded 1.6–2.0 g cm ⁻³	103.5 ± 0.3	50	26.3 ± 0.2	526	83
light occluded <1.6 g cm ⁻³	97.6 ± 0.8	84	9.6 ± 0.2	>1000	165

times are still longer at Halle than at Rotthalmünster and are probably largely due to the more continental climatic conditions and differences in soil texture.

The ¹⁴C analysis of density fractions (for details, *cf.*, Kaiser et al., 2002; Kaiser and Guggenberger, 2003) obtained from the “Steigerwald” Cambisol after ultrasonic dispersion shows that throughout the profile, the light fraction has a bomb-¹⁴C signature, *i.e.*, values >100 pmC as a result of the nuclear weapons testing (Fig. 1). Except in Bw1, the ¹⁴C concentrations are all significantly above that of the atmospheric level at the time of sampling. The high ¹⁴C concentrations indicate OM formed in the 1990s and 1980s. In contrast, the heavy, mineral-associated OM fraction is generally depleted in ¹⁴C relative to the light-fraction OM except in the A horizon (Fig. 1). The ¹⁴C concentration in the heavy, mineral-associated OM fraction decreases with soil depth down to Bw3, likely because of input of recent organic debris close to the surface. At this site, light-fraction OM seems to comprise of mainly young materials not inherently recalcitrant. Bulk-soil ¹⁴C values are intermediate between light and heavy fraction, but largely follow that of the heavy fraction, which indicates most of the C is in the heavy, mineral-associated fraction.

In contrast, all fractions in the Haplic Podzol, except for the light fraction in CB2, are <100 pmC. Considering the atmospheric ¹⁴C concentrations of the past 50 y and the ¹⁴C distribution in the Dystric Cambisol, this could indicate no bomb-

¹⁴C of the litter layer of the last decades has yet reached the mineral soil or that the Haplic Podzol contains old C. The light fraction, which is thought to represent OM not or only weakly protected, is more depleted in ¹⁴C than the heavy, mineral-associated OM fraction, except for the subsoil horizons below the Bs2 horizon. That means the light fraction in the upper horizons contains compounds older than the heavy fraction, possibly old black-C particles. The increase in light-fraction ¹⁴C in the deeper subsoils where the heavy-fraction OM is older suggests plant-derived material carrying a bomb-¹⁴C signature to migrate deeper into the soil than old C particles. However, it is difficult to say to what extent this may indicate the presence of material that is recalcitrant. One possible source of recalcitrant OM is the different vegetation at the two sites (deciduous vs. coniferous). Yet, the difference in chemical composition of plant material of various origins is not large enough to justify the differences in light-fraction ¹⁴C between the study sites.

The analysis of light-fraction particles with a combination of scanning-electron imaging with energy-dispersive X-ray spectroscopy (for details, *cf.*, Kaiser et al., 2002) revealed many fragments with the typical structure of plant tissue, rich in C but depleted in O (Fig. 2), thus identifying the fragments as charred plant debris (black C). Although the evidence is only qualitative, the small ¹⁴C content of the topsoil light fraction at this site likely is due to a contribution of rather old recalcitrant black C.

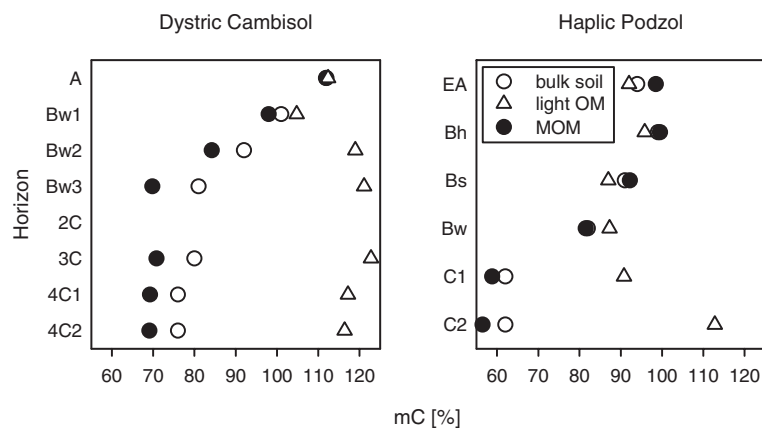


Figure 1: Radiocarbon in bulk samples and density fractions (light OM: <1.6 g cm⁻³; mineral-associated OM (MOM): >1.6 g cm⁻³) of horizons at the two study sites (Fichtelgebirge: Haplic Podzol; Steigerwald: Dystric Cambisol). Note, ¹⁴C in the bulk CB1 and CB2 of the Haplic Podzol was analyzed on a mixed sample composed of material of both horizons (Rumpel et al. 2002). Bars representing the errors of measurements and estimates are smaller than the icons.

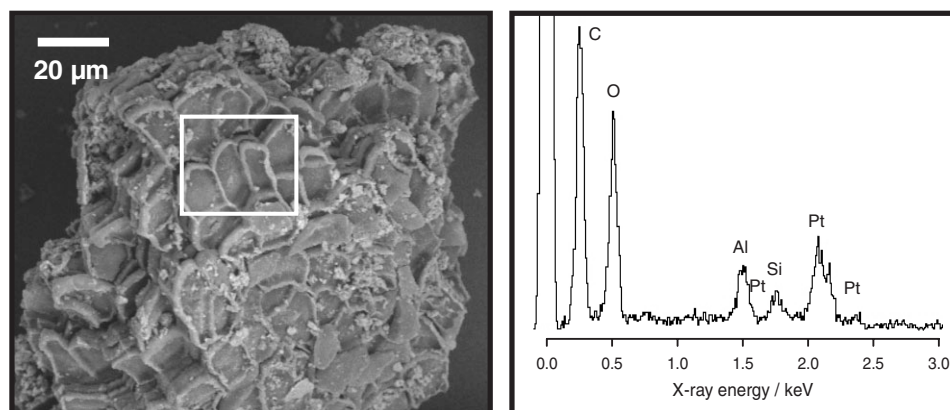


Figure 2: High-resolution scanning-electron microscope image (recorded on a LEO 1530 field-emission instrument) of a charred plant fragment (density fraction <1.6 g cm⁻³) from the Bs2 horizon (30–55 cm depth) of the Haplic Podzol (right). Images result from detection of secondary electrons (Everhart-Thornley detector/In-lens detector). The highlighted area was analyzed by energy-dispersive X-ray spectroscopy (Oxford INCAEnergy) for elemental composition (spectra shown left).

From the same Haplic Podzol, SOM in the Bs horizon and in its textural size fractions displayed very distinct mineralization rates during a 56 d incubation (Fig. 3). Soil organic C mineralization in the unfractionated sample (Bs) was lowest, in the isolated sand fraction more than 4-fold higher. This corresponds well with the much younger mean ^{14}C age of 49 y BP as opposed to 749 y BP in the bulk soil and 719 and 882 y BP in the silt and clay fractions, respectively (Ohm et al., 2007). Accordingly, SOM mineralization in the small-size fractions is lower than in the sand fraction. However, the comparison of bulk-soil data with the summed fraction data clearly showed that the physical-fractionation process itself increased SOM degradability considerably (data not shown).

The addition of fructose or alanine strongly increased the mineralization of the SOM in all fractions (positive priming effects). In general, the priming effects increased with decreasing particle size. The strong priming effects in the silt and clay fraction showed that not only a labile pool of OM is affected but also the more stable pool characterized by high ^{14}C ages (Ohm et al., 2007). This furthermore indicates that the stabilization of the OM in these fractions is not only due to recalcitrance or to interactions with the minerals, but that it may also be caused by a substrate limitation of the degrading microorganisms.

In summary, the results of these studies with physical SOM fractions show that old SOM with long turnover times is generally either associated with soil minerals, *i.e.*, the heavy high-density fraction and the small textural fractions, or it is present as fossil or black C. Other SOM that is not stabilized by interactions with minerals or occluded in aggregates generally has mean turnover times or ^{14}C ages of <50 y and therefore cannot be considered as recalcitrant. This is also evident from other studies, where the free-particulate SOM (fPOM) fraction generally reacts most sensitively to land-use or agricultural-management changes (Christensen, 2001; Jolivet et al., 2003; Marriott and Wander, 2006).

4.3 Chemical SOM fractions

In the analysis of SOM, numerous different chemical-extraction procedures have been developed to isolate fractions with distinct properties. The most classical approach is the

humus-fractionation scheme developed by Kononova (1966), which differentiates humus fractions according to their solubility in acid and base solutions. The classical acid-alkali-acid extraction scheme of radiocarbon dating, which removes first the acid-soluble fulvic acid fraction with HCl and then separates NaOH-soluble humic acids and the insoluble humin fraction, was applied to agricultural soils by Rethemeyer et al. (2005), who found the isolated humic acid fraction is always younger than the humin fraction.

In a pasture soil at Rothalmünster, humic acids in the topsoil have a ^{14}C content exceeding that of the recent atmospheric level (Fig. 4) indicating the contribution of OC from the last approx. 40 y to this fraction. Below 20 cm soil depth, humic acids yield ^{14}C values <100 pMC which suggests a small proportion of C derived from recent plant inputs (Rethemeyer et al., 2005). A considerable decrease in ^{14}C with increasing soil depth of 30% to 54% was observed for the humin fraction with increasing depth in the profile, similar to earlier observations by Paul et al. (1997). This indicates both, a low contribution of young compounds and a relative enrichment of stabilized organic components in the humin fraction with soil depth.

For the identification of recalcitrant SOM fractions, this approach is not very helpful, since SOM treatment with HCl and NaOH may alter the organic compounds in the extracted fractions (MacCarthy, 2001). As for all operationally defined fractionation methods, both fractions still consist of a wide range of different compounds which partly may have been stabilized by physical protection prior to solubilization with NaOH. This is even more the case for the humin fraction, which supposedly consists of resistant, highly condensed humic substances but also of humic acids which are intimately bound to the mineral phase (Stevenson and Cole, 1999).

At the same location at Rothalmünster, ^{14}C was also determined in different soil extracts from an agricultural soil with maize (Fig. 5a). The ^{14}C values in the cold-water and the Napyrophosphate extracts closely follow the depth gradient of ^{14}C in SOM in the bulk soil. Cold water-extractable OM generally has a larger ^{14}C content, *i.e.*, is younger than the bulk

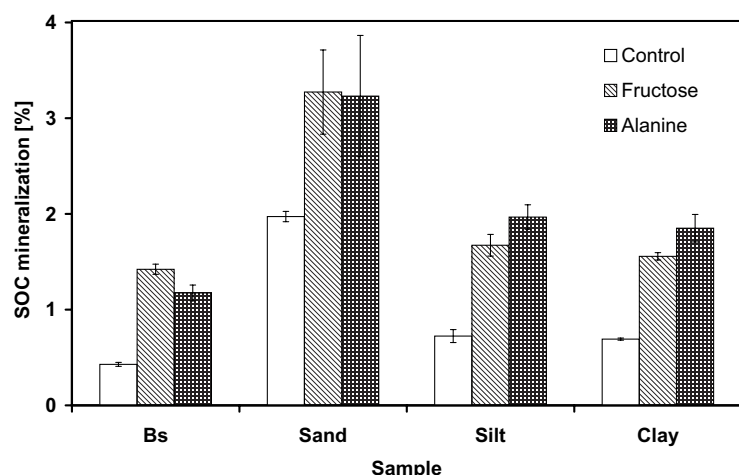


Figure 3: SOC mineralization during 56 d of incubation in the Bs horizon and in the three soil size fractions sand, silt, and clay without amendments (control) and after repeated addition of the ^{14}C -labeled substrates fructose or alanine (Ohm et al., 2007).

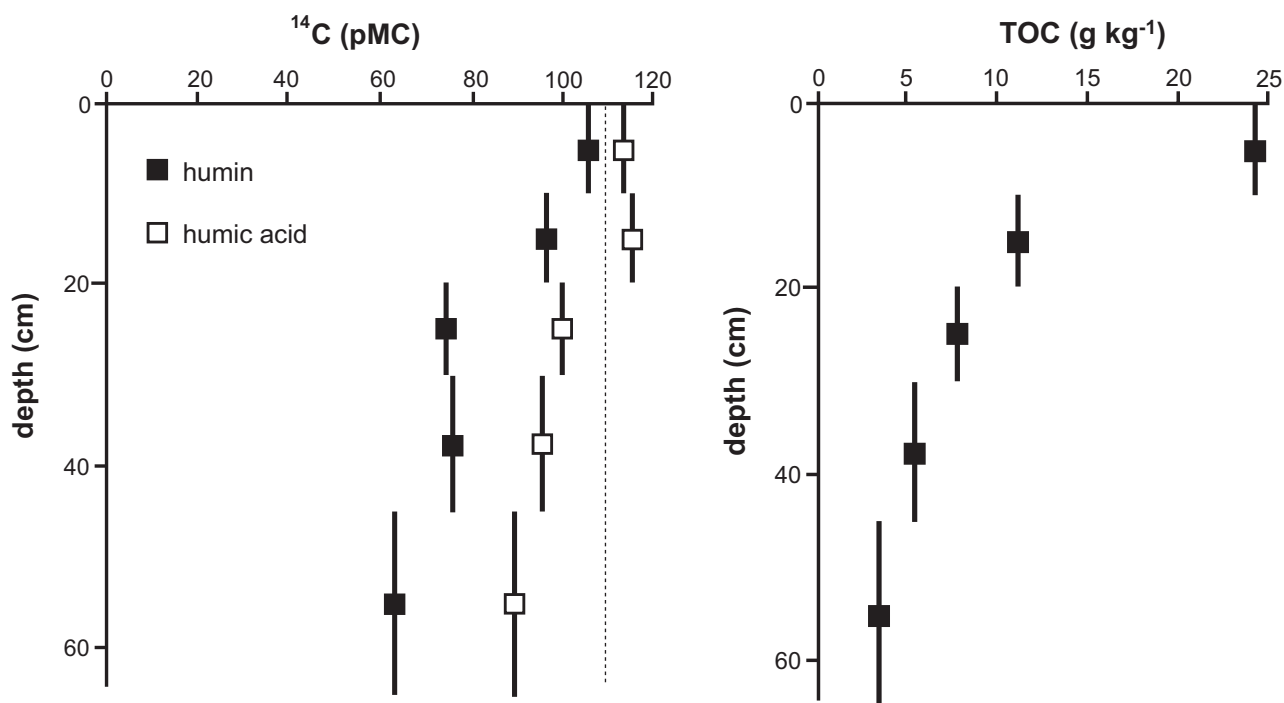


Figure 4: ^{14}C Carbon values of humin and humic acid fractions and total organic-C content (TOC) of the bulk soil under pasture at Rotthalmünster (Rethemeyer et al., 2005). The vertical bars represent the sampling interval and the dashed line the atmospheric ^{14}C level in 2002 (Levin and Kromer, 2004).

soil SOM, especially below 50 cm depth. The ^{14}C values of SOM, including water-soluble SOM, below 60 cm depth are unexpectedly low (approx. 45 pMC, approx. 6,000 y BP) suggesting that this part of the soil profile was only weakly influenced by the transport of SOM down from the surface. The change in ^{14}C content coincides with a boundary at 60 cm depth between a lower layer of periglacially redeposited loess and an upper colluvial layer. Since the easily water-soluble SOM should be mobile, interactions with mineral surfaces probably play a minor role for this apparent long-term stability. Nothing is known about the composition or structure of water-soluble SOM, but it cannot be ruled out that it is partly present as small particles or colloids, especially since extracts were passed through 1 μm quartz filters. Possibly these colloids comprise organic molecules associated with Al(Fe) hydroxides, which have been shown to stabilize DOC against degradation (Kaiser et al., 2002). Another probable explanation for the apparent long persistence of these soluble compounds is that they are newly formed fragments of microbial metabolites of old local SOM.

In the forest profile of the Podzol, bulk SOM in the subsoil has up to 56 pMC higher ^{14}C levels in comparison to the arable soil at Rotthalmünster, reaching a minimal ^{14}C concentration of only 84 pMC in the C1 horizon (Fig. 5b). In addition, the C content in the subsoil (1.15%) is 7-fold higher compared to the Rotthalmünster soil. This suggests a considerable transport of SOM down from the surface. The elevated ^{14}C contents of the forest floor show that it consists largely of SOM that was formed within the last 40 y, when the atmosphere was enriched by bomb- ^{14}C . Nevertheless, a signifi-

cant part of the SOM in the Oa horizon was formed before 1950 as indicated by the ^{14}C values between 100 pMC and that of the atmosphere in summer 2004. In the mineral soil horizons, water-soluble SOM again yields the highest ^{14}C value of about 93 pMC corresponding to a few 100 y BP in the subsoil. Although this is considerably less than in the agricultural soil, such relatively long residence times for the soluble SOM fraction, which can be assumed to be available to microorganisms, indicates that it may consist of recalcitrant compounds or of fragments or microbial metabolites derived from the breakdown of the even older SOM from that depth.

Degradability of dissolved organic matter (DOM) is highly variable, ranging from <5% to >90% of the initial C as measured at laboratory incubations of several days to months (Buyanovsky and Wagner, 1998; Yano et al., 1998; Kalbitz et al., 2003a; Don and Kalbitz, 2005). Typically, degradation can be described by a two-component exponential model, as determined for 13 DOM solutions from various sources (Kalbitz et al., 2003a) with a labile pool being mineralized within days ($k_1 = 0.1\text{--}0.3 \text{ d}^{-1}$) and a more stable pool with turnover times of years ($k_2 = 0.0002\text{--}0.0085 \text{ d}^{-1}$). Differences in biodegradability are therefore mainly due to the size of the labile pool which ranges between 3% and 87% of DOC (Kalbitz et al., 2003a) and the differences in degradation rate of the more stable pool. Aromatic compounds, possibly deriving from lignin, seem to be the most stable constituent of DOM in forest floors and topsoils; solutions containing DOM with a large portion of aromatic components are only poorly biodegradable (McCracken et al., 2002; Kalbitz et al., 2003a; McDowell et al., 2006), and these aromatic constituents

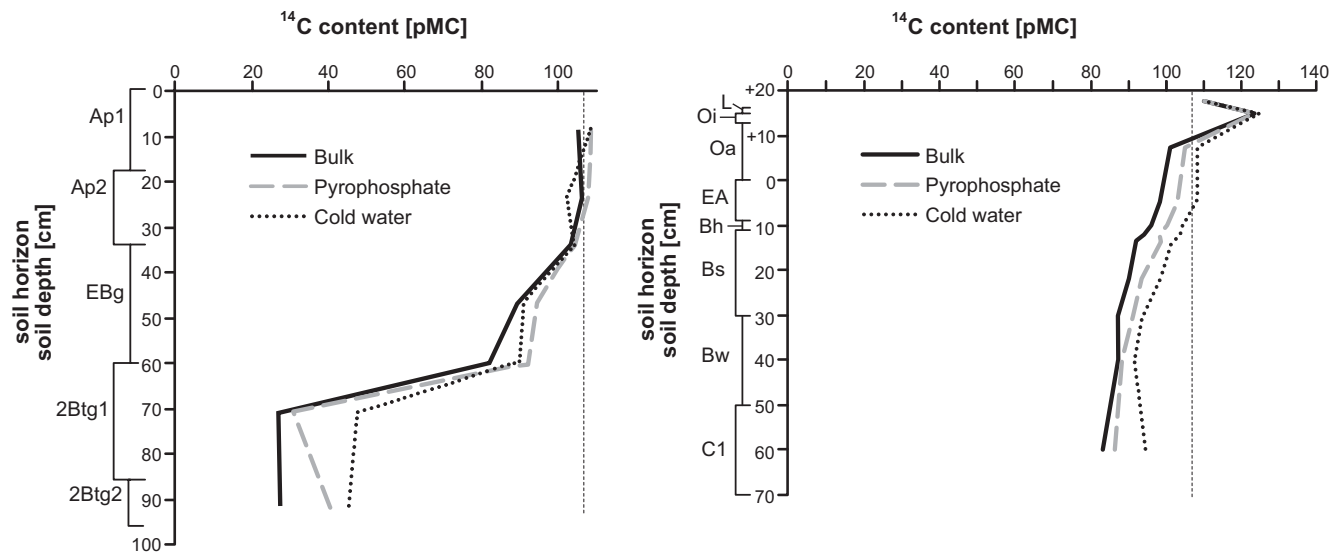


Figure 5: ^{14}C Carbon values for bulk soil and for cold water– and Na-pyrophosphate–extractable SOM in depth profiles from an agricultural soil at Roththalmünster and a forest Podzol (Waldstein). Note the different scale of the x-axis to display the differences in each profile. The vertical dashed line represents the atmospheric ^{14}C level in summer 2004 (Levin, personal communication). The soil extracts were obtained by sequential extraction with pure water at room temperature followed by a 0.1 M Na-pyrophosphate solution at 50°C. The soil-to-solution ratio was 1:5 for a mineral horizon and 1:10 for an O horizon, respectively, and the extracting time was 24 h. All suspensions were centrifuged (3500 g for 10 min), and the supernatant was filtered through a 1 μm pre-combusted quartz filter and acidified to pH < 3.

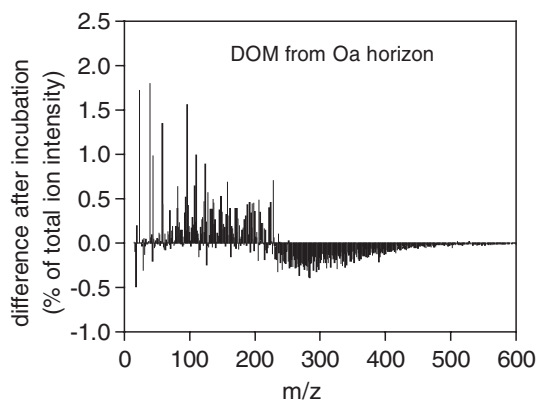


Figure 6: Pyrolysis–field ionization mass spectra of DOM from Oa horizon: differences between spectra before and after 90 d of incubation; positive values indicate enrichment whereas negative values indicate depletion (according to Kalbitz et al., 2003b).

survive biodegradation preferentially (Kalbitz et al., 2003b). However, even poorly degradable DOM obtained from the Oa horizon of a spruce stand underwent substantial compositional changes during 90 d of incubation. Mass spectra from pyrolysis field ionization (Fig. 6) showed a depletion of high-mass compounds and an accumulation of low-mass compounds and a decrease in lignin dimers in favor of monomers (Kalbitz et al., 2003b).

In subsoils, the amount of aromatic compounds in DOM is relatively low because of preferential adsorption and precipitation during percolation of soil solution through the profile (Kaiser and Zech, 1997; Kalbitz et al., 2005; Scheel et al., 2007). However, despite its large content of carbohydrates,

DOM in subsoils is even less degradable than the largely stable DOM from Oa horizons (Boyer and Groffman, 1996; Schwesig et al., 2003). Therefore, lignin-derived compounds are not the only recalcitrant components of DOM. Microbial degradation of DOM results also in the formation of carbohydrates and peptides (Kalbitz et al., 2003b) contributing to stable DOM. At the moment, we have no conclusive answer why these microbial products are not mineralized as fast as carbohydrates initially present in DOM in litter, forest floor, and topsoils (Kalbitz et al., 2003b). Probably, these microbial products are just a reflection of recycling of microbial biomass. On the other hand, the microbial biomass in the subsoils may be simply too low and too patchily distributed to access these compounds.

4.4 Specific compounds

In the context of recalcitrance, lignin is of special interest because it is generally assumed to be highly resistant to microbial degradation due to its size, irregular structure, and nonhydrolyzable bonds and therefore one of the main precursors for stable OM (Kirk and Farrel, 1987; Stevenson, 1994). However, from the data presented in Tab. 3, it is evident that mean residence times of individual lignin monomers in agricultural soils are in the range of one to two decades. These data also indicate that lignin monomers are less persistent in soil than the average of OM found in soils. In this context, it has to be noted that the method primarily yields information on how long the original structural units remain in the soil. Degradation products are not detected and might persist in the soil for longer times. However, incubation studies with isolated lignin also showed that it is easily mineralized at a rate of 2.8% within 26 d (Hamer and Marschner, 2002).

The various phenolic subunits of lignin differ in their turnover rates. Cinnamyl units decompose faster than syringyl units, while vanillyl units are slowest. *Bahri et al.* (2006) found that after 9 y of maize cultivation on a eutric cambisol, more than 60% of cinnamyl phenols in the soil were maize-derived. For the vanillyl units, on the other hand, this proportion was <30%. They propose various explanations for this observation including a higher degree of cross-linking between vanillyl-type monomers. Consequently, variations in degradation rates between different types of lignin can be attributed to differences in recalcitrance between lignin macromolecules. While there may be relative differences in recalcitrance between lignin-monomer units, there is no evidence that absolute recalcitrance is high enough that it would lead to long-term stabilization of lignin in soil.

A recent study by *Rasse et al.* (2006) found that observed changes in lignin isotope ratio can be explained by a two-compartment model. The model assumes an unprotected lignin pool and a stabilized pool. The former contains approx. 92% of the lignin and has a turnover time of <1 y, while the latter contains only 8% of the soil lignin pool, but contains lignin with a longer mean residence time in the soil (18 y in the study by *Rasse et al.* [2006]). Consequently, virtually all lignin detected in soil samples represents this slow pool as lignin in

the fast pool turns over too fast to significantly accumulate in the soil.

Such a two-compartment model can also explain the observation at Rotthalmünster. At this site, the annual input of lignin is estimated to be approx. 89% of the lignin stock in the Ap horizon (Tab. 4), resulting in a mean residence time for lignin in the Ap horizon of only 1.1 y. On the other hand, the isotope data indicate that, after 23 y of continuous maize cropping, 27% of the lignin still derives from the previous C3 vegetation (Tab. 4). These two seemingly contrasting observations can be reconciled if two lignin pools are assumed with similar parameters as determined by *Rasse et al.* (2006) at Les Closeaux. The existence of a large pool (approx. 95% of the annual lignin input) with a rapid turnover (residence time <1 y) and a smaller pool (approx. 5% of the annual lignin input) with substantially slower turnover (residence time of 20 y) explains both the low current lignin stock in the soil (111 g m⁻² [30 cm]) as well as the fraction of 73% maize-derived lignin after 23 y of maize cultivation (unpublished data).

As a conclusion, if >90% of lignin input into soil is rapidly degraded, there is obviously no inherent recalcitrance of the lignin molecule itself. The processes stabilizing the remaining lignin fraction and being responsible for its accumulation,

Table 3: Overview of sites for which lignin turnover has been determined using isotopic labeling and compound-specific isotope analysis of lignin monomers.

Site	Ref.	Labeled plot	Control plot	Duration of experiment	Fraction of new SOC	Fraction of new lignin	Apparent lignin residence time
				[y]	[g g ⁻¹]	[g g ⁻¹] ^a	[y] ^b
Grassland soils							
Eschikon L	(1)	<i>Lolium perenne</i> , elevated CO ₂	<i>Lolium perenne</i> , ambient CO ₂	10	0.34	0.52 (0.36–0.85)	14
Eschikon T	(1)	<i>Trifolium</i> , elevated CO ₂	<i>Trifolium</i> , ambient CO ₂	10	0.32	0.49 (0.32–0.60)	15
Soil under perennial culture							
Hohenheim	(1)	<i>Miscanthus giganteus</i>	Mixed C3 pasture	9	0.37	0.55 (0.44–0.83)	13
Arable soils							
Les Closeaux	(2)	Maize cultivation	Wheat cultivation	9	0.09	0.47 (0.34–0.72)	14
Boigneville	(1)	Maize cultivation	Wheat cultivation	23	0.36	0.65 (0.45–0.93)	22
Rotthalmünster		Maize cultivation	Wheat cultivation	23	0.38	0.73 (0.54–1.00) ^c	18

References: (1): *Heim and Schmidt* (2006); (2): *Dignac et al.* (2005)

^a Weighted average of vanillyl, syringyl, and cinnamyl monomers (range of individual monomers in parentheses)

^b Calculated from fraction of new lignin assuming steady state and mono-exponential decay

^c Vanillic acid not included in calculation due to analytical problems

Table 4: Annual input and turnover of lignin at the maize plot in Rotthalmünster (*Heim*, unpublished). Lignin determined according to *Heim and Schmidt* (2007).

	Biomass input ^a	Lignin-C / C _{org}	Lignin-C input	Lignin-C stock in soil
	[kg C m ⁻² y ⁻¹]	[g kg ⁻¹]	[g C m ⁻² y ⁻¹]	[g C m ⁻² × (30 cm)]
Maize shoots	0.46	135	62.1	
Maize roots	0.17	216	36.6	
Total	0.63		98.8	111.2

^a Data from *Ludwig et al.* (2005)

however, require further research. Particle-size fractionation indicates that disproportionately high contents of old lignin are found in the fine-size fractions <20 μm (Heim et al., unpublished data). Therefore, organo-mineral associations deserve attention as potential protection mechanisms.

Lipids were also thought to be a part of the “stable” C pool in soils and can persist for at least a thousand years, as previously determined for peaty soils (Bol et al., 1996). For a more detailed insight into the stabilization of lipids in soil, the different sources and single components within this heterogeneous fraction must be considered. *N*-alkyl fatty acids predominate among soil lipids, and they can originate from plant residues, soil organisms, and organic fertilizer (Bull et al., 2000). Fatty acid quantification of primary OM and the corresponding soils from the long-term experiment at Halle showed that rye stubble and farmyard manure (FYM) were the main contributors to long-chain fatty acids with a maximum at *n*-C₂₈. Above- and belowground maize residues and soil fauna produced more short-chain (maxima at *n*-C_{14–18}) than long-chain fatty acids (Jandl et al., 2005, 2007). The 89% decomposition of rye-derived short-chain fatty acids (*n*-C_{10:0} to *n*-C_{20:0}) was larger than the 60% decomposition of the corresponding long-chain (*n*-C_{21:0} to *n*-C_{34:0}) fatty acids. Because of their hydrophobic properties, the long-chain compounds resist biodegradation and have longer residence times in soils (Schulten and Schnitzer, 1990). Figure 7 shows that, in addition to the extractable lipids, short-chain and long-chain fatty acids remained nonextracted by organic solvents. These proportions of stronger-bound aliphatics can be directly determined by pyrolysis–field ionization mass spectrometry (Py-FIMS) and must be considered in turnover models of lipids or aliphatics in soil.

Distribution patterns and compound-specific isotope data ($\delta^{13}\text{C}$ values) of major parts of the most abundant plant lipids (alkanes and carboxylic acids) showed that they vary with plant age and plant organ but are clearly differentiated between C3 and C4 plants (Wiesenberg, 2004; Wiesenberg et al., 2004; Wiesenberg and Schwark, 2006).

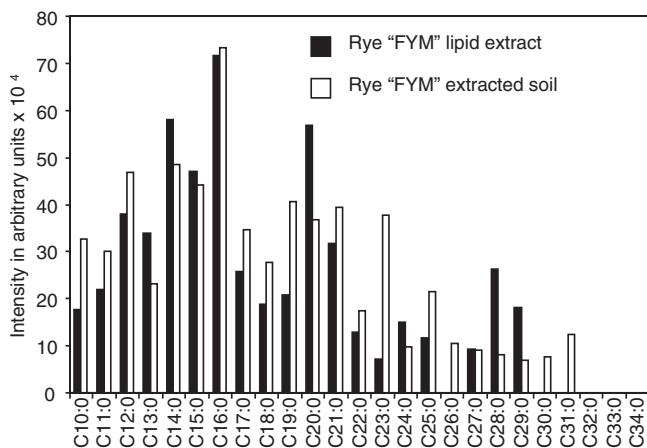


Figure 7: Fatty acid pattern determined by Py-FIMS of a lipid extract compared to the solid extraction residue (Jandl and Leinweber, unpublished data).

In Fig. 8, the replacement of SOC lignin and lipids in various long-term field experiments where a $\delta^{13}\text{C}$ label was introduced via crop change or through $^{13}\text{C}\text{CO}_2$ -labeling of plants (FACE experiments) is merged (Wiesenberg, 2004; Wiesenberg et al., 2004; Heim and Schmidt, 2008; Wiesenberg et al., 2008). During the first one or two decades, a fast replacement of C in soils can be observed for grain-cropped soils on a molecular level. Thereafter, the speed of the replacement decreases, and after three to four decades, the replacement of C is very slow. This means that the C of the “labile” and “intermediate-stable” C pools is mostly replaced after this time.

The turnover of lipids and lignin derivatives is significantly faster than for bulk C, following in the order from fast to slow: carboxylic acids, lignin derivatives, alkanes, SOC. In comparison to bulk SOC, a larger proportion (up to 80%) of the individual SOC components reacts “labile” during several decades. In comparison to arable soils, alkanes remain more stable than SOC in grassland soils (data derived from the 10-year FACE experiment at Eschikon/Switzerland (Wiesenberg et al., 2008)), whereas the turnover of carboxylic acids and lignin is the same as in arable soils. These data clearly show that most lipids are not selectively preserved during the decomposition of SOM and therefore cannot be considered as particularly recalcitrant compounds.

The degradation of the ^{14}C -labeled monomeric catechol in an arable topsoil from Halle was monitored in a long-term laboratory incubation. After 4 y of incubation, 48% of catechol

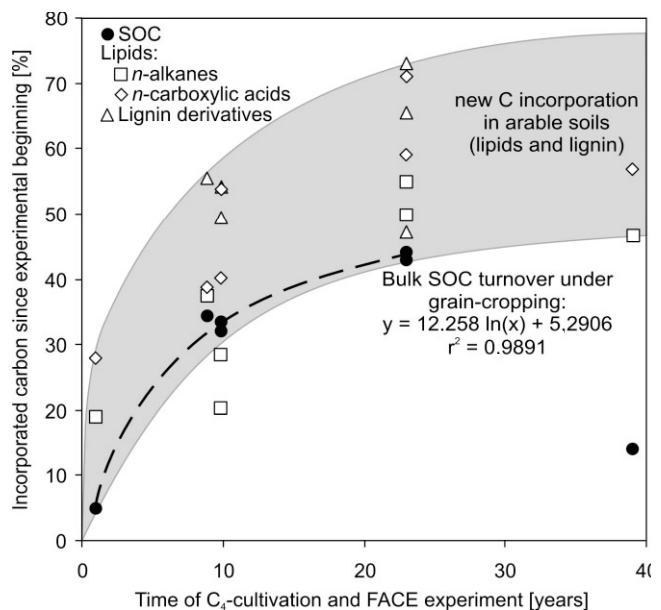


Figure 8: Incorporation of lipids, lignin derivatives, and SOC into soils after crop change (C4 plants for C3 plants) and in FACE experiments with ^{13}C -labeled CO_2 . The dashed line represents the SOC turnover in grain-cropped arable soils and grassland soils. Due to an exceptionally slow turnover, the contaminated silage-cropped soil was not included in this function. The shaded area reflects turnover of lipids in arable soils, whereby alkanes of the grassland soils are characterized by a lower turnover. Data compiled from Wiesenberg et al. (2004), Wiesenberg and Schwark (2006), and Heim et al. (2007).

were mineralized (Fig. 9). Similar to the DOM degradation, the kinetics of catechol mineralization followed a two-exponential model with two first-order components, one labile and one more stable:

$$A_t = 17.2 \exp(-2.49 \times 10^{-2} t) + 82.8 \exp(-4.00 \times 10^{-4} t), \quad (1)$$

with a regression coefficient $R^2 = 0.996$. The model suggests that catechol, like many pesticides, was transformed into two groups of soil C with different degradability, one (17%) is relatively labile to degradation with a half-life of 27.8 d, another (83%) is relatively recalcitrant with a half-life of 4.75 y. The two-component kinetics indicates that this inherently easily degradable compound is stabilized with increasing incubation time, possibly *via* several mechanisms: formation of more stable metabolites or through interactions of this phenolic compound and its metabolites with SOM and soil minerals (McBride, 1987; Vinken et al., 2005). This was confirmed when the soil was analyzed after 4 y, and only 2.4% of the remaining radioactivity were water-extractable, while 46% were extractable with NaOH (Ji et al., unpublished).

In short-term incubations with various soils, ^{14}C -catechol mineralization was highly variable, ranging from 6% to 16% within 26 d in most arable and forest soils (Hamer and Marschner, 2005a). However, in two soil samples from a Podzol, catechol mineralization was as high as 28% and 42% and in a model system with quartz sand and lignin even 58% (Hamer and Marschner, 2002). Clearly, this compound is not inherently recalcitrant but can easily be mineralized if not stabilized by other mechanisms.

Phospholipids are components of microbial cell walls which are only present in living cells and not stable in soils (Zelles, 1999). The ^{14}C analysis of individual microbial phospholipid fatty acids (PLFAs) from the plough horizon and the subsoil at Rotthalmünster and their comparison with PLFA- ^{14}C data from the fossil C-contaminated surface soil at Halle (Fig. 10) suggest that the synthesis of straight-chain monounsaturated PLFAs (*n*-C16:1, *n*-C17:1, *n*-C18:1) is mainly from the assimilation of recent OC by most probably Gram-negative bacteria (Rethemeyer et al., 2004b, 2005). The microbes producing saturated PLFAs (*i/a*-C15:0, *n*-C16:0, and *n*-C17:0) seem to

have a low substrate specificity, because at Rotthalmünster, these PLFAs have a higher bomb- ^{14}C content in the surface soil while in the subsoil, ^{14}C values are below atmospheric levels, which indicates the assimilation of older SOM from the past years to decades and pre-1954 material, respectively. At Halle, the low ^{14}C values of *n*-C17:0 and *cy*-C18:0 PLFAs even indicate that fossil C is part of the diet of the presumably Gram-positive bacteria (Rethemeyer et al., 2004b).

The determination of the $^{13}\text{C} : ^{12}\text{C}$ ratio in pyrolysis products of SOM allows us to calculate mean residence times of single compounds in soils where a C3-C4 crop change has occurred. This is achieved after separation by GC and analysis by mass spectrometry and by combustion isotope-ratio mass spectrometry GC/MS-C-IRMS (Gleixner et al., 1999). For the three long-term field experiment sites at Halle, Rotthalmünster, and Boigneville (Balesdent and Mariotti, 1996),

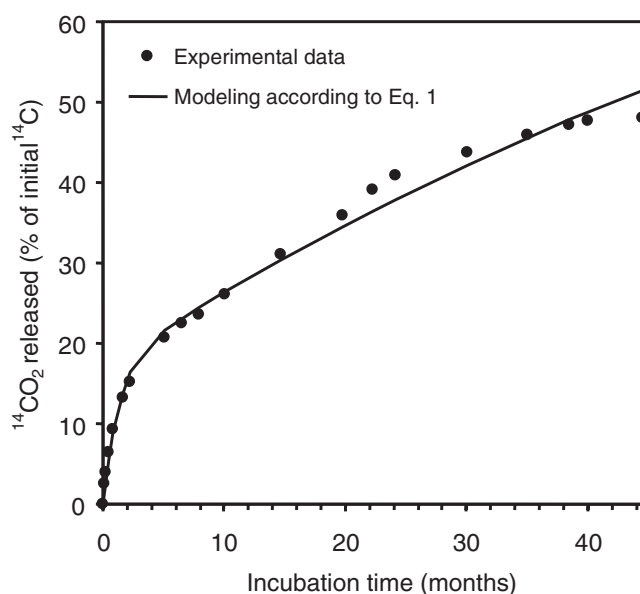


Figure 9: Experimental data and modeling of mineralization of catechol in soil during 4 y of incubation in an agricultural loamy sandy soil (Halle) at a concentration of $100 \mu\text{g}$ catechol (g dry soil) $^{-1}$, at 20°C in dark. Error bars are smaller than the symbols and therefore not shown ($n = 2$).

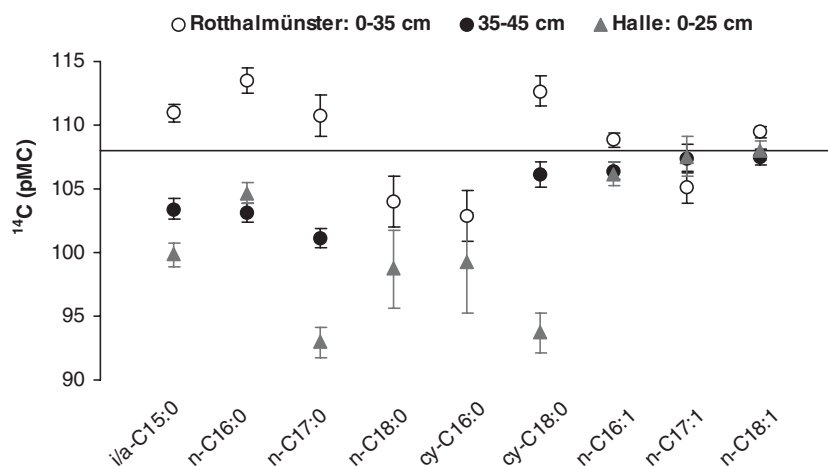


Figure 10: ^{14}C Carbon concentration of individual PLFAs from the surface and the subsoil at Rotthalmünster compared with data of the topsoil at Halle, both planted with C3 crops (Rethemeyer et al., 2004b, 2005). The line represents current atmospheric ^{14}C content of 108 pMC (Levin, personal communication).

ten pyrolysis products appearing in all three sites were grouped by their origin, *i.e.*, carbohydrates (polysaccharides), proteins/chitin, or unspecific source (Fig. 11).

At Boigneville and Rotthalmünster, the average mean residence times of SOC in the soils were very similar and are very similar for the different substrate classes. At Rotthalmünster, the pyrolysis products of carbohydrates had the highest average residence time of 55 (\pm 5) y, followed by proteins/chitin with 43 (\pm 14) y while the unspecific pyrolysis products had the lowest average residence time of 41 (\pm 33) y. In Boigneville, the pyrolysis products of proteins/chitin showed the highest value of 48 (\pm 19) y, carbohydrates had turnover times of 44 (\pm 7) y, and unspecific pyrolysis products had average turnover times of 30 (\pm 14) y. For carbohydrates (polysaccharides) and proteins, these results are unexpectedly high since both substance classes are rapidly degraded when added to soil (Azam et al., 1985; Hamer and Marschner, 2002, 2005a, b). However, independent methods have confirmed these results (Derrien et al., 2006). The two-pool lignin model of Rasse et al (2006) cannot be applied to carbohydrates and proteins as the pyrolysis products from plant material and soil differ in the chemical composition indicating their *in situ* formation. As possible explanations for the observed residence times related to the slow pool, physicochemical protection and biological recycling of organic compounds in soils remain (Gleixner et al., 2001).

At the study site Halle, the calculated average residence times of pyrolysis products were all >100 y (carbohydrate-derived 161 (\pm 32) y, proteins/chitin 284 (\pm 159) y, unspecific 3,880 (\pm 6,332) y). The longer residence times at this site in contrast to Rotthalmünster are presumably caused by the contribution of a substantial amounts of fossil fuel-derived compounds with similar $\delta^{13}\text{C}$ values as C3 plants (Rethemeyer et al., 2004a; Wiesenberg et al., 2004; Brodowski et al., 2005a) For the unspecific pyrolysis products, this produces unrealistically long residence times (see also 4.2). In contrast, these results provide further evidence for the longer

turnover time of *in situ* formed carbohydrates and proteins (Rethemeyer et al., 2004b; Kramer and Gleixner, 2006).

The mean residence times calculated from all individual pyrolysis products do not differ significantly from the residence times of the bulk samples. Consequently, apparent residence times are not caused by the recalcitrance of individual precursors but are more affected by the input and recycling of C in soils (Kramer and Gleixner, 2006).

4.5 Black C

Here, data are presented from a short-term (60 d) and a long-term (2 y) incubation study where the effects of glucose and the presence of microorganisms on the mineralization of charred plant materials were investigated in quartz sand and in soil samples. Details about production of the charred plant materials and the short-term experiment are documented in Hamer et al. (2004). For the long-term experiment, the ground black-C material was mixed at a weight ratio of 1:10 with either quartz sand or with topsoil from the continuous-rye field experiment in Halle. The samples were incubated at 20°C in the dark and a water content of approx. 70% water-holding capacity. The mixtures were incubated (1) with no additional treatment, (2) with glucose additions of 100 mg per 10 g sample every two weeks, or (3) under sterile conditions in a chloroform-saturated atmosphere. Black C was determined after oxidation to benzene polycarboxylic acids as described in Brodowski et al. (2005b).

In the 60 d experiment, the total black-C mineralization in the untreated controls amounted to 0.78%, 0.72%, and 0.26% of the charred maize, rye, and wood during the two incubation periods (Fig. 12). Apparently, some microorganisms were able to live with black C as sole C source. Mean residence times for the two charred straw residues and charred wood of 39 and 76 y, respectively, were calculated by a fit of loss of black C in the controls using a two-component first-order decay equation (Hamer et al., 2004). These are minimum

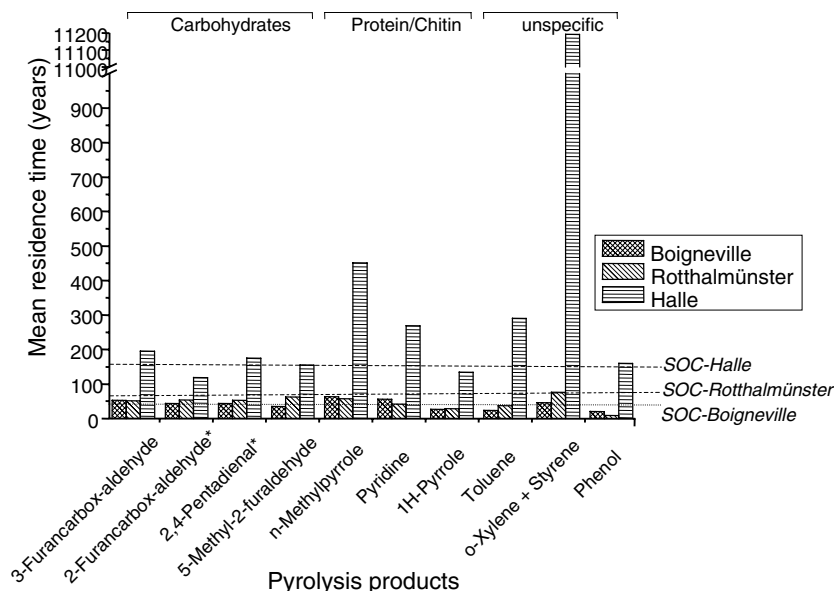


Figure 11: Mean residence times of pyrolysis products of SOM from three long-term agricultural field experiments, fertilized soils. Calculations of MRT are based on component-specific $^{12}\text{C} : ^{13}\text{C}$ ratios after conversion from C3 to C4 plant crops (Balesdent and Mariotti, 1996; Gleixner et al., 2002). Compounds marked with * were not separated by gas chromatography in Boigneville.

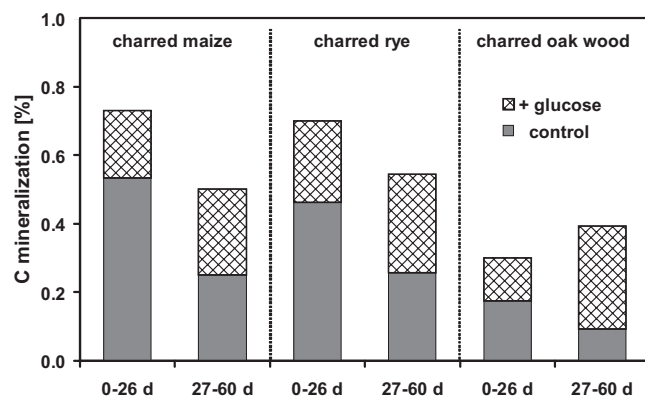


Figure 12: Effects of glucose additions at day 0 and day 26 on the mineralization of charred maize, rye, and oak wood during incubation in quartz sand (data modified from Hamer et al. [2004]).

residence times since they are based on an incubation experiment of only 60 d where probably more easily degradable moieties of BC were degraded preferentially.

The glucose additions greatly enhanced BC mineralization, especially during the second incubation period, when the relative increase compared to the control exceeded 100% in all samples. In total, the two glucose additions accelerated black-C mineralization by 58%, 72%, and 115% for charred maize, rye, and wood, respectively. Since priming effects were more pronounced after the second glucose addition, this indicates that the pool of degradable pyrogenic compounds was not depleted during the first phase of incubation, and we suggest that a more adapted microbial population had been established in the glucose-amended samples to account for this additional black-C mineralization.

As in the short-term experiment conducted by Hamer et al. (2004), glucose additions increased mineralization of black C also in the long-term experiment (Brodowski, unpublished). Additionally, the nonsterile treatments lost more black C than the treatments incubated in a chloroform-saturated atmosphere. These findings supported the hypothesis that especially microorganisms are responsible for degradation of black C in soil and suggest that the enhanced growth of microbial biomass and the resulting increased enzyme production as a response to glucose additions accelerated degradation of black C. This agrees with results from Wengel et al. (2006) as well as Willmann and Fakoussa (1997) who showed that certain fungi can degrade black C if an easily degradable C source is available, and with Rethemeyer et al. (2004a), who demonstrated incorporation of fossil C in PLFAs in Halle. However, Cheng et al. (2006) pointed out that abiotic oxidation processes on black-C surfaces may also occur in soils. They assume that this reduces surface hydrophobicity and thus may consequently enhance microbial degradation.

In the long-term incubation experiment, black-C degradation in the pure-quartz sand system was among the highest. Possibly, when black C is the only available substrate, a microbial consortium capable of black-C degradation was able to develop without competition from other microorganisms. Addi-

tionally, an increased accessibility of black C to microorganisms is also likely to be relevant, since quartz sand does not provide surfaces susceptible to sorptive interactions with particles of black C (Brodowski et al., 2005a) or allow the formation of aggregates, where black C may be protected from microbial attack (Brodowski et al., 2005a, 2006). Hence, the overall stability of black C seems to be controlled by its chemical recalcitrance, by interactions with other soil-inherent compounds, and by physical stabilization, i.e., the sole inclusion within (micro-)aggregates.

In this long-term experiment, the degradation of black C mainly occurred within the first 6 months of incubation (data not shown). The large BC losses of >20% may be due to the use of weakly charred material and incubation under optimum conditions for microbial growth and hence likely overestimates that of the field. Still, also data from chemical oxidations with 0.333 M KMnO_4 for 1 h (Brodowski, unpublished) and the black-C losses upon incubation render the assignment of black C to one single stable C pool questionable.

5 Conclusions

Our results and other published data have shown that various methodological approaches allow for the isolation of SOM fractions and individual compounds of different stability, characterized by their ^{14}C content or by turnover rates determined in natural- ^{13}C -abundance studies or incubation experiments. Generally, SOM that is not or only weakly associated with soil minerals and therefore not stabilized by physical or chemical interactions had the highest turnover rates. This is the case for free light-fraction OM, dissolved and water-extractable OM fraction, but to a certain extent also to Na-pyrophosphate-extractable OM and for SOM found in the sand-sized fraction. Compared to the bulk soil or to other fractions, these fractions contained much younger SOM with mean residence times in the order of years or decades.

The investigated biogenic compounds lignin and lipids were apparently not selectively preserved during SOM degradation since they had similar turnover rates as bulk SOM. In fact, among the SOM not protected by physicochemical interactions, we did not find any class of compounds being more stable than the bulk SOM pool. Surprisingly, a fraction of the carbohydrates and peptides present in DOM appeared not easily degradable, possibly due to microbial resynthesis, and such compounds were also found in other stable SOM fractions.

Black C was the only SOM fraction with long mean residence times of up to and more than a century. Apparent long residence times up to several millennia, calculated for some fractions of Halle SOM, do not reflect the residence time of the *in situ*-formed C compounds, but instead a substantial input of ^{14}C -free OM with a C_3 signature derived from lignite over the last century. If present in soils at higher concentrations, such contaminations can strongly bias the apparent turnover rates of plant-derived SOM to lower values. Charred plant materials can contain considerable amounts of degradable components so that they probably consist of a continuum of relatively low to high recalcitrance. This also seemed to be true for fossil C from lignite mining, as evidenced by the presence of

old C in bacterial PLFAs which also indicates the ability of certain microbes to degrade presumably nondegradable organic matter. In addition to the inherent recalcitrance of these compounds, they may be further stabilized through surface interactions with soil constituents.

Many examples showed that the degradability of SOM, SOM fractions, or specific compounds was greatly increased if easily degradable substrates, such as carbohydrates or amino acids, were supplied to the soil biota. Since these priming effects were most pronounced in SOM of low degradability (bulk Podzol BS, Podzol BS silt fraction, charred wood), the apparent recalcitrance was at least partly caused by substrate limitations of the soil biota and not by physicochemical SOM properties.

In summary, these results do not provide much evidence that the selective preservation of recalcitrant biogenic compounds is a major mechanism for the stabilization of SOM against microbial degradation. Old SOM with low turnover rates was generally only found in association with soil minerals. The only not mineral-associated SOM components that were persistent in soils appear to be black and fossil C. However, the calculated degradation of black C only takes decades to a few centuries. Therefore, the often reported very low turnover rates on the millenium scale for certain SOM fractions and subsoil SOM are most likely not due to selective preservation of primary biogenic compounds or of black C but due to other stabilization mechanisms.

The recalcitrance of pedogenic neoformations, *i.e.*, humic substances *in sensu strictu* was not explicitly investigated within these studies. But due to the low selectivity of the applied fractionation schemes, humic substances were not excluded. All physical and chemical SOM fractions containing free or easily extractable SOM were either much younger or showed higher turnover rates than the bulk soil or other fractions. We therefore conclude that the largest part of SOM that is not stabilized by physical or chemical interactions with other organic or mineral soil constituents is not recalcitrant and therefore does not contribute to the stable SOM pool.

Until today, the fractionation schemes for the separation of different SOM fractions are rather crude and not very selective. The analytical tools are so far also limited to the identification and quantification of only a few compounds. New methodological approaches are therefore needed to isolate and analyze more known and unknown compounds within SOM. New analytical possibilities for the compound-specific isotope analysis such as pyrolysis-GC/MS-IRMS or ultrahigh-resolution mass-spectral analysis (Hockaday et al., 2006) combined with chemical and physical fractionations may be promising tools to identify the more recalcitrant and the easily degradable components of SOM.

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