Lehrstuhl für Bodenkunde
Technische Universität München

# Characterization of refractory soil organic matter in long-term agroecosystem experiments

**Rita Kiem**

Vollständiger Abdruck der von der Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt der Technischen Universität München zur Erlangung des akademischen Grades eines

Doktors der Naturwissenschaften (Dr. rer. nat.)

genehmigten Dissertation.

| | | |
|---|---|---|
| Vorsitzender: | | Univ.-Prof. Dr. Urs Schmidhalter |
| Prüfer der Dissertation: | | |
| | 1. | Univ.-Prof. Dr. Ingrid Kögel-Knabner |
| | 2. | Univ.-Prof. Dr. Martin H. Gerzabek,<br>Universität für Bodenkultur Wien /<br>Österreich |

Die Dissertation wurde am 19.12.2001 bei der Technischen Universität München eingereicht und durch die Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt am 21.02.2002 angenommen.

Der Gras hervorsprossen läßt für das Vieh
und Pflanzen zum Dienst des Menschen,
damit er Brot hervorbringe aus der Erde ...
Wie zahlreich sind deine Werke, o Herr!
Du hast sie alle mit Weisheit gemacht,
die Erde ist voll deines Eigentums ...
Du sendest deinen Lebenshauch aus: Sie werden geschaffen,
du erneuerst die Flächen des Ackers.

(Aus Psalm 104)

# 1 Introduction

## 1.1 Soil organic carbon (SOC) and its refractory compartment

Soils are an important compartment in the global C cycle, since firstly, they represent a major storage pool of organic carbon, and, secondly, the SOC pool is connected with other terrestrial and the atmospheric pool through large annual fluxes (Schlesinger, 1991). Estimates of the C amount stored in soils range between 1200 and 1500 Gt C (Schlesinger, 1991; Trumbore, 1997). About one tenth of the wordwide SOC stock is contained in arable soils (Flach et al., 1997). The role of soils as a C sink nowadays has received attention with regard to the problem of „global change“, i.e. the continuously increasing levels of atmospheric $CO_2$ due to anthropogenic activity, this increase presumably resulting in a global warming. One point in the current discussion is that the C-balance of cultivated soils can be controlled by agronomic practices (e.g. crop rotation, fertilization regime, tillage systems). Many data from long-term field experiments document that management practices have an impact on the dynamics of SOC contents, i.e.whether these soils are a net sink or source for $CO_2$.

In the literature, the bulk of SOC has been divided into „kinetic“ pools, taking into account that organic compounds of varying turnover time are found in soil. In this work, two contrasting pools will be distinguished:

- A *labile* SOC compartment with a turnover time (or mean residence time) of years to several decades; this pool may comprise both an „active“ fraction with very rapid turnover (several years) and part of an „intermediate“ fraction (turnover time of one to several decades), which are defined separately in some studies (see Trumbore, 1997). The labile pool in this work may be identical with the „fast-cycling“ C pool defined by Trumbore (1997).
- A *refractory,* very stable compartment for compounds having a turnover time from hundreds to thousands of years.

Gobal estimates for the fast-cycling C fraction range from 250 to 530 Gt C (Trumbore, 1997). Comparing this value with the total SOC stock, it is obvious that the major part of OC stored in soils is assignable to the refractory pool. Estimates for the size of the refractory C in a particular soil were based on various methods and vary widely (from 15 to 60% of total SOC) (Falloon and Smith, 2000). Within the global C cycle, the refractory SOC is relevant as a C reservoir that is not subject to short/mid-term fluctuations. According to the great turnover times, the refractory (or „passive“) C should not respond to disturbance following changes in land-use (e.g. cultivation of a native site), agronomic practices in arable soils, or a possible climate change at a time-scale of decades (Trumbore, 1997). By contrast, labile C fractions should quickly respond when the C-input/output-balance of a soil is altered in a certain manner.

## 1.2 Stabilization mechanisms for SOC

Stabilization may be defined as the sum of processes/properties that confer biological stability to organic compounds in soil, i.e. resistance against rapid decomposition (Baldock and Skjemstad, 2000). The latter authors use the term „decomposition“ in a broad sense, including the pathways of „structural alteration“ of a compound, „assimilation“ (incorporation of the C into tissues of decomposer organisms), and „mineralization“ (loss of the C from soil in form of $CO_2$). Sollins et al. (1996), in a more strict sense, consider stabilization as a decrease of the potential for SOC loss by mineralization/respiration. There are a variety of mechanisms which retain OC in soil, these mechanisms being effective at different time-scales. According to Sollins et al. (1996), stabilization mechanisms for SOC can be classified in the following way:

### *I. Biochemical recalcitrance*

In this case, stability of organic compounds arises from their specific molecular characteristics (e.g. elemental composition, chemical structure, conformation), due to which the compounds are not readily attacked by microbes/enzymes. A recalcitrant nature has been ascribed, for example, to the following compounds/compound classes: the alkyl C fraction in plant and microbial tissues (Baldock et al., 1992); melanins as synthesized by soil microbes (Sollins et al., 1996); lignin in plant tissues, although Baldock et al. (1992) claim that alkyl C is more recalcitrant; the so-called „black carbon“, i.e. carbonaceous residues produced by incomplete combustion of fossil fuels or plant biomass (Schmidt and Noack, 2000).

### *II. Interaction of OC with the inorganic phase / or with other organic compounds*

„Interaction“ here is used in a broad sense, including the different types of interaction which were proven / or which are hypothesized to protect SOC against decomposition.

### *II a. Interaction with inorganic compounds*

Many studies using various approaches have provided evidence that the interaction of OC with the *surface of mineral particles* is an important mechanism stabilizing OC in soils and sediments. For example, model incubation experiments, as a direct approach to test the influence of minerals on the decomposition of organic compounds, showed a reduced mineralization of substrates in the presence of defined minerals (Sollins et al., 1996). In another approach, OC contents accumulated in soils and sediments were related to mineral surface area (Mayer, 1994); the observed positive correlation between these variables indicated a C stabilization mechanism based on interaction with minerals. Evaluation of data from bare fallows by Rühlmann (1999) indicated that the interaction of OC with minerals within the fine particle-size separates - showing the highest specific surface area - is a relevant mechanism for the long-term storage of SOC. The interaction of organics with the surface of minerals has been denoted as „chemical stabilization“ by Christensen (1996). This is in contrast to the „physical protection“ due to soil structure, which is based on physical effects (physical barriers) (see below).

It is often supposed that this association occurs mainly by „sorption“ of OC onto mineral surfaces (Sollins et al., 1996; concept of „sorptive preservation of OC“ in marine sediments, Keil et al., 1994). A sorption would involve a transfer of the soluble organics from the solution to the solid (mineral) phase. In soils, however, ways of interactions between OC and minerals other than sorption (in a strict sense) may exist and play a role. There are several hypotheses regarding the mechanisms which lead to a preservation of mineral-associated OM. One concept is that enzymes are inhibited in their activity, as the close association of the substrate with mineral surfaces may lead to sterical limitations in the enzyme-substrate interaction (Hedges and Keil, 1995). Since most of the mineral surface area in soils and sediments was found to be present in pores < 8 nm in diameter, Mayer (1994) developed the hypothesis that organic matter is localized within such small intraparticle pores, which can not be entered by enzymes (enzyme size exclusion hypothesis). Another possibility of isolating organic matter from decomposing organisms / enyzmes is the entrapment within the interlamellar space of layer silicates (Theng et al., 1992). However, this phenomenon seems to play a substantial role for OC preservation only in soils with strongly acidic pH and smectitic clay mineralogy.

The importance of an interaction with *polyvalent cations* (mainly $Ca^{2+}$, $Al^{3+}$, $Fe^{3+}$), resulting in the formation of salts/complexes, as a protection mechanism is pointed out by Sollins et al. (1996) and Baldock and Skjemstad (2000). The addition of these cations caused a decrease of mineralization rates for organic substrates in model experiments, in the absence of minerals. This mechanism is believed to be important, e.g., for polysaccharides (Tisdall, 1996).

### II b. Interaction with other organic compounds

It is believed that physical association of potentially labile organic compounds with more *refractory/recalcitrant compounds* results in a stabilization of the former. Entrapment of peptide-material within the network of hydrophobic, recalcitrant biopolymers was proposed by Knicker and Hatcher (1997) to explain the survival of labile peptides in a 4000 year old algal sapropel. Knicker et al. (2000) suggested that an analogous mechanism could play a role for the stabilization of peptides in the clay of a Podzol, which was estimated to have a high content of paraffinic structures. The interaction of labile compounds (e.g. proteins) with polyphenols such as tannins may also result in a reduced availability towards decomposing organisms (Oades, 1988).

### III. Reduced accessibility

Organic matter in soil can be protected against decomposition through physical isolation from organisms provided by the three-dimensional structure of the soil matrix (concept of „physical protection“). The capacity of a soil to stabilize OC by this mechanism depends on its structural properties, such as the degree of aggregation of primary particles, a possible hierarchical build-up of aggregates, and the stability/dynamics of soil structure. Conceptually, two ways of isolation through soil structure can be distinguished (Baldock and Skjemstad, 2000): i) Pore diameters are too small, so that microbes or soil animals can not enter the pores and reach the potential substrates (size exclusion); ii) OM is intimately associated with/coated by soil particles, it is „encapsulated“ within an aggregate structure. According to Baldock and Skjemstad (2000), this encapsulation may take place at different size-scales, ranging from nanometres (occlusion of OM by flocculation of clay particles) to centimetres (occlusion of plant debris within macro-aggregates). For particulate plant debris, the interaction with the soil matrix and its consequences for the turnover of these components have been studied extensively (e.g. Oades and Waters, 1991; Golchin et al., 1994; Jastrow and Miller, 1998). Physical protection by the soil structure is generally considered to result in a medium-term stabilization. Particulate organic matter occluded in aggregates shows turnover times in the range of several decades (Cambardella, 1998). Physically protected OM is thought to correspond to the conceptual pool of „intermediate“ SOM which is described in some SOM models (Elliott et al., 1996).

## 1.3 Objectives

The objective of this work is to characterize the refractory SOC pool in arable soils. Thereby, the following aspects/questions will be considered:

i) Distribution of the refractory SOC between particle-size fractions.

Previous studies have shown that fractionation of soils / soil organic matter (SOM) according to particle-size is suitable for isolating OM-fractions with distinctly different turnover properties. This fractionation procedure will be adopted in the present work in order to separate the refractory C from more labile C pools in the samples.

ii) Structural composition of refractory SOC.

Compositional characteristics of refractory C will be examined by analyzing the gross composition of OC ($^{13}$C NMR spectroscopy), by focusing on two different compound classes which are quantitatively important for SOM, i.e. lignin and polysaccharides, and by applying techniques from coal petrography to evaluate the contribution of airborne contaminants (e.g. coal, „black carbon“). It appeared important to include the last aspect into the present work, since most of the study sites are localized in industrialized regions.

iii) Elucidation of long-term stabilization mechanisms for OC.

Among the main stabilization mechanisms outlined before, the interaction of OC with mineral surfaces is thought to guarantee a stabilization at the long-term, and, thus, the present work will focus on this mechanism. The importance of organo-mineral interactions for C stabilization in a soil will be assessed mainly from the relationship between OC contents and mineral surface area across the particle-size separates; thereby, the surface area of the total mineral matrix and of defined fractions (silicates and oxides) will be considered.

Chemical recalcitrance is another mechanism leading to the stability of organic compounds in soil. Airborne contaminants such as coal and combusted particles are generally considered to be recalcitrant. This work will reveal the quantitative importance of such recalcitrant compounds for the refractory SOC pool.

## 1.4 Experimental and methodological approach

### 1.4.1 Experimental approach

Soil samples were obtained from eight long-term agroecosystem experiments, located in Germany, Poland, and the Czech Republic. A detailed overview of soil properties and other characteristics of the study sites is given in Table 1 of chapter 4. In agroecosystem experiments usually a variety of different fertilization/management practices are established, resulting in different SOC levels in the various experimental plots over the long-term. Moreover, the plots, depending on their SOC level, differ in the relative contribution of labile versus refractory compounds to the total SOC pool: In plots with higher SOC levels (resulting from higher organic inputs) labile C makes up a higher proportion of total SOC (Janzen et al., 1997).

The experimental approach here is the comparative study of SOC in two contrasting treatment types from the long-term experiments, i.e. „C-depleted plots“ (unmanured plots and bare fallows) and „fertilized plots“ (plots with the highest input of fertilizers at a site, receiving a combination of mineral and organic fertilizers). Given the lower amounts of organic input compared with the fertilized plots (unmanured plots) or the missing input (bare fallows), the „C-depleted plots“ are depleted in total SOC relative to the fertilized counterparts. This depletion can mainly be explained by a lower amount of labile SOC. In contrast to the labile C, the size of the refractory C pool should not be affected by the type of soil management at a decadal scale. As a consequence of this different behavior of the two pools, the SOC of the C-depleted plots results to be relatively enriched in refractory compounds (for a schematic illustration of this concept see Fig. 1 in chapter 2). The „labile“ C pool, as defined in this study, may include compounds of the „intermediate“ fraction (turnover time up to several decades). Except for two experiments, the duration of the long-term experiments in this study ranges from 30 to nearly 100 years (see Table 1, chapter 4). Therefore, it is considered that intermediate compounds have largely been turned over and do not accumulate within the C-depleted plots.

### 1.4.2 Methods

#### *1.4.2.1 Physical fractionation techniques (particle-size fractionation and density fractionation for selected size separates)*

Particle-size fractionation involved i) ultrasonic dispersion by a two-step method (60 J ml$^{-1}$ followed by 440 J ml$^{-1}$), and ii) isolation of the particle-size separates by combining different methods: wet sieving (for fractions 2000-20 µm), hydrodynamic sorting of particles by the SPLITT-system (for fractions 20-0.2 µm), and centrifugation (for the fraction < 0.2 µm).

Applying 440 J ml$^{-1}$ in the second sonication step should result in a complete dispersion of aggregates, to obtain „primary organo-mineral complexes“. The analysis of these primary particles should elucidate the effect of mineral surfaces on C stabilization. As far as the author is aware, this is the first time that SPLITT-fractionation is applied to soil samples. The applicability of this new fractionation technique for soils will be evaluated.

Selected particle-size separates were further fractionated on the basis of density: i) For separates 2000-20 µm, density fractionation allowed the OM to be concentrated within the light fraction (≤ 1.8 or 1.9 g $cm^{-3}$). This was done as a pretreatment prior to coal petrographic analysis and $^{13}C$ NMR spectroscopy. ii) In separates < 6 µm, the average OC loading of mineral surfaces was determined for the fraction > 2 g $cm^{-3}$, after removing the light material (≤ 2 g $cm^{-3}$).

#### *1.4.2.2 Techniques for characterizing SOC in bulk soils and particle-size fractions*

##### *1.4.2.2.1 Elemental analysis (organic carbon and total nitrogen)*

##### *1.4.2.2.2 Solid-state $^{13}C$ NMR (nuclear magnetic resonance) spectroscopy*

Solid-state NMR spectra were obtained by using the cross polarization technique with variable amplitude of the $^{1}H$-excitation pulse (VA CP = variable amplitude cross polarization). The samples were measured after enrichment of the OC, either by treatment with 10% HF (bulk samples and separates < 63 µm) or by concentration of OC within the light fraction (separates 2000-63 µm). Apart from conventional CP experiments, measurements using the dipolar dephasing (DD) technique were performed for selected separates to obtain specific information on the structure of aryl C.

##### *1.4.2.2.3 Biochemical compound classes*

a) Lignin

The amount of lignin and its degree of oxidative alteration were assessed from the phenol monomers (vanillyl, syringyl, cinnamyl units) released by alkaline CuO oxidation.

b) Cellulosic and non-cellulosic polysaccharides

Polysaccharides were investigated by two different methods: i) The amount of non-cellulosic and cellulosic polysaccharides was determined by a two-fold hydrolysis procedure: hydrolysis of non-cellulosic sugars with 1 M HCl, and of total sugars with 12 M + 1 M $H_2SO_4$; (cellulose calculated by difference: total sugars - non-cellulosic sugars); colorimetric determination of sugar monomers by reaction with MBTH („MBTH-method“). ii) The composition of non-cellulosic polysaccharides was investigated by hydrolysis with TFA (trifluoroacetic acid) followed by gas chromatographic analysis of sugar monomers („method for analysis of individual sugars“).

#### *1.4.2.2.4 Coal petrographic analysis*

The coal petrographic examination for separates 2000-20 µm of the Thyrow and Bad Lauchstädt soils was carried out by Dr. Bertrand Ligouis at the University of Tübingen (Institut für Geologie und Paläontologie). High contributions of aromatic C in $^{13}$C NMR spectra of these samples pointed towards a possible presence of airborne carbonaceous particles. This analysis included: separation of the organic matter from minerals by density fractionation (density cutoff 1.9 g cm$^{-3}$), embedding of the OM in expoxy resin, light microscopic analysis (reflected white light and fluorescence mode).

### ***1.4.2.3 Techniques for characterizing the mineral matrix in particle-size fractions < 6 µm***

#### *1.4.2.3.1 Specific surface area of minerals*

Surface area was determined by sorption of $N_2$ to mineral surfaces after the OM had been removed from the separates by oxidation (data evaluation by the BET approach). For two sites (Thyrow, Skierniewice), a differentiation between the surface area contributed by oxides and silicates was obtained, by treating the separates with dithionite for a selective removal of the oxides (difference approach: decrease of surface area caused by dithionite-treatment was attributed to the oxides).

#### *1.4.2.3.2 Iron oxides*

The content of total iron oxides was determined by extraction with dithionite-citrate-bicarbonate ($Fe_d$), the content of poorly crystalline oxides (mainly ferrihydrite) by extraction with oxalate ($Fe_o$).

#### *1.4.2.3.3 Composition of silicates*

For a better understanding of the surface area data, the composition of the silicate fraction in clay samples was investigated by means of x-ray diffraction analysis (Thyrow, Skierniewice).

## 1.5 References

Baldock, J.A., Skjemstad, J.O., 2000. Role of the mineral matrix and minerals in protecting natural organic materials against decomposition. Organic Geochemistry 31, 697-710.

Baldock, J.A., Oades, J.M., Waters, A.G., Peng, X., Vassallo, A.M., Wilson, M.A., 1992. Aspects of the chemical structure of soil organic materials as revealed by solid-state $^{13}C$ NMR spectroscopy. Biogeochemistry 16, 1-42.

Cambardella, C.A., 1998. Experimental verification of simulated soil organic matter pools. In Lal, R., Kimble, J.M., Follett, R.F., Stewart, B.A. (Eds.), Soil Processes and the Carbon Cycle. Advances in Soil Science. CRC Press, Boca Raton. pp. 519-526.

Christensen, B.T., 1996. Carbon in primary and secondary organomineral complexes. In: Carter, M.R., Stewart, B.A. (Eds.), Structure and Organic Matter Storage in Agricultural Soils. Advances in Soil Science. CRC Press, Boca Raton. pp. 97-165.

Elliott, E.T., Paustian, K., Frey, S.D., 1996. Modeling the measurable or measuring the modelable: a hierarchical approach to isolating meaningful soil organic matter fractionations. In: Powlson, D.S., Smith, P., Smith, J.U. (Eds.), Evaluation of Soil Organic Matter Models Using Existing Long-term Datasets, NATO ASI Series. Springer Verlag, Berlin. pp. 161-179.

Flach, K.W., Barnwell, Jr., T.O., Crosson, P., 1997. Impacts of agriculture on atmospheric carbon dioxide. In: Paul, E.A., Elliott, E.T., Paustian, K., Cole, C.V. (Eds), Soil Organic Matter in Temperate Agroecosystems - Long-term Experiments in North America. CRC Press, Boca Raton. pp. 3-13.

Golchin, A., Oades, J.M., Skjemstad, J.O., Clark, P., 1994. Study of free and occluded particulate organic matter in soils by solid state $^{13}C$ CP/MAS NMR spectroscopy and scanning electron microscope. Australian Journal of Soil Research 32, 285-309.

Hedges, J.I., Keil, R.G., 1995. Sedimentary organic matter preservation: an assessment and speculative synthesis. Marine Chemistry 49, 81-115.

Janzen, H.H., Campbell, C.A., Ellert, B.H., Bremer, E., 1997. Soil organic matter dynamics and their relationship to soil quality. In: Gregorich, E.G., Carter, M.R. (Eds.), Soil Quality for Crop Production and Ecosystem Health. Developments in Soil Science vol. 25. Elsevier, pp. 277-291.

Jastrow, J.D., Miller, R.M., 1998. Soil aggregate stabilization and carbon sequestration: feedbacks through organomineral associations. In: Lal, R., Kimble, J.M., Follett, R.F., Stewart, B.A. (Eds.), Soil Processes and the Carbon Cycle. Advances in Soil Science. CRC Press, Boca Raton. pp. 207-223.

Keil, R.G., Montiucon, D.B., Prahl, F.G., Hedges, J.I., 1994. Sorptive preservation of labile organic matter in marine sediments. Nature 370, 549-552.

Knicker, H., Hatcher, P.G., 1997. Survival of protein in an organic-rich sediment: possible protection by encapsulation in organic matter. Naturwissenschaften 84, 231-234.

Knicker, H., Schmidt, M.W.I., Kögel-Knabner, I., 2000. Nature of organic nitrogen in fine particle size separates of sandy soils of highly industrialized areas as revealed by NMR spectroscopy. Soil Biology & Biochemistry 32, 241-252.

Mayer, L.M., 1994. Relationship between mineral surfaces and organic carbon concentrations in soils and sediments. Chemical Geology 114, 347-363.

Oades, J.M., 1988. The retention of organic matter in soils. Biogeochemistry 5, 35-70.

Oades, J.M., Waters, A.G., 1991. Aggregate hierarchy in soils. Australian Journal of Soil Research 29, 815-828.

Rühlmann, J., 1999. A new approach to estimating the pool of stable organic matter in soil using data from long-term field experiments. Plant and Soil 213, 149-160.

Schlesinger, W.H., 1991. Biogeochemistry - An Analysis of Global Change. Academic Press, San Diego.

Schmidt, M.W.I., Noack, A.G., 2000. Black carbon in soils and sediments: Analysis, distribution, implications, and current challenges. Global Biogeochemical Cycles 14, 777-793.

Sollins, P., Homann, P., Caldwell, B.A., 1996. Stabilization and destabilization of soil organic matter: mechanisms and controls. Geoderma 74, 65-105.

Theng, B.K.G., Tate, K.R., Becker-Heidmann, P., 1992. Towards establishing the age, location, and identity of the inert soil organic matter of a Spodosol. Zeitschrift für Pflanzenernährung und Bodenkunde 155, 181-184.

Tisdall, J.M., 1996. Formation of soil aggregates and accumulation of soil organic matter. In: Carter, M.R., Stewart, B.A. (Eds.), Structure and Organic Matter Storage in Agricultural Soils. Advances in Soil Science. CRC Press, Boca Raton. pp. 57-96.

Trumbore, S.E., 1997. Potential response of soil organic carbon to global environmental change. Proceedings of the National Academy of Science 94, 8284-8291.

# 2 Distribution of refractory OC between particle-size fractions

## Abstract

The aim of this section is to assess the storage of refractory compounds across particle-size separates. The contents and distribution of organic carbon (OC) and nitrogen (N) in size fractions were examined for C-depleted and fertilized plots from three long-term agroecosystem experiments. In two of the long-term experiments, the relative retention of OC in separates < 20 µm was considerably higher than in separates 2000-20 µm (OC contents in depleted plots compared with fertilized plots). Highest residual contents of OC were found in fractions < 6 µm. In the third experiment, additionally to the very fine fractions, separates 250-20 µm retained a high proportion of OC. The behavior of N was analogous to that of OC: the highest relative residues in the depleted plots were found in fine separates. These results indicate that in the investigated arable soils, C and N compounds associated with fine separates are most stable. Refractory OC in arable soils may be largely stored in fine particle-size fractions.

## 2.1 Introduction

The turnover time (or mean residence time) of organic compounds in soil ranges from several years to millenia. From this range at least three pools of soil organic carbon (SOC) are derived. Each is characterized by an average turnover time, which increases from the "active"/"labile" pool (several years) to the "intermediate" pool (years to decades) to the "passive"/"refractory" compartment of organic carbon (hundreds to thousands of years) (Elliott et al., 1996; Parton et al., 1987). The division of SOC into different kinetic pools – roughly into a *labile*, fast-cycling and a *refractory* pool – is essential for modelling the dynamics of the bulk SOC or SOM (soil organic matter) for a given site/area. Based on the large difference in turnover time between the labile and the refractory pool, these two pools show a different temporal response to changes in land-use, soil management or climate. In contrast to the labile C pool, which is affected by variations of the mentioned factors within years/decades, the refractory C shows no short- to medium-term response (Christensen, 1996; Falloon and Smith, 2000). Consequently, at a global scale, the refractory SOC pool is important for the role of soils as a long-term $CO_2$ sink (Trumbore, 1997).

To separate and characterize refractory C compounds from soil, several workers used chemical fractionation approaches, as reviewed by Falloon and Smith (2000). Residues after acid hydrolysis were often found to be older than the bulk OC, indicating that non-hydrolyzable OC compounds represent a stable fraction of SOC. Augris et al. (1998) attempted to identify refractory compounds within the non-hydrolyzable, insoluble organic matter residue of a forest soil. Accordingly, OC compounds resistant to chemical degradation are thought to be also resistant against biological attack. On the other hand, physical fractionation of soils according to particle-size has been shown to yield SOM pools with different properties regarding chemical composition and turnover (Christensen, 1996). The characterization of the chemical structure and the nature of SOM in the range of size fractions suggested an SOM continuum: it ranges from recent plant residues in the sand, through partially degraded residues in the silt, to highly processed organic material, presumably dominated by microbially-derived compounds, in the clay (Baldock et al., 1992; Guggenberger et al., 1995; Schulten and Leinweber, 2000). Assessing the dynamics of OC associated with different size separates by various approaches (e.g. natural $^{13}$C abundance technique, mineralization experiments) showed that OC in silt and clay is generally turned over more slowly than the C in sand fractions (e.g. Christensen, 1996; Feller and Beare, 1997; Shang and Tiessen, 1997). However, comparing silt- and clay-associated OC, several authors reported a higher stability for silt-OC (Anderson and Paul, 1984; Christensen, 1996) and suggested a possible accumulation of recalcitrant compounds within fractions 50/20-2 µm. Other workers found OC in the clay to be the most stable C (Feller and Beare, 1997). As particle-size fractionation basically separates compounds having distinctly different turnover properties, this approach seems to be suitable to separate and identify refractory components of SOC.

The present study compares soils from two different treatment types in long-term agroecosystem experiments (Fig. 1): *fertilized* plots, receiving both mineral fertilizer and farmyard manure, and plots *depleted* in OC as compared to the fertilized ones (“C-depleted plots”). The latter group encompasses i) plots with the same crop rotation as the fertilized soils, but not receiving any kind of fertilizers (unmanured plots), and ii) bare fallows. In the bare fallow, there is no OM-input into the soil, whereas in the unmanured plots the input is much reduced compared with the fertilized ones, owing to the lack of farmyard manure and reduced crop productivity. As shown schematically in Fig. 1, under the management conditions of the depleted plots the amount of SOC declines over the course of several decades compared with the level in the fertilized counterparts. At the decadal scale, changes in SOC in response to management practices are mainly due to changes in the labile C pool, whereas the refractory pool should not be affected (Rühlmann, 1999). Due to the preservation of refractory compounds, the SOC pool of the depleted plots should be relatively enriched in these compounds compared with the fertilized counterparts.

The aim of this paper is to characterize the distribution of refractory OC among particle-size fractions in arable soils by focusing on C-depleted soils from long-term experiments and comparing them with the respective fertilized counterparts.

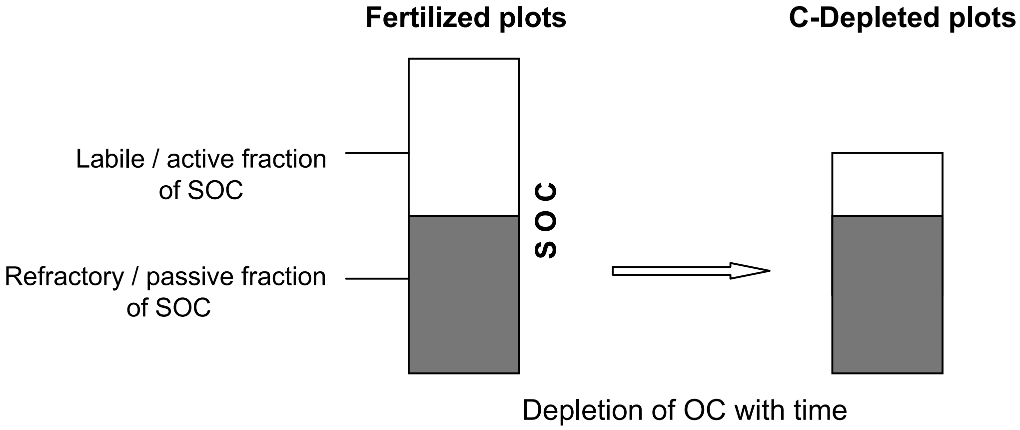


**Fig. 1:** Behavior of the labile and refractory SOC pools in the course of C-depletion when comparing fertilized plots with C-depleted plots from long-term agroecosystem experiments.

## 2.2 Materials and methods

### 2.2.1 Long-term experimental sites and soil sampling

Soil samples were obtained from three European long-term agroecosystem experiments running for at least 40 years (Table 1). The sites are similar with respect to climatic features, having a long-term mean annual temperature of 7.9 to 8.6 °C and a mean annual precipitation ranging from 490 to 527 mm. The soils from Thyrow and Skierniewice are classified as Luvisols and are characterized by high contents of sand (2000-63 µm). The soil from Bad Lauchstädt, a Haplic Chernozem which has developed from loess material, is dominated by silt (63-2 µm) and has a higher clay content than the soils from the other sites. In Table 1, the sites are arranged according to increasing clay contents.

At the different sites, the following types of experimental treatments were chosen:

a) *Fertilized* plots with the application of NPK, farmyard manure, and lime for the sandy soils of Thyrow and Skierniewice (treatment I in Table 1);

b) Plots *depleted* in organic matter, either unmanured plots without any fertilization/liming (treatment II) or bare fallows (treatment III at Bad Lauchstädt).

**Table 1:** Characteristics of the long-term agroecosystem experiments.

| **Site** | **MAT** [a] (°C) | **MAP** [b] (mm) | **Soil type** (FAO) | **Texture** [c] (%) | | | **Start of experiment** | **Crop rotations** | **Treatments** | **Organic carbon** ($g\ kg^{-1}$)<br>Plots | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Sand | Silt | Clay | | | | **Fertilized** | **Depleted** |
| Thyrow (Germany) [d] | 8.6 | 520 | Albic Luvisol | 82 | 15 | 3 | 1937 | Potatoes – spring barley – maize – spring barley | I) Fertilized NPK + farmyard manure (15 t $ha^{-1}$ $y^{-1}$) + lime | 6.8 | |
| | | | | | | | | | II) No fertilization/liming | | 3.2 |
| Skierniewice (Poland) [e] | 7.9 | 527 | Luvisol | 72 | 22 | 6 | 1923 | Fertilized plots (I): potatoes – spring barley – red clover – winter wheat – rye<br>Unmanured plots (II): arbitrary rotation | I) Fertilized NPK + farmyard manure (6 t $ha^{-1}$ $y^{-1}$) + lime | 8.8 | |
| | | | | | | | | | II) No fertilization/liming | | 4.4 |
| Bad Lauchstädt (Germany) [f] | 8.6 | 490 | Haplic Chernozem | 7 | 70 | 23 | 1902 | Sugarbeet – spring barley – potatoes - winter wheat | I) Fertilized NPK + farmyard manure (15 t $ha^{-1}$ $y^{-1}$) | 24 | |
| | | | | | | | | | II) No fertilization | | 16 |
| | | | | | | | 1956 | — | III) Bad fallow | | 15 |

[a] Mean annual temperature

[b] Mean annual precipitation

[c] Results of textural analysis (chemical dispersion/sedimentation): sand (2000-63 µm), silt (63-20 µm), clay (< 2 µm); mean values of the various plots from a site

[d] Ellmer et al. (2000)

[e] Mercik et al. (2000)

[f] Körschens et al. (1998)

In the bare fallow (Bad Lauchstädt), weed plants were removed manually avoiding the use of herbicides. According to Table 1, the OC level of the depleted soils is about half that of the fertilized soils at Thyrow and Skierniewice, whereas it amounts to 62 and 67% in the unmanured and bare fallow soil, respectively, at Bad Lauchstädt.

Except for the bare fallow, each treatment was replicated on several plots in the field: At Thyrow and Bad Lauchstädt, four replications were conducted, at Skierniewice three and five replications for the unmanured and fertilized treatment, respectively. In summer 1998, ten soil samples were taken (from a depth 0-20 cm) at each of the replication plots of a particular treatment. All the subsamples were thoroughly mixed afterwards. The mixed samples were air-dried and components > 2 mm were removed by dry sieving.

### 2.2.2 Particle-size fractionation (ultrasonic dispersion / SPLITT)

#### *2.2.2.1 Dispersion and fractionation in the range 2000-250 µm*

Soil samples were dispersed by the two-step procedure used by Amelung et al. (1998). Soil suspensions (with a soil-to-water ratio of 1:3 for the sandy soils from Thyrow and Skierniewice, and 1:5 for Bad Lauchstädt) were ultrasonicated at 60 J ml$^{-1}$ using a probe-type sonicator (Branson Sonifier 250, Branson) (Fig. 2). The power output was 50 W, as determined calorimetrically according to North (1976). For Thyrow and Skierniewice a more narrow soil-to-water ratio was adopted to ensure a good dispersion effiency in the presence of high sand contents (see Christensen, 1985). According to Amelung and Zech (1999), the first step of low-energy sonication is aimed at dispersing macroaggregates (> 250 µm) and releasing occluded particulate OM, which is removed - together with free particulate OM - prior to the second sonication step. This removal of coarse OM should avoid a physical disruption and subsequent redistribution of the OM, which would otherwise occur during the second high-energy sonication step. In the present study, the fraction 2000-250 µm, including particulate organic matter, was isolated by wet sieving. The suspension < 250 µm (soil-to-water ratio of 1:5 for Thyrow and Skierniewice, and 1:10 for Bad Lauchstädt) was subjected to ultrasonication a second time, applying 440 J ml$^{-1}$ for complete dispersion of microaggregates < 250 µm (Fig. 2). During this ultrasonication step the temperature of the suspension was kept at 20-30 °C using a water cooling jacket.

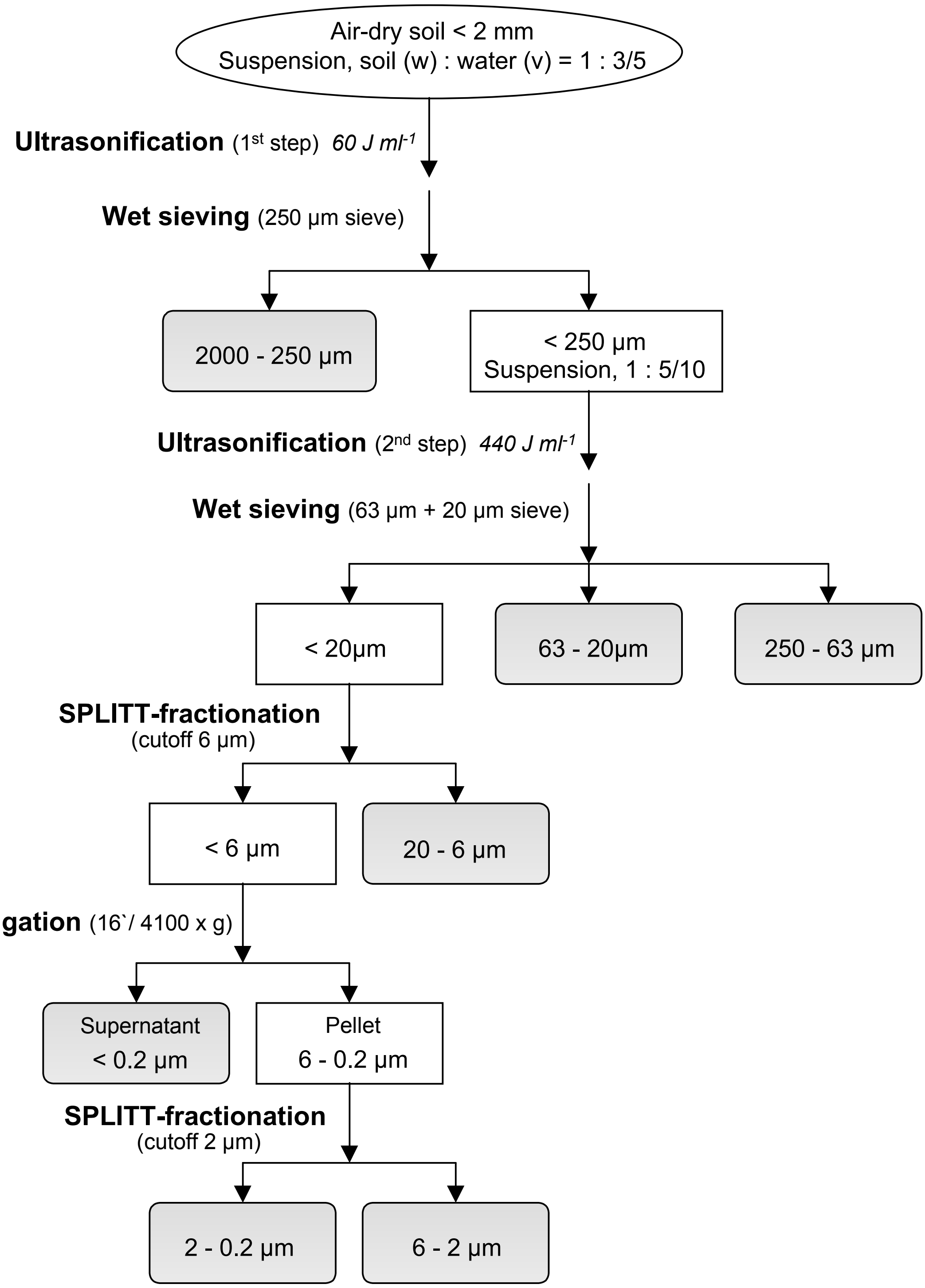


**Fig. 2:** Flow-chart for the particle-size fractionation procedure (ultrasonic dispersion/SPLITT).

### *2.2.2.2 Fractionation in the range < 250 µm*

After complete dispersion of the samples < 250 µm, they were separated into six particle-size fractions following the size limits of the German classification system (AG Boden, 1994) and adopting a combination of several techniques (Fig. 2):

### *2.2.2.2.1 Wet sieving*

Wet sieving of the suspension < 250 µm, using a 63 and 20 µm sieve to obtain the fractions 250-63 µm (fine sand) and 63-20 µm (coarse silt).

### *2.2.2.2.2 SPLITT-fractionation*

SPLITT-fractionation of the suspension < 20 µm, employing two size cutoffs, 6 and 2 µm, to gain the fractions 20-6 µm (medium silt), 6-2 µm (fine silt) and 2-0.2 µm (coarse clay).

SPLITT- or split-flow thin-cell fractionation is based on a hydrodynamically controlled settling of particles in a very thin channel. This technique has so far been used in the preparative separation of homogeneous materials, e.g. silica particles (Jiang et al., 1997) and in the fractionation of marine sediments (Keil et al., 1994). A detailed description of the fundamentals of SPLITT-fractionation is given by Giddings (1985). The dimensions of the used SPLITT cell (model SF1000HC, FFFractionation LLC, Salt Lake City) were 20 cm length, 4 cm breadth, and 380 µm thickness. Briefly, the cell has two inlets, an upper one (a`) for continuously introducing a suspension of particles, and a lower one (b`) for the flow of particle-free/deionized water (carrier). At the end of the flow channel there are two outlets, the upper (a) and lower (b) one, where the two size-separated fractions are collected. The theoretical size cutoff obtained in a SPLITT run can be calculated as a function of volumetric flow rates at the inlets and outlets ($V_{(a`,\ b`,\ a,\ b)}$ in ml min$^{-1}$), channel dimensions (length x breadth), and settling characteristics of the particles as defined by Stokes` Law (Jiang et al., 1997). As the particles are moved down the very thin channel at a certain speed (depending on the flow rates), they settle to a certain extent depending on their density, size, and shape, analogously to the sedimentation in a static field. Assuming similar density and shape, the settling behavior of particles is mainly controlled by particle-size: coarser particles should settle faster than finer ones and thus exit from the bottom outlet (b) of the cell, while the finer particles should remain in the upper zone of the cell and be collected from outlet a. In order to obtain a desired cutoff, we calculated the appropriate flow rates assuming a density of 2.65 g cm$^{-3}$ and spherical shape for the particles. The smaller the cutoff, the lower the flow rates have to be to allow sedimentation/separation of finer particles in the cell, and consequently the lower the sample throughput is. The lower separation boundary of our SPLITT cell is reached around 0.5 µm. In the range < 0.5 µm, gravitational forces are too weak to obtain a separation of particles within the cell of the used thickness (Keil et al., 1994).

The separation resolution in a SPLITT run is determined by i) the particle concentration in the suspension (above a certain particle density in the suspension, resolution decreases), and ii) the relation of flow rates, especially the ratio of $V_a$ to $V_{a'}$, which should ideally not be smaller than 1:4 or 1:3 (Jiang et al., 1997). The two SPLITT runs were performed using the following conditions: The particle concentration was adjusted to 0.8 to 1 % (w/v) in the suspension; the average flow rates in the 1st run (*cutoff 6 µm*) were 5 mL $min^{-1}$ ($V_{a'}$), 21 mL $min^{-1}$ ($V_{b'}$), 20 mL $min^{-1}$ ($V_a$) and 6 mL $min^{-1}$ ($V_b$); the flow rates in the 2nd run (*cutoff 2 µm*) were 1.1 mL $min^{-1}$ ($V_{a'}$), 3.2 mL $min^{-1}$ ($V_{b'}$), 3.0 mL $min^{-1}$ ($V_a$) and 1.3 mL $min^{-1}$ ($V_b$). These settings represented a compromise between resolution and sample throughput/analysis time.

As shown in Fig. 2, the suspension < 6 µm isolated in the 1st SPLITT run was centrifuged to remove particles < 0.2 µm (see section 2.2.2.2.3). The pellet remaining after centrifugation, representing the fraction 6-0.2 µm, was redispersed by ultrasonication (5 min at 50 W) and subjected to a 2nd SPLITT run (cutoff 2 µm). This time, NaCl was added to both the suspension and the carrier to a final concentration of 20 mM. $Na^+$ served to prevent intense flocculation of particles during the run (7-8 h). In the absence of NaCl, floccules were formed, which led to strong sedimentation in the cell and had an overall negative effect on the separation quality. A preliminary experiment revealed that the presence of 20 mM NaCl had no effect on the concentration of dissolved organic carbon (DOC) in soil suspensions < 6 µm as monitored over a period of 24 h. Thus, NaCl did not induce the extraction of organic substances. The alteration of density and viscosity brought about by 20 mM NaCl compared with deionized water should be negligible (Weast, 1969). Thus, the physical conditions of sedimentation/separation should be the same as in water. Finally, NaCl was removed from the suspensions 2-0.2 µm and 6-2 µm by repeated cycles of centrifugation and addition of deionized water, until the electric conductivity in the supernatant was < 10 µS $cm^{-1}$.

#### *2.2.2.2.3 Centrifugation*

Centrifugation of the suspension < 6 µm at 4100 x g for 16 min to gain the fraction < 0.2 µm (fine clay).

After centrifugation, the supernatant (< 0.2 µm) was saved, the residue resuspended in deionized water and centrifuged again. This was repeated until a clear supernatant was obtained after centrifugation (four times for Thyrow and Skierniewice, seven times for Bad Lauchstädt). Particles < 0.2 µm in the combined supernatants were flocculated by adding $MgCl_2$, followed by centrifugation to obtain a complete settling. After discarding the supernatant, the pellet was washed several times with water to eliminate excessive $MgCl_2$.

The combination of the procedures described in this section for particle-size fractionation, in the following, is summarized as “ultrasonic dispersion / SPLITT” method. All the isolated fractions were freeze-dried.

### 2.2.3 Textural analysis (chemical dispersion / sedimentation)

The procedure involved removal of SOM by $H_2O_2$-treatment, followed by chemical dispersion of the samples with sodium pyrophosphate ($Na_4P_2O_7$), and wet sieving plus gravitational sedimentation for determination of particle-size fractions (Gee and Bauder, 1986). After treating soil material with 10 % (v/v) $H_2O_2$ solution at 50°C for several days, the residue was washed and freeze-dried. 12.5 mM $Na_4P_2O_7$ solution was added to the freeze-dried sample at a soil-to-solution ratio of 1:20. The suspension was shaken end-over-end for 16 h. Then the suspension was passed through a 63 and 20 µm sieve, collecting the fraction < 20 µm in a 1 L cylinder. After a period of time $t$, necessary for particles of 2 and 6 µm diameter to cover a distance $h$ in the cylinder, aliquots of the suspension were taken by a pipette at the depth $h$. The settling times for 2 and 6 µm particles were calculated according to Stokes` Law. The aliquots were dried at 105°C for 24 h, together with the fractions obtained by wet sieving (2000-63 and 63-20 µm). The mass proportions of the various fractions were expressed relative to the SOM-free sample mass. The proportion of the fraction 20-6 µm was calculated by difference to 100%. This combination of dispersion and particle-size separation techniques will be denoted as “chemical dispersion / sedimentation” method.

### 2.2.4 Carbon and nitrogen analyses

C and N contents were determined by dry combustion in a Vario EL elemental analyzer (Elementar Analysen-Systeme, Hanau, Germany). Prior to elemental analysis, aliquots of bulk samples and particle-size fractions were ground in a ball mill. To determine the proportion of inorganic carbon, bulk samples were analyzed after ignition at 550°C for 3 h (removal of organic matter). Inorganic carbon was not detected in any of the samples.

For the soil samples from each treatment, the OC distribution across the size fractions was calculated, normalizing the OC amounts in the various fractions to the recovered sum of OC. Additionally, the OC amounts in the particle-size fractions of the depleted plot were expressed as a percentage of the bulk OC content of the fertilized plot for a given site:

$$\frac{\text{OC}_{\text{fraction, depleted}} \times \text{Mass}_{\text{fraction}}}{\text{OC}_{\text{bulk soil, fertilized}} \times 100} = \text{OC}_{\text{fraction, residual}} \left(\%\text{ of bulk OC}_{\text{fertilized}}\right) \quad (1)$$

| | |
|---|---|
| $\text{OC}_{\text{fraction, depleted}}$ | OC content of a given particle-size fraction in the depleted plot (g $\text{kg}^{-1}$ fraction) |
| $\text{Mass}_{\text{fraction}}$ | Mean mass yield of that fraction at the experimental site (% soil mass) |
| $\text{OC}_{\text{bulk soil, fertilized}}$ | OC content of bulk samples from the fertilized plot (g $\text{kg}^{-1}$ soil) |
| $\text{OC}_{\text{fraction, residual}}$ | "Residual" OC in a given particle-size fraction in the depleted plot (% of bulk OC of the fertilized plot) |

### 2.2.5 Statistics

Statistical analyses (*t*-test and linear regression analysis) were performed with the software SigmaPlot 4.00 (SPSS Corp.).

## 2.3 Results and discussion

### 2.3.1 Comparison of fractionation procedures: ultrasonic dispersion / SPLITT *versus* chemical dispersion / sedimentation

Table 2a shows the mass balances for the soils from the three sites obtained by the procedure of ultrasonic dispersion/SPLITT-fractionation. The results were similar for the differently managed plots at a study site with respect to the mean mass yields and the standard deviations for the different size fractions. Only the mass proportion of the fine sand (250-63 µm) in the unmanured soil from Bad Lauchstädt was found to be significantly lower than that in the fertilized counterpart (paired *t*-test). For all soils, except the fertilized soil from Skierniewice, fractionation in the range < 20 µm (SPLITT plus centrifugation) generally resulted in a higher variability than in the range 2000-20 µm (wet sieving) (see relative standard deviations in Table 2a). This is because the former fractionation techniques are more complex, involving a number of sequential steps. The total mass recovery of the procedure was highly reproducible and ranged from 92 to 97%.

**Table 2a:** Mass yields of particle-size fractions obtained by the ultrasonic dispersion/SPLITT method for the contrasting treatments from the study sites.

| | **Thyrow** | | | | **Skierniewice** | | | | **Bad Lauchstädt** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Fertilized** | | **Depleted** | | **Fertilized** | | **Depleted** | | **Fertilized** | | **Depleted** [c] | |
| **Particle-size fractions** (µm) | **Mass yields** [a] (%) | **Rel. SD** [b] (%) | **Mass yields** (%) | **Rel. SD** (%) | **Mass yields** (%) | **Rel. SD** (%) | **Mass yields** (%) | **Rel. SD** (%) | **Mass yields** (%) | **Rel. SD** (%) | **Mass yields** (%) | **Rel. SD** (%) |
| 2000 - 250 | 39 | 12 | 37 | 8 | 31 | 0 | 34 | 2 | 3.1 | 55 | 1.1 | 7 |
| 250 - 63 | 42 | 10 | 44 | 7 | 40 | 4 | 39 | 1 | 5.5 | 2 | 3.9 | 5 |
| 63 - 20 | 6.3 | 7 | 6.9 | 15 | 10 | 4 | 8.3 | 21 | 33 | 5 | 35 | 2 |
| 20 - 6 | 3.1 | 30 | 3.2 | 12 | 5.6 | 10 | 5.2 | 22 | 23 | 5 | 25 | 4 |
| 6 - 2 | 2.6 | 7 | 2.3 | 23 | 3.7 | 11 | 3.4 | 9 | 9.1 | 6 | 7.0 | 23 |
| 2 – 0.2 | 2.5 | 24 | 1.9 | 18 | 3.8 | 5 | 3.7 | 29 | 11 | 26 | 10.4 | 29 |
| < 0.2 | 0.5 | 27 | 0.7 | 14 | 1.0 | 7 | 1.1 | 19 | 9.5 | 19 | 10.4 | 25 |
| Mass recovery [d] (%) | 96 | 1 | 97 | 3 | 94 | 2 | 95 | 3 | 94 | 0 | 92 | 1 |

[a] Mean value (*n* = 3)
[b] Relative standard deviation of the mass yields (*n* = 3)
[c] Unmanured plot
[d] Mass recovered in the fractions as % of initial mass

**Table 2b:** Comparison of mass yields obtained by ultrasonic dispersion/SPLITT and chemical dispersion/sedimentation for the contrasting treatments from Thyrow and Bad Lauchstädt.

| | **Thyrow** | | | | | | **Bad Lauchstädt** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Fertilized** | | | **Depleted** | | | **Fertilized** | | | **Depleted** [d] | | |
| | **Mass yields** | | $\frac{\textbf{Meth. 1}}{\textbf{Meth. 2}}$ [c] | **Mass yields** | | $\frac{\textbf{Meth. 1}}{\textbf{Meth. 2}}$ | **Mass yields** | | $\frac{\textbf{Meth. 1}}{\textbf{Meth. 2}}$ | **Mass yields** | | $\frac{\textbf{Meth. 1}}{\textbf{Meth. 2}}$ |
| **Particle-size fractions** (μm) | Meth. 1 [a] (%) | Meth. 2 [b] (%) | | Meth. 1 (%) | Meth. 2 (%) | | Meth. 1 (%) | Meth. 2 (%) | | Meth. 1 (%) | Meth. 2 (%) | |
| 2000 - 63 | 81 | 82 | 1.0 | 81 | 82 | 1.0 | 8.6 | 9.8 | 0.9 | 5.0 | 9.8 | 0.5 |
| 63 - 20 | 6.3 | 7.6 | 0.8 | 6.8 | 7.3 | 0.9 | 33 | 33 | 1.0 | 35 | 33 | 1.0 |
| 20 – 6 [e] | 3.1 | 4.4 | 0.7 | 3.2 | 5.4 | 0.6 | 23 | 29 | 0.8 | 25 | 29 | 0.9 |
| 6 - 2 | 2.6 | 2.8 | 0.9 | 2.3 | 2.3 | 1.0 | 9.1 | 5.0 | 1.8 | 7.0 | 5.0 | 1.4 |
| < 2 [f] | 3.0 | 2.9 | 1.0 | 2.6 | 2.5 | 1.1 | 20 | 23 | 0.9 | 21 | 23 | 0.9 |

[a] Method 1: ultrasonic dispersion / SPLITT

[b] Method 2: chemical dispersion / sedimentation

[c] $\frac{\text{Yields}_{\text{Method 1}}}{\text{Yields}_{\text{Method 2}}}$

[d] Unmanured plot

[e] Method 2: $\text{yield}_{20\text{-}6} = (100 - \Sigma\ \text{yields}_{2000\text{-}20;\ <6})$

[f] Method 1: $\text{yield}_{<2} = \text{yield}_{2\text{-}0.2} + \text{yield}_{<0.2}$

Table 2b compares, for the soils from Thyrow and Bad Lauchstädt, the mass yields obtained by ultrasonic dispersion/SPLITT with those of textural analysis (chemical dispersion/sedimentation). The results for sand (2000-63 µm) and coarse silt (63-20 µm) obtained by the two methods agree well, with one exception (unmanured soil, Bad Lauchstädt). The fractions 2000-20 µm were separated by wet sieving in both approaches. In the chemical dispersion/sedimentation method, the proportion of medium silt (20-6 µm) was not directly determined, but calculated by difference to 100% mass. In this way, errors/losses in the mass balance for the other fractions may lead to errors in the estimation of this size fraction. For all samples, the theoretically calculated proportion of the fraction 20-6 µm (chemical dispersion/sedimentation) was higher than the value obtained by direct isolation of the fraction (ultrasonic dispersion/SPLITT). The combination of SPLITT-fractionation and centrifugation led to mass yields of clay (< 2 µm) which corresponded well with the results from gravitational sedimentation (chemical dispersion/sedimentation). As for the fine silt (6-2 µm), SPLITT-fractionation yielded exactly the same mass proportions as chemical dispersion/sedimentation (Thyrow), or slightly higher values (Bad Lauchstädt).

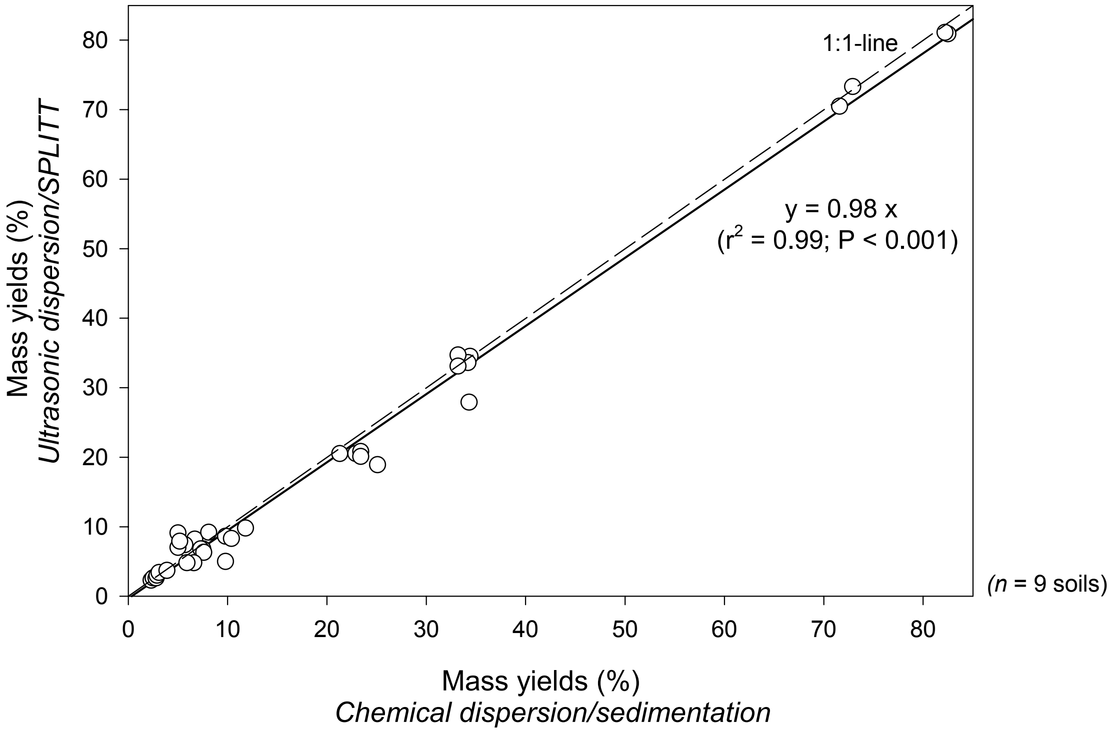


**Fig. 3:** Comparison of mass yields obtained by ultrasonic dispersion/SPLITT and chemical dispersion/sedimentation.

Fig. 3 plots the results obtained by ultrasonic dispersion/SPLITT of nine soil samples (plots from Thyrow, Skierniewice, Bad Lauchstädt) against the chemical dispersion/sedimentation method. Data include the mass proportions of the four size classes sand, coarse silt, fine silt, and clay. The medium silt was not included in this comparison, for reasons mentioned above. The regression line is close to a 1:1-relationship, indicating that the two techniques, though differing in the type of dispersion and particle-size separation, yielded approximately the same gross particle-size distribution. Regarding the ultrasonic dispersion technique used, these results confirm that an ultrasonication energy of 440 J $mL^{-1}$ applied to soil suspensions is sufficient to obtain the same dispersion effect as by chemical dispersion of soils after removal of OM (Schmidt et al., 1999). On the other hand, Schmidt et al. (1999) did not find a redistribution of OM between particle-size fractions at this energy level. Regarding the use of SPLITT-fractionation in the range < 20 µm, our data show that, although SPLITT is based on hydrodynamic sorting of particles, the outcome of separation is similar to that of conventional sedimentation analysis. Keil et al. (1994), using the SPLITT system to sort marine sediments, also noted that the mass distributions agreed with those obtained from standard methods for textural analysis (in the range < 64 µm).

The SPLITT technique provides several advantages and should therefore be considered as a useful alternative to traditional fractionation methods such as gravitational sedimentation or centrifugation: i) The system can be applied for separation in a wide particle-size range (40 to 0.2 µm), in contrast to centrifugation which is limited to smaller size-cutoffs (around 2 µm). ii) Complete fractionation of a sample takes only a few days (depending on the number of size classes), in contrast to gravitational sedimentation. This approach avoids a potential decomposition/transformation of SOM during prolonged incubation of soil suspensions at room temperature. Our data on different soils covering a range of OC contents show that SPLITT-fractionation can be regarded a suitable technique for the fractionation of soil organo-mineral associates.

### 2.3.2 Contents and distribution of organic carbon

As mentioned before, the two treatments from a site did not differ with respect to the mass yields of the size separates. Thus, the extent of C-depletion in the various separates may be described by calculating the ratio of the OC content in the depleted plot to the respective content in the fertilized plot (Table 3). This ratio, expressed as percentage, gives the relative remainder of OC in a particular fraction in the depleted plot. For comparison, the ratios obtained for the bulk soil OC contents are also given. A relative decrease in OC content is

**Table 3:** Contents of organic carbon in bulk samples and particle-size fractions of the contrasting treatments from the three study sites.

| | **Organic carbon** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Thyrow** | | | **Skierniewice** | | | **Bad Lauchstädt** | | | | |
| **Particle-size fractions** (µm) | Fertilized | Depleted | **Depleted / Fertilized** [a] | Fertilized | Depleted | **Depleted / Fertilized** | Fertilized | Depleted | | **Depleted / Fertilized** | |
| | | | | | | | | Unmanured | Bare fallow | Unmanured | Bare fallow |
| | (g kg$^{-1}$) | | (%) | (g kg$^{-1}$) | | (%) | (g kg$^{-1}$) | | | (%) | |
| bulk soil | 6.9 | 3.2 | 47 | 8.8 | 4.4 | 51 | 24 | 16 | 15 | 67 | 62 |
| 2000 - 250 | 2.5 | 0.6 | 22 | 1.9 | 1.0 | 53 | 27 | 14 | 4.8 | 51 | 18 |
| 250 - 63 | 1.2 | 0.5 | 41 | 0.6 | 0.7 | 107 | 16 | 4.8 | 8.2 | 29 | 51 |
| 63 - 20 | 12 | 3.9 | 32 | 5.8 | 4.0 | 70 | 7.4 | 2.1 | 2.2 | 28 | 30 |
| 20 - 6 | 15 | 8.1 | 54 | 7.5 | 6.6 | 88 | 6.7 | 4.1 | 4.3 | 61 | 64 |
| 6 - 2 | 47 | 28 | 59 | 51 | 28 | 55 | 63 | 43 | 31 | 68 | 49 |
| 2 – 0.2 | 55 | 36 | 66 | 57 | 30 | 52 | 62 | 55 | 38 | 88 | 60 |
| < 0.2 | 73 | 48 | 65 | 63 | 43 | 68 | 43 | 38 | 34 | 88 | 79 |

[a] $$\frac{\text{OC content}_{\text{depleted}} \times 100}{\text{OC content}_{\text{fertilized}}}$$

evident for all fractions in the C-depleted soils at the three sites, except for the fine sand (250-63 µm) from Skierniewice. At Thyrow and Bad Lauchstädt (unmanured soil and bare fallow), residual OC contents generally were higher in fractions < 20 µm than in coarser ones (Table 3). The residual level in these fractions was in most cases higher than that for the bulk OC, whereas the ratios for the coarser fractions were below the bulk soil values. The highest proportion of OC – relative to the content in the fertilized plot – was retained in the coarse and fine clay for both sites (fractions < 2 µm): 65 to 66% at Thyrow, and 60 to 88% in the depleted soils from Bad Lauchstädt. Comparing the proportions of residual OC contents across the range of fractions, it can be inferred that the OC stored in fine fractions (< 6 µm) exhibited the highest stability among the fractions; this is especially evident for the C in the fine clay (< 0.2 µm).

The Skierniewice soil shows a slightly different pattern of C-depletion in the size fractions (Table 3). In contrast to the other sites, in coarse-intermediate fractions (250-6 µm) 70 to 100% of the OC content were preserved, and the relative retention of OC within fine fractions (< 6 µm) was not higher as compared with coarser fractions. This led to a smaller discrepancy between the behavior of coarse and fine fractions compared with the other sites. It may be hypothesized that, apart from fine separates, also coarse-intermediate fractions comprised a certain proportion of refractory compounds.

Considering OC contents across the size fractions, Table 3 shows that for all the sites, regardless of treatment, highest contents always occurred in the fine fractions. Whereas OC contents steadily increased from sand to silt to clay in the sandy soils from Thyrow and Skierniewice (with highest values in fine clay), at Bad Lauchstädt the OC peak was in the coarse clay / fine silt (6-2 µm). These data agree with results from a number of arable soils, as reviewed by Christensen (1996), where the highest C concentrations were noted either in the fine silt (5-2 µm) or in the clay (< 2 µm). Apart from the feature that arable soils contain the highest OC contents in fine fractions – these fractions being enriched in C relative to the bulk soil – Christensen (1996) found that the ratio of OC content in a clay/silt separate relative to that of the whole soil (enrichment factor) is inversely related to the content of this separate within the bulk soil. The lower the clay content, for example, the more the clay fraction would be enriched in C relative to the bulk soil. The soils of the present study, which differ in textural composition, seem to fit to this trend: For example, the fine clay of the sandy Thyrow soil was enriched 11.6-fold (fertilized plot) and 15-fold (depleted plot) relative to the bulk soil; the OC content of the coarse clay from Bad Lauchstädt was 2.6 times (fertilized plot) and

2.5 times (bare fallow) that of the bulk soil, this soil showing a higher total clay content than that from Thyrow (Table 1).

The different extent of C decrease in the various particle-size fractions modified the C distribution in the depleted versus fertilized plots (Fig. 4a). In the soils from Thyrow and Bad Lauchstädt, the C distribution was clearly shifted towards finer separates along with C-depletion. In Thyrow, the proportion of OC associated with total clay increased by 12% compared with the fertilized soil, and the percentage of C in the fine silt by 4%, whereas the proportion of OC in the fractions > 6 µm decreased (- 15%). In sum, the fractions < 6 µm accounted for 65% of total OC in the depleted soil from Thyrow (+ 15%). Similarly, in Bad Lauchstädt the proportion of total OC in the clay (< 2 µm) increased by 12% in the depleted versus fertilized soil. This was offset by a decrease in the proportion of C in separates > 6 µm. In the depleted soil, the fractions < 6 µm made up 86% of total OC (+ 11%). The characteristics of C distribution in the depleted plots from the two study sites – compared with the fertilized counterparts – indicate that the refractory OC is mostly associated with fractions < 6 µm. Since most OC stored in the range < 6 µm was found within the clay (< 2 µm) (see Fig. 4a), clay separates may be particularly relevant for refractory C storage. In Skierniewice, changes in the C distribution were less pronounced, and unlike the other two sites, the distribution was not shifted towards finer particle-size separates (Fig. 4a). In the depleted soil, the percentage of C stored in fractions 6-0.2 µm slightly decreased (- 8%), which was offset by an increase in silt and sand fractions > 6 µm (+ 6%), when compared with the fertilized soil. This result may indicate a contribution of organic compounds from sand / silt fractions (250-6 µm) to the refractory C pool in this soil. Apart from this contribution, however, some refractory OC seems to be associated with fine fractions (< 6 µm) because in the depleted soil the sum of fine fractions still accounted for 66% of total SOC.

Fig. 4b summarizes the data on the C distributions in the contrasting treatments from the three study sites. The C amounts left in the various fractions of the depleted plot are expressed as a percentage of the bulk OC of the fertilized counterpart (“residual” OC amounts) (see equation 1). The distribution in the depleted plot is compared with the standard C distribution for the fertilized soil. For better comparison, a dotted line is introduced to separate the fractions finer / coarser than 6 µm. Fig. 4b points out the different features in the C distribution for the three differently textured soils: In fertilized plots at Skierniewice and Bad Lauchstädt, most OC (72 and 75%, respectively) was associated with fine fractions < 6 µm. In Thyrow, however, which had the lowest content of fine particles among the soils, the fractions 2000-6 µm were more important for the C balance than at the other sites, and accounted for 50% of the bulk C (fertilized soil). However, the C in the coarse fractions declined more than in fractions < 6 µm in this soil, as mentioned above. At Bad Lauchstädt, the major part of OC in the fertilized soil was stored in fractions < 6 µm, and much of the OC in these fractions was preserved in the depleted plot. This contributed to the high residual OC in the bulk soil here (70%) compared with the other sites. Furthermore, it becomes apparent that at Thyrow and Bad Lauchstädt the depletion in total SOC was mainly attributable to the decrease of C in coarse-intermediate fractions. At Skierniewice, in contrast, the OC in fine fractions (except for the fine clay) declined to a higher extent than that associated with coarser fractions (2000-6 µm) (Fig. 4b).

Several workers who followed the C dynamics upon cultivation of native soils reported a markedly different behavior of coarse and fine fractions in the course of an overall C-depletion (Balesdent et al., 1988; Dalal and Mayer, 1986; Preston et al., 1994; Tiessen and Stewart, 1983). The OC decrease – relative to the content in the virgin soil – was generally most extensive in coarse fractions and lowest in fine fractions (< 5/2 µm). Using the $^{13}C$ natural abundance approach, several studies directly proved that C in clay / fine separates generally had the slowest turnover among the separates, whereas C in coarse/sand-sized OM was turned over more rapidly, often within several years (Balesdent et al., 1988; Feller and Beare, 1997). In the present study, the relative depletion in OC at Thyrow and Bad Lauchstädt was strongest for coarse fractions. Assuming a labile nature for OM in these fractions, this depletion may directly reflect the reduced OM-input in the depleted plots. Due to the duration of the experiments, OM-input had been lower in the depleted plots for several decades. On the other hand, in Thyrow and Bad Lauchstädt, the extent of C-depletion was lowest for fine fractions, and thus OC in the fine separates was considered to represent a fraction of stable C. This conclusion agrees with the trends reported by Balesdent et al. (1988) and Feller and Beare (1997), i.e. increasing OC turnover times from coarse to fine separates. Accordingly, OC in the fine separates shows the highest turnover time and is thus the most stable C within a soil. Such a stable C pool in fine fractions may be because the interaction of organic compounds with mineral surfaces protects organic matter against biological attack (Baldock and Skjemstad, 2000; Sollins et al., 1996). The entrapment of OM within micro-aggregates, formed for example by flocculation of clay particles, may further contribute to the protection of OM associated with fine particles (Baldock and Skjemstad, 2000).

In Skierniewice, the high residual amounts of C in sand/silt fractions of the depleted plot contradict a rapid turnover in coarse fractions, if one assumes that the overall OM-input to the

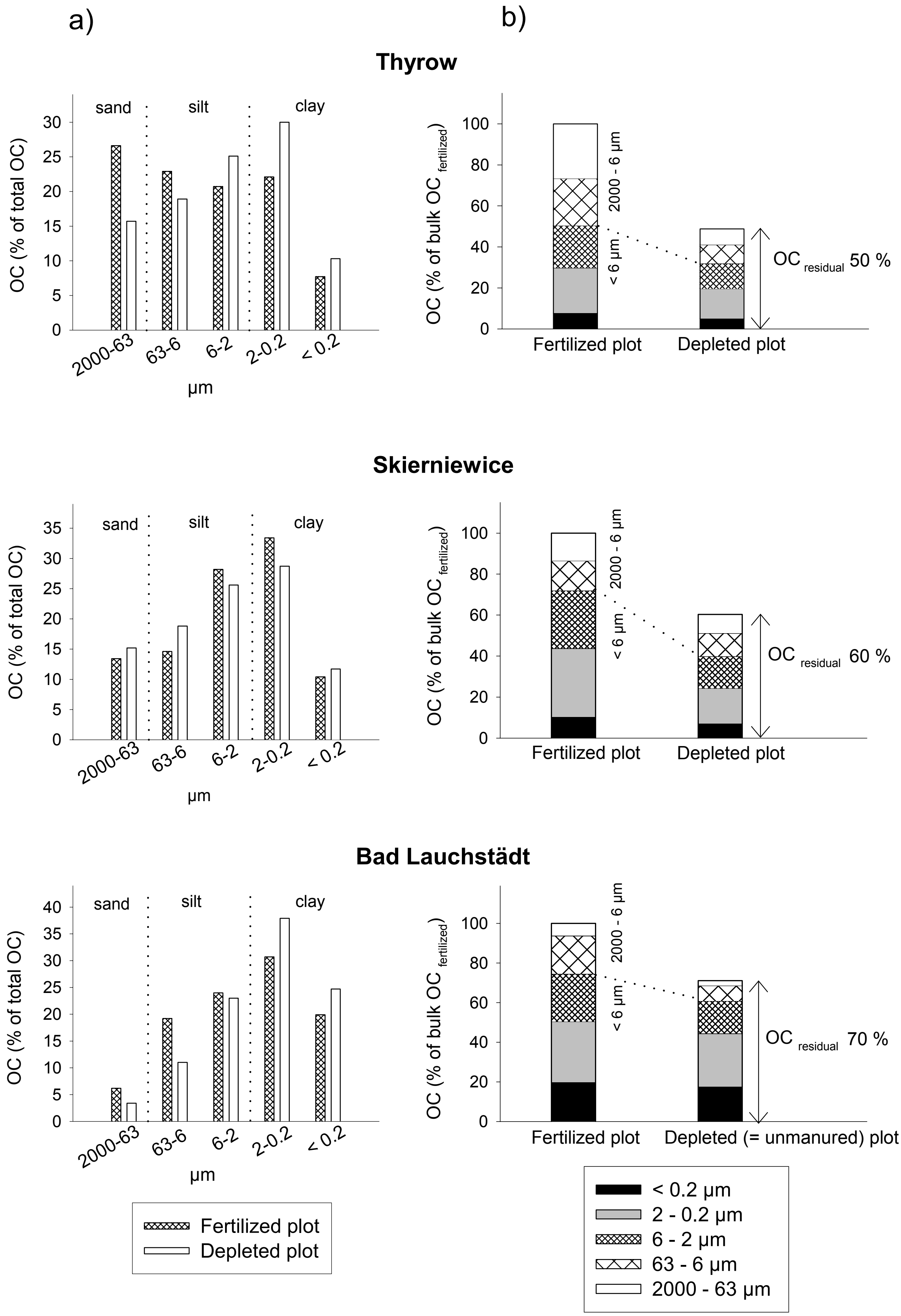


**Fig. 4:**

a) OC distribution in the contrasting treatments of the long-term experiments.

b) Residual amounts of OC in particle-size fractions of the depleted plots in comparison with the fertilized counterparts.

depleted plot had been considerably reduced over the last years/decades. These high C residues may be indicative of chemically recalcitrant OM within these fractions.

The present findings differ from some of the literature data as far as the fine clay is concerned and the stability of C in clay versus silt. Several authors (e.g. Anderson and Paul, 1984; Tiessen and Stewart, 1983) reported the OC in fine clay had a more labile character than that associated with coarse clay / fine silt. This is not supported by our data, when the relative extent of C-depletion in the fine clay (versus coarser fractions) is considered. Moreover, our results contradict Christensen (1996), who states that in temperate soils the silt fraction (20-2 µm) has the highest OC stability, whereas the clay fraction (< 2 µm) contains a higher proportion of labile C relative to the silt. This statement is, among others, based on C mineralizability in short-term laboratory experiments (Christensen, 1987), as well as on the retention of substrate-derived C in an 18 year-incubation experiment in the field (Christensen and Sørensen, 1985). In contrast to these short- or medium-term experiments, OC in the silt fraction of our long-term field experiments showed a lower persistence/stability than OC associated with the clay fraction.

### 2.3.3 Nitrogen contents and carbon-to-nitrogen ratios

Table 4 presents total N contents along with the ratios of N contents in the depleted soils relative to the values in the fertilized counterparts. The effect of OM-depletion on N contents in the size separates revealed a pattern similar to that for OC. In Thyrow and Bad Lauchstädt, the proportions of residual N contents in the fractions < 20 µm were higher than for the range 2000-20 µm, with maximum values in the clay. Thus, N compounds associated with fine fractions can be considered to show the greatest stability. In Skierniewice, apart from the fine clay (< 0.2 µm), coarse-intermediate fractions retained a high proportion of the N relative to the fertilized plot (Table 4). Analogous to the pattern of OC contents across the fractions, the lowest N contents within a given soil sample were present in coarse fractions (sand / coarse silt), and highest N contents were found in the clay, either in the fine clay (Thyrow, Skierniewice) or in the coarse clay (Bad Lauchstädt) (Table 4).

All soil samples were characterized by a gradually decreasing C/N-ratio when progressing from sand to silt to clay (Table 5), the fine clay material having the narrowest ratio. A narrowing C/N-ratio is thought to indicate an increasing decomposition of the organic matter, i.e. a progressive degradation of plant litter, accompanied by an increasing contribution of microbial biomass (of a C/N-ratio 8-12) and its metabolites to the OM, when progressing

**Table 4:** Contents of total nitrogen in bulk samples and particle-size fractions of the contrasting treatments from the three study sites.

| | **Total nitrogen** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Thyrow** | | | **Skierniewice** | | | **Bad Lauchstädt** | | | | |
| **Particle-size fractions** (µm) | Fertilized | Depleted | **Depleted / Fertilized** [a] | Fertilized | Depleted | **Depleted / Fertilized** | Fertilized | Depleted | | **Depleted / Fertilized** | |
| | | | | | | | | Unmanured | Bare fallow | Unmanured | Bare fallow |
| | (g kg$^{-1}$) | | (%) | (g kg$^{-1}$) | | (%) | (g kg$^{-1}$) | | | (%) | |
| bulk soil | 0.61 | 0.27 | 44 | 0.76 | 0.39 | 51 | 1.97 | 1.32 | 1.12 | 67 | 57 |
| 2000 - 250 | 0.16 | 0.02 | 12 | 0.09 | 0.03 | 31 | 1.3 | 0.64 | 0.21 | 51 | 16 |
| 250 - 63 | 0.07 | 0.02 | 20 | 0.03 | 0.02 | 67 | 0.80 | 0.18 | 0.17 | 22 | 21 |
| 63 - 20 | 0.88 | 0.21 | 23 | 0.31 | 0.16 | 53 | 0.53 | 0.13 | 0.03 | 24 | 5 |
| 20 - 6 | 1.1 | 0.50 | 43 | 0.52 | 0.38 | 73 | 0.51 | 0.36 | 0.24 | 70 | 47 |
| 6 - 2 | 4.1 | 2.2 | 53 | 4.4 | 2.5 | 57 | 5.2 | 3.7 | 2.2 | 71 | 41 |
| 2 – 0.2 | 5.2 | 2.9 | 57 | 5.3 | 2.8 | 53 | 5.4 | 4.8 | 2.9 | 90 | 54 |
| < 0.2 | 9.2 | 5.5 | 60 | 8.0 | 5.4 | 68 | 4.5 | 3.6 | 3.1 | 80 | 68 |

[a] $$\frac{\text{N content}_{\text{depleted}} \times 100}{\text{N content}_{\text{fertilized}}}$$

**Table 5:** C-to-N-ratios in particle-size fractions of the contrasting treatments from the three study sites.

| | **C-to-N-ratios** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Thyrow** | | **Skierniewice** | | **Bad Lauchstädt** | | |
| **Particle-size fractions** (µm) | **Fertilized** | **Depleted** | **Fertilized** | **Depleted** | **Fertilized** | **Unmanured** | **Bare fallow** |
| 2000 - 250 | 16 | 27 | 20 | 35 | 22 | 22 | 24 |
| 250 - 63 | 17 | 33 | 22 | 36 | 20 | 27 | 50 |
| 63 - 20 | 14 | 19 | 19 | 25 | 14 | 16 | 86 |
| 20 - 6 | 13 | 16 | 15 | 18 | 13 | 11 | 18 |
| 6 - 2 | 11 | 13 | 12 | 11 | 12 | 12 | 14 |
| 2 – 0.2 | 11 | 12 | 11 | 11 | 12 | 11 | 13 |
| < 0.2 | 8 | 9 | 8 | 8 | 9 | 10 | 11 |

from coarse to fine separates (Baldock et al., 1992; Guggenberger et al., 1995). When comparing the two treatments, the ratios were higher in the range 2000-6 µm of the depleted soils, but remained nearly unchanged in fine fractions < 6 µm, except for a slight increase in the bare fallow (Bad Lauchstädt) (Table 5). The greatest deviations occurred in the bare fallow from Bad Lauchstädt, with exceptionally high values in the fractions 250-20 µm. The higher values in coarse-intermediate fractions of the unmanured plots from the three sites (compared with the fertilized ones) may be explained by the lack of farmyard manure input in the former. For example, the organic manure applied to the fertilized plots in Bad Lauchstädt has a C/N-ratio of 17 to 18. In contrast, organic matter entering the soil in the unmanured plots consists only of plant litter; most crops probably have a C/N-ratio higher than in manure (e.g. 50 to 100 for cereal straw). The high ratios in fractions 250-20 µm of the bare fallow – with no recent organic input – suggest that refractory compounds are present which are highly depleted in N relative to C. Examples for (recalcitrant) compounds which can show a high C/N-ratio are charcoal, as well as carbonaceous particles (coal, soot) which were detected in soils of industrialized areas (Schmidt et al., 1996; Rumpel et al., 1998). Whether such compounds are present in the bare fallow should be determined by appropriate methods in future work (see chapter 5).

In contrast to coarser fractions, there was no substantial change in the C/N-ratios in fractions < 6 µm along with OM-depletion. As mentioned above, both C and N in the fine fractions showed the highest stability of all size separates. The unchanged C/N-ratio in the depleted plots indicates that N was stabilized to a similar extent as OC within the fine fractions. The present findings are in line with the studies of Tiessen and Stewart (1983) and Preston et al. (1994), who showed that OC and N declined / or were retained to a similar degree within fine separates during a process of SOC-depletion.

## 2.4 Conclusions

- Comparing OC contents in size separates of the depleted versus fertilized plots revealed the highest residual level of OC in separates < 6 µm (for two experiments) or 250-20 µm (for one experiment). Thus, the most stable OC may be associated with fine separates, although coarse-intermediate separates may also contain refractory C compounds.
- Since the C distribution shifted towards fine fractions in the depleted plots compared with the fertilized plots (for two experiments), the fine fractions apparently contained most of the refractory SOC.

- Analogously to OC, the N associated with fine fractions (< 6 µm) appeared to show the highest stability among the fractions, when the relative retention of N in the depleted plots is regarded. C and N compounds in the fine fractions may thus be attributed to a pool of refractory organic matter.

## 2.5 References

AG Boden, 1994. Bodenkundliche Kartieranleitung, 4. Auflage. E. Schweizerbart`sche Verlagsbuchhandlung, Stuttgart.

Amelung, W., Zech, W., Zhang, X., Follett, R.F., Tiessen, H., Knox, E., Flach, K.-W., 1998. Carbon, nitrogen, and sulfur pools in particle-size fractions as influenced by climate. Soil Science Society of America Journal 62, 172-181.

Amelung, W., Zech, W., 1999. Minimisation of organic matter disruption during particle-size fractionation of grassland epipedons. Geoderma 92, 73-85.

Anderson, D.W., Paul, E.A., 1984. Organo-mineral complexes and their study by radiocarbon dating. Soil Science Society of America Journal 48, 298 - 301.

Augris, N., Balesdent, J., Mariotti, A., Derenne, S., Largeau, C., 1998. Structure and origin of insoluble and non-hydrolyzable, aliphatic organic matter in a forest soil. Organic Geochemistry 28, 119-124.

Baldock, J.A., Oades, J.M., Waters, A.G., Peng, X., Vassallo, A.M., Wilson, M.A., 1992. Aspects of the chemical structure of soil organic materials as revealed by solid-state $^{13}$C NMR spectroscopy. Biogeochemistry 16, 1-42.

Baldock, J.A., Skjemstad, J.O., 2000. Role of the mineral matrix and minerals in protecting natural organic materials against decomposition. Organic Geochemistry 31, 697-710.

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

Christensen, B.T., 1985. Carbon and nitrogen in particle size fractions isolated from Danish arable soils by ultrasonic dispersion and gravity-sedimentation. Acta Agriculturae Scandinavica 35, 175-187.

Christensen, B.T., 1987. Decomposability of organic matter in particle-size fractions from field soils with straw incorporation. Soil Biology & Biochemistry 19, 429-435.

Christensen, B.T., 1996. Carbon in primary and secondary organomineral complexes. In: Carter, M.R., Stewart, B.A. (Eds.), Structure and Organic Matter Storage in Agricultural Soils. Advances in Soil Science. CRC Press, Boca Raton, pp. 97-165.

Christensen, B.T., Sørensen, L.H., 1985. The distribution of native and labelled carbon between soil particle size fractions isolated from long-term incubation experiments. Journal of Soil Science 36, 219 - 229.

Dalal, R.C., Mayer, R.J., 1986. Long-term trends in fertility of soils under continuous cultivation and cereal cropping in Southern Queensland. III. Distribution and kinetics of soil organic carbon in particle size fractions. Australian Journal of Soil Science 24, 293-300.

Elliott, E.T., Paustian, K., Frey, S.D., 1996. Modeling the measurable or measuring the modelable: a hierarchical approach to isolating meaningful soil organic matter fractionations. In: Powlson, D.S., Smith, P., Smith, J.U. (Eds.), Evaluation of Soil Organic Matter Models Using Existing Long-term Datasets, NATO ASI Series. Springer Verlag, Berlin, pp. 161-179.

Ellmer, F., Peschke, H., Köhn, W., Chmielewski, F.-M., Baumecker, M., 2000. Tillage and fertilizing effects on sandy soils. Review and selected results of long-term experiments at Humboldt-University Berlin. Journal of Plant Nutrition and Soil Science 163, 167-272.

Falloon, P.D., Smith, P., 2000. Modelling refractory soil organic matter. Biology and Fertility of Soils 30, 388-398.

Feller, C., Beare, M.H., 1997. Physical control of soil organic matter dynamics in the tropics. Geoderma 79, 69-116.

Gee, G.W., Bauder, J.W., 1986. Particle-size analysis. In: American Society of Agronomy, Soil Science Society of America (Eds.), Methods of Soil Analysis, Part 1. Physical and Mineralogical Methods - Agronomy Monographs no. 9. Madison, USA, pp. 383-411.

Giddings, J.C., 1985. A system based on split-flow lateral transport thin (SPLITT) separation cells for rapid and continuous particle fractionation. Separation Science and Technology 20, 749-768.

Guggenberger, G., Zech, W., Haumaier, L., Christensen, B.T., 1995. Land-use effects on the composition of organic matter in particle-size separates of soils: II. CPMAS and solution $^{13}$C NMR analysis. European Journal of Soil Science 46, 147-158.

Jiang, Y., Kummerow, A., Hansen, M., 1997. Preparative particle separation by continuous SPLITT fractionation. Journal of Microcolumn Separations 9, 261-273.

Keil, R.G., Tsamakis, E., Fuh, C.B., Giddings, J.C., Hedges, J.I., 1994. Mineralogical and textural controls on the organic composition of coastal marine sediments: Hydrodynamic separation using SPLITT-fractionation. Geochimica et Cosmochimica Acta 58, 879-893.

Körschens, M., Weigel, A., Schulz, E., 1998. Turnover of soil organic matter (SOM) and long-term balances - tools for evaluating sustainable productivity of soils. Zeitschrift für Pflanzenernährung und Bodenkunde 161, 409 - 424.

Mercik, S., Stepien, W., Labetowicz, J., 2000. The fate of nitrogen, phosphorus and potassium in long-term experiments in Skierniewice. Journal of Plant Nutrition and Soil Science 163, 273-278.

North, P.F., 1976. Towards an absolute measurement of soil structural stability using ultrasound. Journal of Soil Science 27, 451-459.

Parton, W.J., Schimel, D.S., Cole, C.V., Ojima, D.S., 1987. Analysis of factors controlling soil organic matter levels in Great Plains grasslands. Soil Science Society of America Journal 51, 1173-1179.

Preston, C.M., Newman, R.H., Rother, P., 1994. Using $^{13}$C CPMAS NMR to assess effects of cultivation on the organic matter of particle size fractions in a grassland soil. Soil Science 157, 26-35.

Rühlmann, J., 1999. A new approach to estimating the pool of stable organic matter in soil using data from long-term field experiments. Plant and Soil 213, 149-160.

Rumpel, C., Knicker, H., Kögel-Knabner, I., Hüttl, R.F., 1998. Airborne contamination of immature soil (Lusatian mining district) by lignite-derived material: its detection and contribution to the soil organic matter budget. Water, Air and Soil Pollution 105, 481-492.

Schmidt, M.W.I., Knicker, H., Hatcher, P.G., Kögel-Knabner, I., 1996. Impact of brown coal dust on the organic matter in particle-size fraction of a Mollisol. Organic Geochemistry 25, 29-39.

Schmidt, M.W.I., Rumpel, C., Kögel-Knabner, I., 1999. Evaluation of an ultrasonic dispersion procedure to isolate primary organomineral complexes from soils. European Journal of Soil Science 50, 87-94.

Schulten, H.-R., Leinweber, P., 2000. New insights into organic-mineral particles: composition, properties and models of molecular structure. Biology and Fertility of Soils 30, 399-432.

Shang, C., Tiessen, H., 1997. Organic matter lability in a tropical Oxisol: evidence from shifting cultivation, chemical oxidation, particle-size, density, and magnetic fractionation. Soil Science 162, 795-807.

Sollins, P., Homann, P., Caldwell, B.A., 1996. Stabilization and destabilization of soil organic matter: mechanisms and controls. Geoderma 74, 65-105.

Tiessen, H., Stewart, J.W.B., 1983. Particle-size fractions and their use in studies of soil organic matter: II. Cultivation effects on organic matter composition in size fractions. Soil Science Society of America Journal 47, 509-513.

Trumbore, S.E., 1997. Potential response of soil organic carbon to global environmental change. Proceedings of the National Academy of Science 94, 8284-8291.

Weast, R.C. (Ed.), 1969. Handbook of Chemistry and Physics. The Chemical Rubber Company.

# 3 Organic carbon in relation to mineral surface area and iron oxides in fractions < 6 µm

## Abstract

The objective of this section is to assess the role of mineral surfaces for the long-term stabilization of OC in the arable soils, with special emphasis on iron oxides. In fractions < 6 µm of samples from three long-term experiments, OC was studied in relation to total mineral surface area, surface area contributed by oxides and silicates, and the content and type of iron oxides (dithionite and oxalate extractable iron, $Fe_d$ and $Fe_o$). In two sandy soils, OC contents were linearly related to total mineral surface area and the content of the two iron oxide fractions ($Fe_d$ and $Fe_o$). The surface area developed by the silicates was low and thus the surface area contribution from oxides was dominant in fractions < 6 µm. In contrast to the sandy soils, in the loamy soil OC was not correlated with surface area or the iron oxide content. However, the different soils agreed with respect to the behavior of C in density fractions: losses of OC occurred mainly from the light fraction ($\leq 2$ g $cm^{-3}$), whereas C in the heavy fraction ($> 2$ g $cm^{-3}$) proved to be stable. For the sandy soils, mineral surface area appears to control the storage of OC in fine fractions. Given the dominant surface area contribution from oxides, OC storage here may primarily depend on the oxides. The C-depleted plots in particular show that surface area controls the accumulation of refractory C. The interaction of organic compounds with the mineral phase, mainly with the surface of oxides, may be a major mechanism for the long-term stabilization of OC in these sandy soils. Also in the loamy soil an interaction with minerals seems to be important for stabilizing OC, although there is no proportional relation between OC and surface area across the fractions.

## 3.1 Introduction

The interaction of organic substances with mineral surfaces is recognized as one mechanism which protects organic matter (OM) in soil against decomposition (Sollins et al., 1996). The extent to which OM interacts with minerals depends on both the physical and chemical properties of the mineral fraction as well as on the morphology and chemical structure of the organic material (Baldock and Skjemstad, 2000). Regarding the properties of the mineral fraction, the surface area available to organic molecules is an important physical aspect, being mainly a function of particle-size and -shape, surface roughness, and presence of internal surfaces in expandable layer silicates (e.g. Ransom et al., 1998). The chemical nature of the mineral phase determines the type and the density of active binding sites with which organic materials can interact (Baldock and Skjemstad, 2000). A variety of different binding mechanisms were described for charged and uncharged organic molecules, especially regarding the interaction with clay minerals and (iron) oxides (Cornejo and Hermosin, 1996; Oades, 1989). These physical and chemical aspects indicate that protection of OM through interaction with minerals should play a major role within fine particle-size fractions. These fractions exhibit a specific surface area (SSA) several orders of magnitude higher than that of the sand fraction, and contain clay minerals and oxides with reactive surfaces. Long-term incubation experiments in the field (Jenkinson, 1977; Ladd et al., 1985) and short-term laboratory experiments (Saggar et al., 1999) demonstrated that the proportion of substrate-derived C retained in the bulk soil at the end of incubation is positively related to the clay content. The content of fine particles / clay is therefore probably crucial for the preservation of organic carbon (OC) in soils. Saggar et al. (1996) concluded that not the clay content itself but rather the total surface area determines a soil's capacity to stabilize OC. The actual surface area of a soil is controlled by the content of particle-size fractions having a high SSA (clay) and by the nature of the minerals present in the clay (Saggar et al., 1996).

An interaction of OC with iron oxides may be quantitatively important in soils. Iron oxides can develop a large specific surface area, up to several hundred $m^2$ $g^{-1}$, given their small particle-size. Moreover, various mechanisms exist by which organics can interact with their surface. These mechanisms include electrostatic interaction for charged molecules, specific adsorption by ligand exchange through carboxyl and hydroxyl functional groups of organic compounds, as well as hydrogen bonding and hydrophobic effects (Cornell and Schwertmann, 1996). Several empirical studies indicated that organic compounds do interact with iron oxides, resulting in a preservation of soil organic carbon (SOC). Examples include: a greater DOC (dissolved organic carbon) sorption with increasing aluminum and iron oxide coverage of the mineral matter from B horizons of forest soils (Kaiser and Guggenberger, 2000), evidence that goethite was closely associated with humic acids in Oxisols (Fontes et al., 1992), and a positive correlation between iron oxide content and OC in bulk samples and particle-size fractions of various soils (Shang and Tiessen, 1998; Kaiser and Guggenberger, 2000).

The bulk of SOC consists of compounds with widely varying turnover times. This has led to a theoretical classification according to turnover time, whereby two major pools of SOC may be roughly distinguished, namely the *labile* and *refractory* pool (Hsieh, 1994; Trumbore, 1997). The *labile* (or active) pool encompasses compounds having a turnover time of years to decades. Those organic substances which are stabilized against decomposition over the long-term, resulting in turnover times of hundreds to thousands of years, belong to the *refractory* (or passive) pool.

A feature of long-term agroecosystem trials is that different levels of SOC are found in the various experimental plots, depending on the type of soil management employed (Körschens, 1998). Assuming a homogeneous mineralogy within the experimental site and soil mineralogy not being affected by management-induced changes in soil properties (e.g. pH, SOC level) at a time-scale of decades, these field experiments provide an opportunity to study SOC storage in relation to the properties of the mineral matrix. The present study examines samples from two contrasting treatments of long-term field experiments: *fertilized* plots and *C-depleted* plots (unmanured plots and bare fallows), the latter being depleted in total SOC compared with the fertilized counterparts. The hypothesis is that labile compounds are primarily depleted, whereas refractory compounds accumulate within the SOC residue left in the C-depleted plots (Rühlmann, 1999). The depleted plots are therefore relatively enriched in refractory compounds compared with the fertilized plots. Due to this enrichment, the C-depleted plots seem to be especially suitable for studying the role of mineral surfaces for the long-term stabilization of SOC. In the previous section (2.3.2) it was shown that most of the refractory SOC is associated with fine particle-size separates (< 6 µm).

The objectives of this study are i) to evaluate the role of the mineral surface area for long-term OC stabilization in arable soils by investigating the relationship between surface area and OC in fractions < 6 µm, and ii) to assess the importance of iron oxides with respect to the surface area-dependent stabilization.

## 3.2 Materials and methods

### 3.2.1 Soils from long-term agroecosystem experiments

Samples were obtained from three long-term agroecosystem experiments located in Germany and Poland (Table 1). The soils from Thyrow and Skierniewice are classified as Luvisols, the soil at Bad Lauchstädt as Haplic Chernozem (FAO, 1998). There is a gradient of increasing clay and silt contents from Thyrow to Skierniewice to Bad Lauchstädt and an opposite trend for sand contents. With respect to textural composition, the soils from Thyrow and Skierniewice will be denoted as sandy soils, Bad Lauchstädt as loamy soil. The average contents of particle-size fractions < 6 µm are 5, 10 and 28% in Thyrow, Skierniewice and Bad Lauchstädt, respectively. The same set of contrasting experimental treatments was chosen at each site (Table 1): a) *Fertilized* plots (treatment I in Table 1) and b) plots *depleted* in SOC relative to the fertilized soils, either unmanured plots (treatment II) or bare fallows (treatment

**Table 1:** Characteristics of the long-term agroecosystem experiments.

| **Site** | **Soil type** (FAO) | **Texture**[a] (%) | | | **Start of experiment** | **Crop rotations** | **Treatments** | **Organic carbon** (g kg$^{-1}$) | | **pH**[b] | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Sand | Silt | Clay | | | | Fertilized | Depleted | Fertilized | Depleted |
| Thyrow (Germany) | Albic Luvisol | 82 | 15 | 3 | 1937 | Potatoes – spring barley – maize – spring barley | I) Fertilized NPK + farmyard manure (15 t ha$^{-1}$ y$^{-1}$) + lime | 6.8 | | 6.1 | |
| | | | | | | | II) No fertilization/liming | | 3.2 | | 4.1 |
| Skierniewice (Poland) | Luvisol | 72 | 22 | 6 | 1923 | Fertilized plots (I): potatoes – spring barley – red clover – winter wheat – rye<br>Unmanured plots (II): arbitrary rotation | I) Fertilized NPK + farmyard manure (6 t ha$^{-1}$ y$^{-1}$) + lime | 8.8 | | 6.1 | |
| | | | | | | | II) No fertilization/liming | | 4.4 | | 4.5 |
| Bad Lauchstädt (Germany) | Haplic Chernozem | 7 | 70 | 23 | 1902 | Sugarbeet - spring barley – potatoes - winter wheat | I) Fertilized NPK + farmyard manure (15 t ha$^{-1}$ y$^{-1}$) | 24 | | 7.2 | |
| | | | | | | | II) No fertilization | | 16 | | 7.5 |
| | | | | | 1956 | — | III) Bare fallow | | 15 | | N.D. |

[a] Textural analysis by chemical dispersion, followed by wet-sieving and gravitational sedimentation (Gee and Bauder, 1986): sand (2000-63 µm), silt (63-20 µm), clay (< 2 µm)

[b] pH measured in a suspension of soil in 0.01 M $CaCl_2$ (soil-to-$CaCl_2$ solution = 1:2.5) (Schlichting et al., 1995)

N.D. Not determined

III at Bad Lauchstädt). In the two sandy soils (Thyrow, Skierniewice) the pH is around 4 in the depleted plots and 6 in the fertilized plots due to liming. The plots in Bad Lauchstädt have a pH of around 7. In summer 1998, samples were taken from a depth 0-20 cm, air-dried, and the mineral/organic components > 2 mm removed by dry sieving.

### 3.2.2 Particle-size fractionation

The procedure involved ultrasonic dispersion of the soil samples by the two-step method used by Amelung et al. (1998), and in the range < 6 µm separation of the size fractions by a combination of SPLITT-fractionation (6-0.2 µm) (Keil et al., 1994) and centrifugation (< 0.2 µm). Briefly, a suspension of air-dried soil < 2 mm (soil-to-water ratio 1:3 for Thyrow/Skierniewice, 1:5 for Bad Lauchstädt) was ultrasonicated at 60 J ml$^{-1}$ using a probe-type ultrasonic disintegrator (Branson Sonifier 250, Branson). Primary particles 2000-250 µm, including particulate organic matter, released by the first dispersion step were removed by wet sieving. According to Amelung and Zech (1999), the removal of particulate OM after a first step of weak dispersion is recommended in order to avoid artefacts resulting from a disruption of particulate OM during high-energy ultrasonification. Subsequently, the suspension < 250 µm (soil-to-water ratio 1:5 Thyrow / Skierniewice, 1:10 for Bad Lauchstädt) was ultrasonicated at 440 J ml$^{-1}$ for complete dispersion of the samples.

Three separates < 6 µm were isolated: fine silt (6-2 µm), coarse clay (2-0.2 µm) and fine clay (< 0.2 µm). The separation was performed by two successive SPLITT runs and centrifugation. The suspension < 20 µm, gained by wet sieving, was subjected to a first step of SPLITT-fractionation employing a cutoff of 6 µm. This yielded the fractions 20-6 and < 6 µm. From the latter fraction, particles < 0.2 µm were isolated by centrifugation. The fraction < 0.2 µm was recovered by flocculation of particles after adding $MgCl_2$. The pellet, representing the fraction 6-0.2 µm, was redispersed and separated into the size classes 6-2 and 2-0.2 µm by using the SPLITT cell a second time (cutoff 2 µm). In this SPLITT run, NaCl was added to a final concentration of 20 mM in the suspension 6-0.2 µm because NaCl minimized flocculation of particles during the fractionation procedure. All fractions were freeze-dried. Prior to carbon analysis, aliquots of the samples were ground in a ball mill.

### 3.2.3 Density fractionation

Selected particle-size separates, i.e. 6-2 µm and 2-0.2 µm from Thyrow and Bad Lauchstädt, were separated into a light (≤ 2 g cm$^{-3}$) and a heavy (> 2 g cm$^{-3}$) subfraction using a sodium polytungstate solution [$Na_6(H_2W_{12}O_{40})$ $x$ $H_2O$, Sometu, Berlin, Germany] of a density 2 g $cm^{-3}$. To obtain a quantitative separation, the steps of centrifugation, subsequent removal of the floating material and redispersion of the pellet by ultrasonification were repeated four times. The density fractions were washed and freeze-dried.

### 3.2.4 Determination of C

C contents of bulk samples, particle-size separates and their density fractions were determined by dry combustion in a Vario EL elemental analyzer (Elementar Analysen-Systeme, Hanau, Germany). In the bulk samples, the proportion of inorganic carbon was assessed by elemental analysis after ignition of the samples at 550°C for 3 h. Inorganic carbon was not detected in any of the samples.

### 3.2.5 Determination of specific surface area (SSA)

The surface area (SA) of particle-size fractions < 6 µm was determined by the $N_2$-adsorption BET approach, using an Autosorb 1 surface area analyzer (Quantachrome Corporation). The SA of previously outgassed samples was derived from the adsorption isotherm of $N_2$ at 77 K, applying the BET equation to the data in the $p/p_0$ range of 0.05 to 0.30 (multi-point BET) (Brunauer et al., 1938). To remove physically adsorbed surface water, all samples were outgassed under vacuum (40 mbar) at 70°C for 23 ± 1 h at the outgass station of the instrument prior to $N_2$ adsorption.

#### *3.2.5.1 SSA of particle-size fractions after various treatments*

SSA was measured of the particle-size fractions without any chemical treatment as well as after sequential treatment for the removal of organic matter and oxides, respectively (Fig. 1). The SSA measured of bulk fractions ($SSA_{\text{bulk}}$) is given as $m^2$ $g^{-1}$ bulk fraction. The SA analyzed after oxidation of organic matter was attributed to the total mineral matrix ($SSA_{\text{minerals}}$, $m^2$ $g^{-1}$ mineral matter in a fraction). SSA before and after removal of organic matter were compared as follows:

$$\Delta\, SSA \quad = \quad SSA_{\text{minerals}} - SSA_{\text{bulk}} \qquad (1)$$

| | |
|---|---|
| $SSA_{\text{minerals}}$ | SSA of total minerals ($m^2$ $g^{-1}$ mineral matter) |
| $SSA_{\text{bulk}}$ | SSA of untreated size fraction ($m^2$ $g^{-1}$ bulk fraction) |

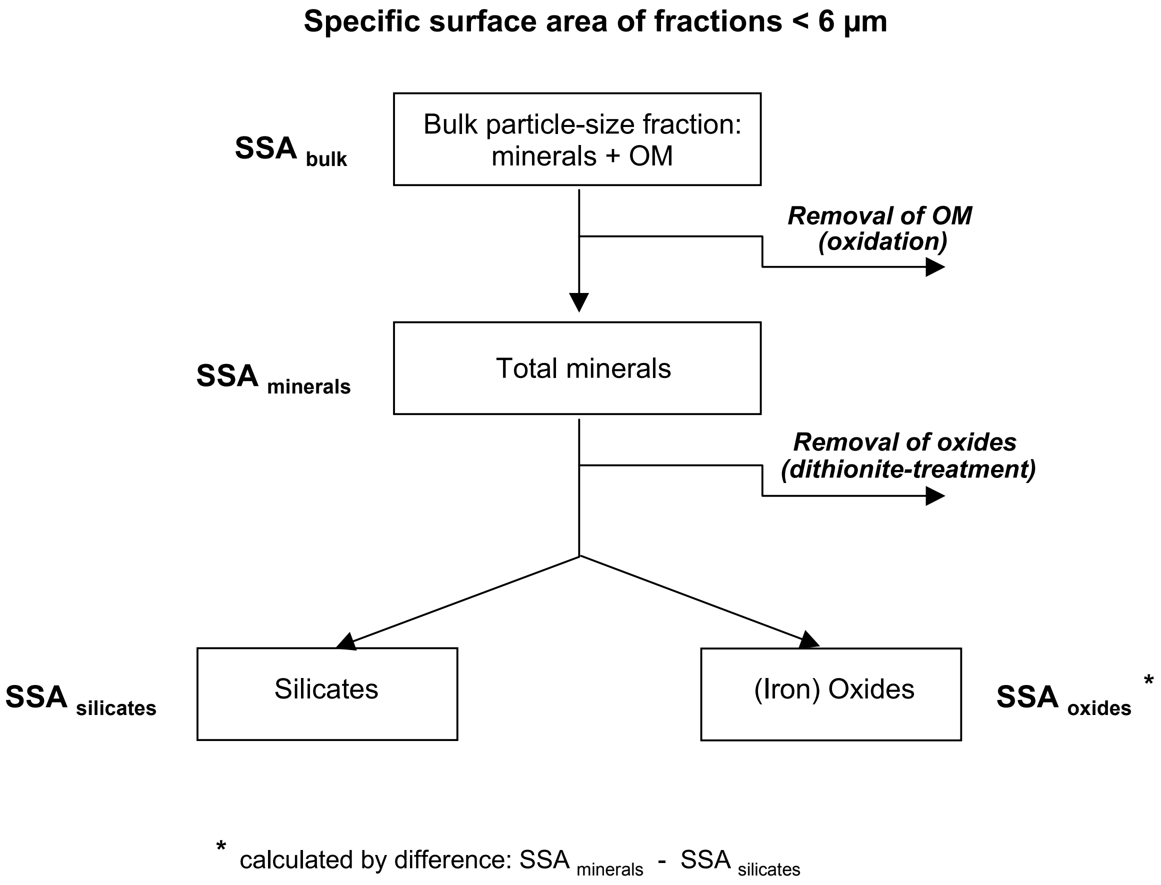


**Fig. 1:** Treatments of fractions < 6 µm of the sandy soils (Thyrow, Skierniewice) for analysis of specific surface area.

For the sandy soils (Thyrow, Skierniewice), the OM-free fractions were treated with dithionite to remove oxides. The SSA measured after dithionite-treatment is considered to be an estimate for the contribution of silicates to the total mineral surface area (SSA $_{\text{silicates}}$). In the calculation of this SSA value, the mass loss caused by the dithionite extraction was taken into account:

$$\text{SSA}_{\text{silicates}} = \frac{\text{SSA}_{\text{residue}} \times (100 - \%\ \text{Mass loss})}{100} \qquad (2)$$

| | |
|---|---|
| SSA $_{\text{silicates}}$ | SSA of silicates ($m^2\ g^{-1}$ (total) mineral matter) |
| SSA $_{\text{residue}}$ | SSA measured for the residue after dithionite-treatment ($m^2\ g^{-1}$ residue) |

The contribution of oxides to the SSA of the mineral phase was assessed from the decrease of surface area following the dithionite-treatment:

$$\text{SSA}_{\text{oxides}} = \text{SSA}_{\text{minerals}} - \text{SSA}_{\text{silicates}} \tag{3}$$

| | |
|---|---|
| $\text{SSA}_{\text{oxides}}$ | SSA of oxides ($m^2\ g^{-1}$ mineral matter) |
| $\text{SSA}_{\text{minerals}}$ | SSA of total minerals ($m^2\ g^{-1}$ mineral matter) |
| $\text{SSA}_{\text{silicates}}$ | SSA of silicates ($m^2\ g^{-1}$ mineral matter) (see equation 2) |

Component-specific surface area values (SSA`) were obtained by relating the SA assigned to a component (oxides/silicates) to the content of this component in a given particle-size fraction:

$$\text{SSA`}_{\text{oxides/silicates}} = \frac{\text{SSA}_{\text{oxides/silicates}} \times 100}{\text{Content}_{\text{oxides/silicates}}} \tag{4}$$

| | |
|---|---|
| $\text{SSA`}_{\text{oxides/silicates}}$ | Component-specific SSA ($m^2\ g^{-1}$) |
| $\text{SSA}_{\text{oxides/silicates}}$ | ($m^2\ g^{-1}$ mineral matter) (equations 2 and 3) |
| $\text{Content}_{\text{oxides}}$ | Mass loss in the dithionite-treatment (% of mineral matter) |
| $\text{Content}_{\text{silicates}}$ | Calculated by difference to total mineral matter (100 - % mass loss) (% of mineral matter). |

#### *3.2.5.2 Removal of organic matter*

For oxidation of organic matter, the method proposed by Meier and Menegatti (1997) was slightly modified. Samples were treated with the oxidant sodium peroxodisulfate ($Na_2S_2O_8$) in the presence of sodium hydrogen carbonate ($NaHCO_3$) acting as a buffer which keeps the pH around 7-8 during the whole reaction. 200 mg of sample were briefly redispersed in water by ultrasonication, then $Na_2S_2O_8$ and $NaHCO_3$ were added and the suspension volume was adjusted to appr. 150 mL. The mass ratio of sample-to-oxidant was 1:40. Samples were incubated at 80°C for 16 h, subsequently washed with deionized water and freeze-dried. On average, OC contents of the peroxodisulfate-treated samples were 3 g $kg^{-1}$, corresponding to 93% of the initial OC to be removed by the procedure.

### *3.2.5.3 Removal of oxides (sandy soils)*

Basically, the procedure was the same as used to quantify dithionite-extractable Fe (see 3.2.6) (dithionite-citrate-bicarbonate (DCB) extraction). Briefly, 200 mg of OM-free fraction material were mixed with 0.3 M sodium citrate solution, 1 M sodium hydrogen carbonate solution and sodium dithionite crystals. After shaking the suspension for 16 h, the supernatant was discarded and the bleached residue was washed with water and freeze-dried.

The reductant dithionite is supposed to remove essentially oxyhydroxides of Fe, but may in part also dissolve oxides of Mn, Al and Si (Anderson and Jenne, 1970; Bigham et al., 1978; Turchenek and Oades, 1979). In this study, the mean ratio of mass loss (caused by the dithionite-treatment) to $Fe_d$ contents was 3. For iron oxides, an empirical conversion factor of 2 is used to calculate the total mass of oxides from $Fe_d$ values (Cornell and Schwertmann, 1996). The higher "conversion factor" of 3 obtained in this study may be explained by the fact that, in addition to iron oxides, traces of other minerals were extracted, and/or Fe was partly substituted by Al in the oxides.

### 3.2.6 Determination of dithionite and oxalate extractable Fe ($Fe_d$ and $Fe_o$)

For the determination of dithionite-extractable Fe ($Fe_d$), the methods of Mehra and Jackson (1960) and Homgren (1967) were followed. An aliquot of 200-300 mg of the fraction material was shaken with a mixture of 40 mL 0.3 M sodium citrate solution ($Na_3C_6H_5O_7$), 10 mL 1.0 M sodium hydrogen carbonate solution ($NaHCO_3$) and 1 g of sodium dithionite ($Na_2S_2O_4$) for 16 h at room temperature. After centrifugation the supernatant was saved. The residue was mixed with 40 mL of 0.05 M $MgSO_4$ solution to exchange the sorbed Fe with Mg, and centrifuged a second time. The Fe in the combined supernatants was measured by atomic absorption spectroscopy after appropriate dilution.

The oxalate-extractable fraction of Fe ($Fe_o$) was determined according to Schwertmann, (1964). A 100-200 mg sample was shaken with 50 mL of an acidic oxalate solution (pH around 3) for 2 h in the dark. The solution contained 0.2 M oxalate and was prepared by mixing a 0.113 M ammonium oxalate $(COONH_4)_2$ and a 0.087 M oxalic acid solution $(COOH)_2$. The extracts were filtered and the diluted filtrates analyzed for Fe by atomic absorption spectroscopy.

### 3.2.7 X-ray diffraction analysis

X-ray diffraction (XRD) analyses of the coarse clay (2-0.2 µm) samples from Thyrow and Skierniewice were performed on a Philips PW 1070 diffractometer using Co K-alpha radiation. Samples were examined free of OM and iron oxides, using the same procedures for removing these compounds as described in section 3.2.5. Analysis was done of Ca-saturated samples, both as random powder and as oriented specimens after glycerol-treatment. Semiquantitative characterization of layer silicates in the glycerol-treated samples was carried out using the intensity correction factors given by Stanjek et al. (1992). Curve-fitting and calculations were done by using the software XFit4Mac.

### 3.2.8 Statistics

Correlation and linear regression analyses were performed with the software SigmaPlot 4.00 (SPSS Corp.).

## 3.3 Results and discussion

### 3.3.1 Organic carbon in relation to total mineral surface area

In all the soil samples, the specific surface area of the OM-free separates increased with decreasing particle-size (Table 2). Regarding the magnitude of SSA values, Bad Lauchstädt was different from the sandy soils (Thyrow/Skierniewice). Especially in the clay (fractions < 2 µm), the soil from Bad Lauchstädt showed a higher surface area than the two other soils. The differences in SA may indicate a divergent mineralogical composition of the clay fraction in Bad Lauchstädt versus the sandy soils from Thyrow/Skierniewice.

#### *3.3.1.1 Sandy soils (Thyrow, Skierniewice)*

When relating the mineral surface area in the fractions to the corresponding OC contents, two different pictures emerged (Figs. 2a and 2b): In the sandy soils (Thyrow and Skierniewice), OC and surface area were highly positively correlated, whereas there was no systematic relationship between the two variables in the different samples of the loamy soil (Bad Lauchstädt). As the data points from Thyrow and Skierniewice fitted well to the same regression scheme, we evaluated the results of the two sites together. At the two sites, the OC content in fractions < 6 µm was best described as a linear function of the mineral surface area for both treatments (Fig. 2a). The two curves in Fig. 2a representing the contrasting treatments have a nearly identical slope. The slope indicates that, starting from the theoretical C amount as given by the intercept, an increase of surface area by 1 $m^2$ resulted in a gain of 0.25 / 0.26 mg OC across the fractions. Apparently, C-depletion did not alter the slope of the curve, i.e. the degree to which OC contents are explained by SA, but resulted in a decrease of the y-intercept by 22 units (Fig. 2a). This decrease would correspond to an average OC decrease of 22 mg per g fraction mass across the separates in the depleted plots.

According to our experimental concept, that amount of OC which decreases in the depleted plots compared with the fertilized plots may be assigned to a fraction of *labile* C accumulated in the fertilized soils. On the other hand, the OC retained in the depleted plots is supposed to comprise mainly *refractory* compounds, whose turnover is not affected by changes in soil management at the short-/mid-term. This concept may be applied both to the bulk OC (bulk samples) and to the OC of defined physical fractions of the soils. Therefore, the findings illustrated in Fig. 2a may be interpreted in relation to the different OC pools: the lower line (depleted plots) would be a good approximation of *refractory* OC, whereas the distance between the upper and lower curve (difference between the intercepts) would represent *labile* OC.

**Table 2:** Specific surface area of the total mineral matrix in the different experimental plots from the three long-term experiments.

| | **SSA $_{\text{minerals}}$** ($m^2$ $g^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | **Thyrow** | | **Skierniewice** | | **Bad Lauchstädt** | |
| **Particle-size fractions** (µm) | **Fertilized** | **Depleted** | **Fertilized** | **Depleted** | **Fertilized** | **Depleted** [a] |
| 6 - 2 | 21 | 17 | 14 | 19 | 28 | 23 |
| 2 – 0.2 | 33 | 31 | 19 | 19 | 74 | 78 |
| < 0.2 | 100 | 89 | 76 | 73 | 157 | 145 |

[a] Unmanured plot

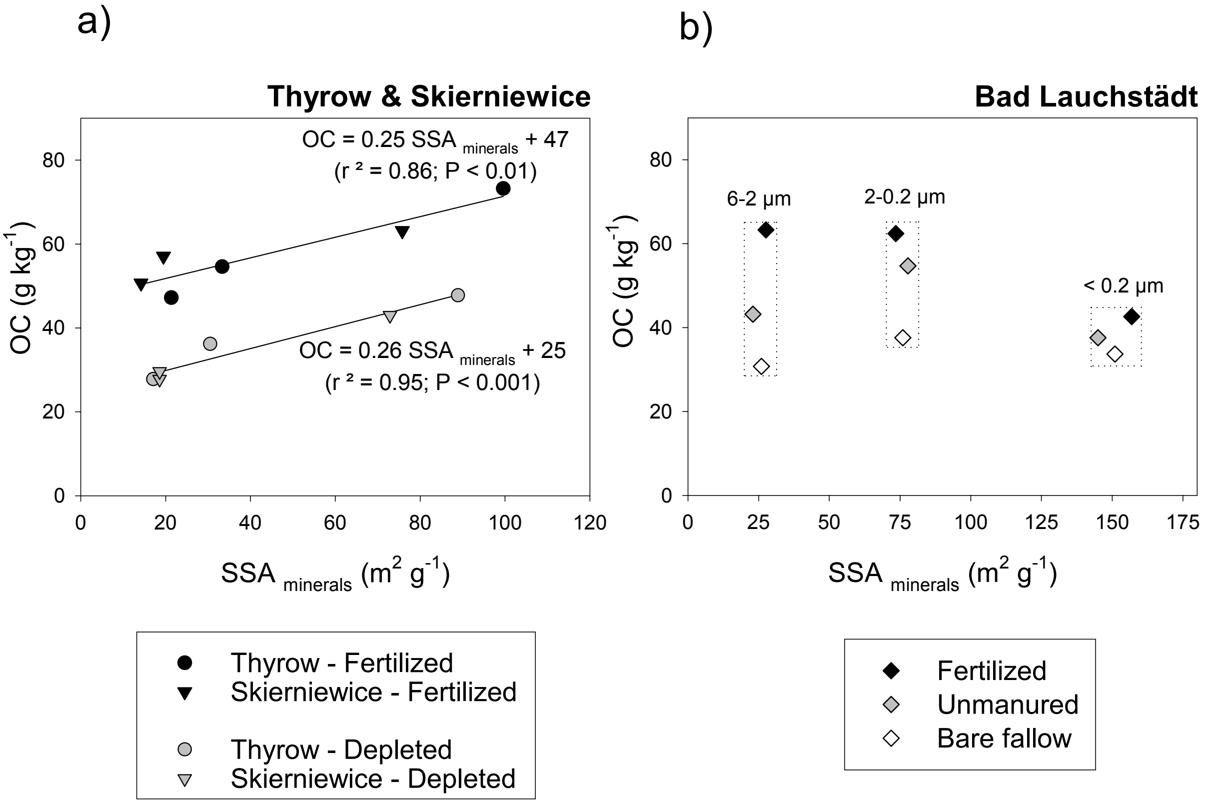


**Fig. 2:** OC contents versus SSA of mineral matrix (SSA $_{\text{minerals}}$) in fractions < 6 µm of the sandy soils (Thyrow, Skierniewice) (a), and of the loamy soil (Bad Lauchstädt) (b).

Examining the regression curve for the depleted plots (Fig. 2a), it follows that the amount of *refractory* OC is related to the size of the mineral SA. According to this, the amount of OC stabilized at the long-term in the fine separates depends on the available SA, and SA plays an important role for the formation of a refractory pool of SOC. The dependency of OC storage on SA suggests that the organic compounds closely interact with minerals. The regression equation for the depleted plots, however, has a rather high intercept (25 mg OC $g^{-1}$) in the relation between SSA and OC, and only part of the total OC contents can be explained on the basis of SA. This suggests that part of the OC in the fine fractions is closely associated with mineral surfaces, as proposed above, but some OC compounds do not show this close interaction. According to Keil et al. (1994) and Mayer (1994b), if most of the OC in the various fractions is associated with the mineral matrix, the regression line goes through the origin and the slope of the curve can be directly interpreted in terms of OC loading per unit of surface area.

The distance between the two regression lines may be a measure for the amount of *labile* C. This value was similar across the fractions and thus independent of the fractions` surface area. Since the amount of *labile* C was not related to the SSA of the fractions, one hypothesis is that the labile C in the fertilized soils of Thyrow and Skierniewice had no close physical association with minerals.

The interpretation that the labile and refractory C pool differ in their degree of association with the mineral phase was further examined by means of density fractionation for the fractions 6-0.2 µm from Thyrow (Table 3). The decrease of OC contents for the whole particle-size separates was clearly due to a decline of OC in the light fraction (≤ 2 g $cm^{-3}$). By contrast, the OC concentration in the heavy fraction (> 2 g $cm^{-3}$) remained unchanged in the depleted versus fertilized plot. Due to this drop in light fraction C, the depleted plot exhibited a higher (or the major) proportion of total OC in the heavy fraction (Table 3). Density fractions ≤ 2 g $cm^{-3}$ mainly comprise OM which is not complexed with minerals, whereas in fractions > 2 g $cm^{-3}$ the organic material is present in organo-mineral associates (Turchenek and Oades, 1979; Christensen, 1992). These data show that the amount of mineral-associated OC was not affected by the overall SOC-depletion, whereas the OC not (closely) associated with minerals declined under the C-depleting management conditions. For fractions 6-0.2 µm from Thyrow, density fractionation confirmed that the refractory C can mainly be identified as C being intimately associated with soil minerals, whereas the labile C lies within light OM fractions.

Relating the OC contents of the density fractions > 2 g $cm^{-3}$ to the mineral SA of the separates yields similar OC loading values for the different treatments, i.e. 0.7 to 0.8 mg C $m^{-2}$ (2-0.2 µm) and 0.5 mg C $m^{-2}$ (6-2 µm), respectively. These values fall within the monolayer-equivalent level of C coverage defined by Mayer (1994a, 1994b) for sediments and soils, ranging between 0.5 and 1.0 mg C $m^{-2}$ SA. The OC-to-SA ratios of numerous samples from different environments lie within this range, and Mayer (1994a) thus suggested this level as a common measure for the (maximum) extent to which mineral surfaces are able to protect C. Our results show that OC present at an OC-to-SA ratio of 0.5 to 0.8 mg C $m^{-2}$ in fractions < 6 µm can be stabilized at the long-term in the soil from Thyrow. Furthermore, we conclude that this level of OC loading represents a maximum with respect to the protection capacity of the minerals of this soil. This assumption is based on the finding that the average C loading of mineral surfaces was very similar in the fertilized and the C-depleted plots at this site, although the C-input rates are much higher in the fertilized plot. The excess of C in the fertilized soil was instead accumulated in light density fractions.

**Table 3:** Distribution of OC between the density fractions ≤ 2 and > 2 g $cm^{-3}$ in particle-size separates from Thyrow and Bad Lauchstädt.

| | **Fertilized** | | **Depleted** | | **Δ (Depleted - Fertilized)** |
|---|---|---|---|---|---|
| | **OC** | | **OC** | | **OC $_{Depleted}$ – OC $_{Fertilized}$** |
| **Particle-size/density fractions** | (g $kg^{-1}$) [a] | (% of total OC in fraction) | (g $kg^{-1}$) | (% of total OC in fraction) | (g $kg^{-1}$) |
| Thyrow, 6-2 µm | | | | | |
| whole particle-size fraction | 47 | | 28 | | - 19 |
| ≤ 2 g $cm^{-3}$ | 39 | 82 | 21 | 76 | - 18 |
| > 2 g $cm^{-3}$ | 8 | 18 | 7 | 24 | - 1 |
| Thyrow, 2-0.2 µm | | | | | |
| whole particle-size fraction | 55 | | 36 | | - 19 |
| ≤ 2 g $cm^{-3}$ | 33 | 60 | 10 | 28 | - 23 |
| > 2 g $cm^{-3}$ | 22 | 40 | 26 | 72 | + 4 |
| Bad Lauchstädt [b], 6-2 µm | | | | | |
| whole particle-size fraction | 63 | | 43 | | - 20 |
| ≤ 2 g $cm^{-3}$ | 34 | 54 | 15 | 35 | - 19 |
| > 2 g $cm^{-3}$ | 29 | 46 | 28 | 65 | - 1 |
| Bad Lauchstädt [b], 2-0.2 µm | | | | | |
| whole particle-size fraction | 62 | | 55 | | - 7 |
| ≤ 2 g $cm^{-3}$ | 11 | 18 | 10 | 18 | - 1 |
| > 2 g $cm^{-3}$ | 51 | 82 | 45 | 82 | - 6 |

[a] OC expressed in terms of the whole particle-size fraction

[b] C-depleted plot (Bad Lauchstädt) = unmanured plot

### *3.3.1.2 Loamy soil (Bad Lauchstädt)*

No positive correlation between SA and OC content across the three fractions is discernable for any treatment at Bad Lauchstädt (Fig. 2b). At this site, OC levels gradually decrease from the fertilized plot to the unmanured plot to the bare fallow (Fig. 2b, Table 1). In the fertilized soil, OC was highest in the fractions 6-0.2 µm. In the unmanured soil, similar contents were associated with the fine silt (6-2 µm) and the fine clay (< 0.2 µm), although these fractions differed considerably in SA, the highest content being associated with coarse clay (2-0.2 µm). Comparing the unmanured soil with the fertilized one, the decline in OC was most pronounced in the fine silt. Further C-depletion in the bare fallow caused OC to drop in fine silt and coarse clay, but did not affect the fine clay (Fig. 2b). The total decline of C (bare fallow versus fertilized soil) was higher in the fractions 6-0.2 µm than in the fine clay (average of minus 29 mg $g^{-1}$ for the former fractions, minus 9 mg $g^{-1}$ for the latter fraction). In the bare fallow, the OC contents of the three fractions were similar.

The inconsistent SA-OC trend in the Bad Lauchstädt samples may have several reasons. It may be expained by one or more of the following hypotheses: i) In the fine silt/coarse clay, a higher proportion of the total OC may be present in light density fractions - representing OC which is not or weakly associated with minerals - compared with the fine clay. ii) Average OC loadings (mg C $m^{-2}$ SA) may differ among the three fractions, possibly resulting from differences in mineralogical composition. From Ransom et al. (1998) it can be inferred that various clay minerals vary in the OC loading rates of their surfaces. iii) In the fine clay, part of the available SA may not be occupied by OC and the theoretical protection capacity of mineral surfaces may not be fully exploited. Such an interpretation is based on the OC-to-SA ratio of 0.3 mg C $m^{-2}$ noted for the fine clay, which is lower than the values for the fine clay at the other sites (Table 4). Assuming that most of the OC here is associated with minerals, this ratio would approximate the average OC loading per unit SA. This value would be below the monolayer-equivalent range (0.5-1.0 mg C $m^{-2}$), representing a level of OC loading frequently found in soils and sediments Mayer (1994a).

For two of the separates (fine silt, coarse clay) the partitioning of OC into a light ($\leq 2$ g $cm^{-3}$) and heavy ($> 2$ g $cm^{-3}$) subfraction is given in Table 3. In the coarse clay (2-0.2 µm) the decrease of OC was small. In the fine silt (6-2 µm) the loss of OC was higher, affecting mainly the light fraction, whereas C in the heavy fraction remained stable. As a consequence, in the C-depleted plot a higher percentage of the fraction`s OC was found in the heavy fraction compared with the fertilized plot. The persistence noted for that C fraction which is associated with minerals (heavy fraction) indicates that the interaction with minerals is an important mechanism for C stabilization in the loamy soil. Although there is no consistent relationship between mineral SA and total OC contents (C in light + heavy fractions) in the loamy soil, density fractionation gave similar results as for the sandy soil (Thyrow): C associated with the mineral phase has a refractory nature in contrast to C which is not so closely associated with minerals.

The ratios of OC-to-SA calculated for the heavy fractions were identical for the contrasting treatments, amounting to 1.1 mg C $m^{-2}$ in the 6-0.2 µm fraction and to 0.6 mg C $m^{-2}$ in the 2-0.2 µm fraction. The finding of identical ratios for the two treatments is in line with the sandy soil (Thyrow). The values are within the monolayer-equivalent range. The similarity of the ratios in the contrasting treatments suggests that i) on the one hand, an association of OC with minerals leads to a long-term stabilization (no decline in the C-depleted plots), and ii) on the other hand, mineral surfaces are "saturated" with OC at an average coverage of 0.6 (coarse clay) or 1.1 mg C $m^{-2}$ (fine silt) (no increase in the fertilized plots). Analogous to Thyrow, the higher total OC amounts in the fertilized plot are due to an accumulation of C in the light fraction. The data from the loamy soil also show that the different particle-size separates of a particular soil may vary to some extent in the mean OC loading of mineral surfaces, possibly originating from differences in the mineralogical composition.

#### *3.3.1.3 Sandy soils versus loamy soil*

Several workers observed that different soil types differ with respect to the pattern of OC contents across the size separates, as summarized in Turchenek and Oades (1979). The latter proposed that soils may be classified into two groups according to their physico-chemical properties and their pattern of OC storage in size separates: i) a group of acidic soils with low clay contents, characterized by highest OM contents in the finest fraction, and ii) a group of soils with high clay content and high base status, in which the fine silt/coarse clay are richer in OM than the fine clay. The soils of the present study fit this classification: The sandy soils (Thyrow, Skierniewice), having a pH of 4 in the absence of liming, showed an increase of OC contents with decreasing particle-size and would fall into the first group. The loamy soil (Bad Lauchstädt), with a pH of 7, showed highest OC concentrations in fractions 6-0.2 µm (fertilized and unmanured plots) and may be assigned to the second group. In fractions < 6 µm of the sandy soils, the increase of OC was related to the increase in mineral SA, suggesting that the direct interaction of organic compounds with mineral surfaces is a dominant stabilization mechanism. According to Oades (1989), in soils of the second group (with high Ca saturation), organic compounds are associated with clay minerals through Ca bridges, leading to the build-up of stable micro-aggregates (< 20 µm) which are enriched in OM. These aggregates might not be completely broken down during ultrasonification, e.g. aggregated clay particles may be isolated in the fine silt fraction which would explain high OC contents in the latter fraction**.** In the loamy soil from Bad Lauchstädt, such resistant micro-aggregates may be present, in which OC is stabilized through both an interaction with minerals and entrapment within the aggregates.

#### *3.3.1.4 Ratios of organic carbon–to–surface area*

For all sites, the amount of OC in a separate normalized to the respective mineral surface area gradually decreased from fine silt to coarse clay to fine clay (Table 4). In the fine silt, the OC amount theoretically attributable to a unit of surface area was 3 times (Thyrow), 3 to 4 times (Skierniewice), and 6 to 7 times (Bad Lauchstädt) that of the respective ratio of the fine clay. A decrease of OC-to-SA ratios with diminishing particle-size in the range of silt and clay separates was reported by e.g. Broersma and Lavkulich (1980), Feller et al. (1992). The higher (theoretical) OC loading in coarser fractions may be explained by a higher proportion of OC in light fractions in the silt versus finer (clay) separates (Christensen, 1996). A high proportion of C in light density fractions would increase the OC-SA ratio for the whole particle-size fraction.

### 3.3.2 Effect of OM-removal on surface area

Removing OM increased the specific surface area of all particle-size fractions in all soil samples (Figs. 3a and 3b) (see equation 1). In the sandy soils (Thyrow and Skierniewice) the gain in SA was linearly related to the decrease in OC contents across the fractions in both treatments (Fig. 3a), whereas there was no proportionality between OC-decrease and SA-increase in the loamy soil (Bad Lauchstädt) (Fig. 3b).

An increase in the $N_2$-BET surface area following removal of OM has been frequently observed for bulk soil samples (Burford et al., 1964; Pennell et al., 1995; Torres Sanchez and Falasca, 1997; Theng et al., 1999). Feller et al. (1992) explained this effect by postulating that OM binds clay particles together, and thus part of the mineral surfaces would be “hidden” within the aggregates. Another explanation is that organic matter coats the mineral surface, thereby preventing a sorption of $N_2$ onto those surfaces (Burford et al., 1964). This hypothesis was supported by the work of De Jonge and Mittelmeijer-Hazeleger (1996), who determined

**Table 4:** Ratios of OC-to-mineral surface area in the different experimental plots from the three long-term experiments.

| | **OC-to- SSA $_{\text{minerals}}$ (mg C $m^{-2}$)** | | | | | |
|---|---|---|---|---|---|---|
| | **Thyrow** | | **Skierniewice** | | **Bad Lauchstädt** | |
| **Particle-size fractions** (µm) | **Fertilized** | **Depleted** | **Fertilized** | **Depleted** | **Fertilized** | **Depleted** [a] |
| 6 - 2 | 2.2 | 1.6 | 3.6 | 1.5 | 2.3 | 1.9 |
| 2 – 0.2 | 1.6 | 1.2 | 2.9 | 1.6 | 0.8 | 0.7 |
| < 0.2 | 0.7 | 0.5 | 0.8 | 0.6 | 0.3 | 0.3 |

[a] Unmanured plot

**Table 5:** Contents of $Fe_d$ and $Fe_o$, and $Fe_o$-to-$Fe_d$ ratios in the contrasting treatments from Thyrow and Skierniewice.

| | **Thyrow** | | | | | | **Skierniewice** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Fertilized** | | | **Depleted** | | | **Fertilized** | | | **Depleted** | | |
| **Particle-size fractions** (µm) | **$Fe_d$** (g $kg^{-1}$) | **$Fe_o$** (g $kg^{-1}$) | **$Fe_{o/d}$** | **$Fe_d$** (g $kg^{-1}$) | **$Fe_o$** (g $kg^{-1}$) | **$Fe_{o/d}$** | **$Fe_d$** (g $kg^{-1}$) | **$Fe_o$** (g $kg^{-1}$) | **$Fe_{o/d}$** | **$Fe_d$** (g $kg^{-1}$) | **$Fe_o$** (g $kg^{-1}$) | **$Fe_{o/d}$** |
| 6 - 2 | 11 | 8.3 | 0.73 | 11 | 8.2 | 0.73 | 8.5 | 6.9 | 0.81 | 9.4 | 7.9 | 0.84 |
| 2 – 0.2 | 17 | 12 | 0.71 | 16 | 12 | 0.75 | 12 | 9.6 | 0.83 | 10 | 8.9 | 0.89 |
| < 0.2 | 32 | 23 | 0.72 | 32 | 23 | 0.72 | 21 | 14 | 0.68 | 21 | 15 | 0.74 |

that $N_2$ at 77 K is unable to penetrate into the microporous network in humic substances, but is limited to the "external" surface. Accordingly, organic matter represents a diffusion barrier for $N_2$, preventing the access of $N_2$ to minerals. The $N_2$-BET surface area of organic matter itself was found to be very low, e.g. $\leq 1$ $m^2$ $g^{-1}$ (Chiou, 1990). Based on these ideas, the net change in SA obtained after OM-oxidation could be an estimate for that mineral SA which was previously covered with/"blocked" by OM. Thus, studying the effect of OM-removal on surface area may provide information on the association between OM and minerals.

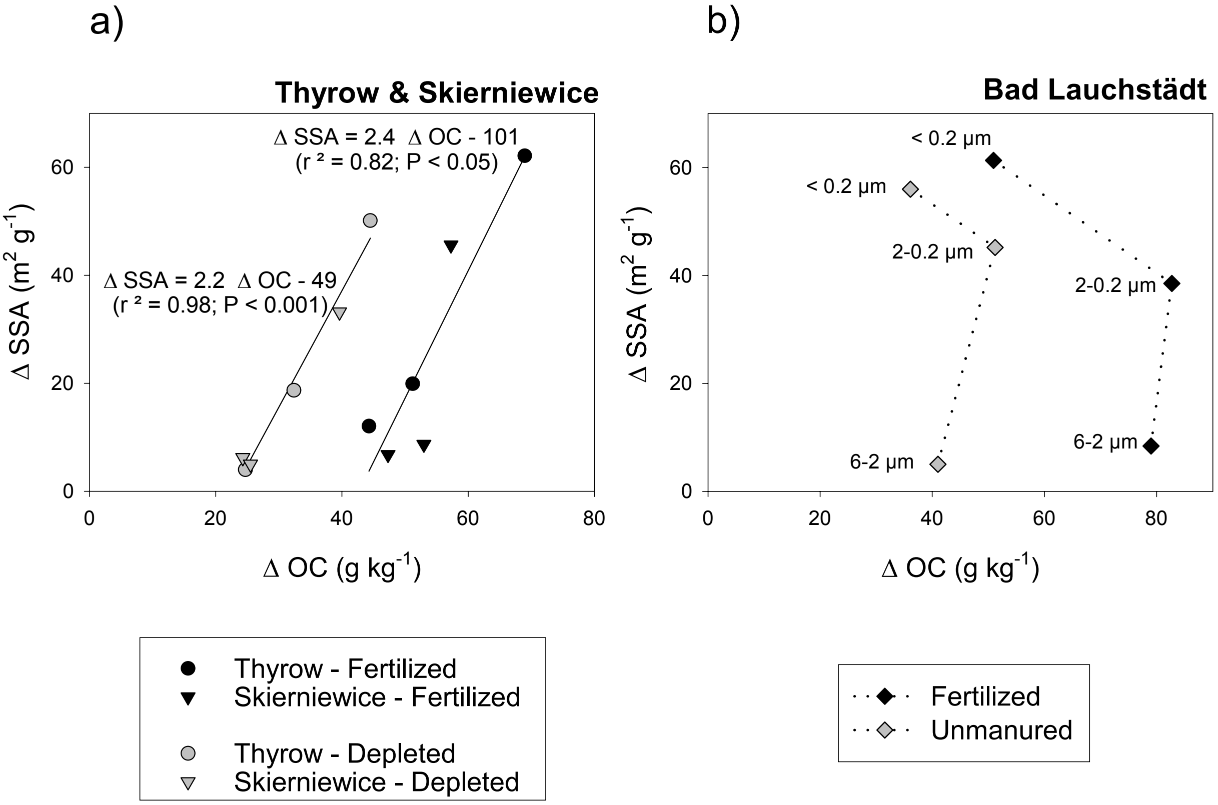


**Fig. 3:** Increase of SSA (Δ SSA) in relation to the decrease of OC contents (Δ OC) following oxidation of organic matter for fractions < 6 µm of the sandy soils (Thyrow, Skierniewice) (a), and of the loamy soil (Bad Lauchstädt) (b).

For the different experimental sites, the patterns noted for the effect of OM-removal on SA were equivalent to those found in the relation of OC to total SSA (section 3.3.1). For the sandy soils (Thyrow, Skierniewice) both approaches indicate that i) OC contents and the mineral SA are linearly related to each other in fine separates, but also that ii) a certain amount of OC cannot be solely explained based on SA, as indicated by the intercept on the OC-axes (y-axis in Fig. 2a, x-axis in Fig. 3a). According to the slope of the regression equations (Fig. 3a), an OC decrease of 1 mg would result in the "exposure" of 2.2 and 2.4 $m^2$ of mineral SA in the depleted and fertilized plots, respectively. The reciprocal values of the slopes would correspond to a theoretical OC "coverage" of 0.4 / 0.5 mg C $m^{-2}$ SA. Analogously to Fig. 2a, the intercept on the axis representing OC contents (this time x-axis) lies at approximately 20 and 40 mg C $g^{-1}$ fraction for the depleted and fertilized soils, respectively. Theoretically, removing these OC amounts would yield no gain of surface area. For the loamy soil (Bad Lauchstädt), the effect of OM-removal on SA confirms i) the lack of a systematic relationship between SA and total OC contents in the separates, and ii) the contrasts between the fine silt and fine clay fractions with respect to the ratio of total OC-to-SA (Fig. 3b). In both treatments, removing OC in the fine silt only slightly increased SA. In the fine clay, less OC was removed but this had a much greater effect in terms of SA. This would be consistent with a higher (theoretical) OC loading per unit SA in the fine silt versus fine clay (as seen in Table 4, section 3.3.1.4).

### 3.3.3 Organic carbon in relation to oxides

#### *3.3.3.1 Contents of $Fe_d$ and $Fe_o$*

In all soil samples, the contents of both $Fe_o$ and $Fe_d$ steadily increased with decreasing particle-size, thereby maintaining a similar $Fe_o/Fe_d$ ratio across the three size separates (Tables 5 and 6). This ratio is a measure for the proportion of poorly crystalline oxides ($Fe_o$) within total iron oxides ($Fe_d$). The value of $Fe_o$ is considered to reflect mainly the amount of ferrihydrite, the difference between $Fe_d$ and $Fe_o$ ($Fe_d - Fe_o$) representing well-ordered / crystalline iron oxides (Cornell and Schwertmann, 1996). In the sandy soils (Thyrow, Skierniewice), the ratios were of similar magnitude: poorly crystalline oxides (ferrihydrite) accounted for 70 to 90% of the total iron oxides and thus dominating over crystalline phases (Table 5). The opposite is true for the loamy soil (Bad Lauchstädt), where $Fe_o$ made up 30 to 40% of $Fe_d$ (Table 6). Regarding the distribution of iron oxides among the fractions, note that, based on their size (e.g. goethite 50-10 nm, ferrihydrite 5-2 nm), all iron oxides should appear within the fine clay. The recorded presence in coarser fractions may indicate that the association of oxides with coarser minerals in soil was not completely destroyed by the ultrasonic treatment.

Summarizing the data of both treatments from Thyrow and Skierniewice, a strong positive correlation between the contents of $Fe_d$ and the specific surface area in the fractions < 6 µm was found ($SSA_{minerals} = 3.7\ Fe_d - 19$; $r^2 = 0.91$; $P < 0.001$). Also in the samples from Bad

**Table 6:** Contents of iron oxides ($Fe_d$ and $Fe_o$) and OC as well as $Fe_o$-to-$Fe_d$ ratios in the contrasting treatments from Bad Lauchstädt.

| | **Bad Lauchstädt** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Fertilized** | | | | **Depleted** [a] | | | |
| **Particle-size fractions** (µm) | **$Fe_d$** ($g\ kg^{-1}$) | **$Fe_o$** | **$Fe_{o/d}$** | **OC** ($g\ kg^{-1}$) | **$Fe_d$** ($g\ kg^{-1}$) | **$Fe_o$** | **$Fe_{o/d}$** | **OC** ($g\ kg^{-1}$) |
| 6 - 2 | 8.5 | 3.3 | 0.39 | 63 | 9.7 | 3.0 | 0.31 | 31 |
| 2 – 0.2 | 16 | 5.6 | 0.36 | 62 | 15 | 4.8 | 0.33 | 38 |
| < 0.2 | 21 | 6.8 | 0.32 | 43 | 22 | 6.7 | 0.30 | 34 |

[a] Bare fallow

**Table 7:** Contributions from oxides and silicates to the total mineral surface area in the fertilized plots from Thyrow and Skierniewice.

| | **Thyrow** | | | | **Skierniewice** | | | |
|---|---|---|---|---|---|---|---|---|
| **Particle-size fractions** (µm) | **$SSA_{oxides}$** | **$SSA_{silicates}$** ($m^2\ g^{-1}$ fraction) | **$SSA_{minerals}$** | **$SSA_{oxides}$** [a] (% of $SSA_{minerals}$) | **$SSA_{oxides}$** | **$SSA_{silicates}$** ($m^2\ g^{-1}$ fraction) | **$SSA_{minerals}$** | **$SSA_{oxides}$** (% of $SSA_{minerals}$) |
| 6 - 2 | 16 | 5 | 21 | 75 | 9 | 5 | 14 | 61 |
| 2 – 0.2 | 26 | 7 | 33 | 78 | 10 | 9 | 19 | 52 |
| < 0.2 | 82 | 18 | 100 | 82 | 60 | 16 | 76 | 79 |

[a] $$\frac{SSA_{oxides} \times 100}{SSA_{minerals}}$$

Lauchstädt, $Fe_d$ values and SSA were correlated ($SSA_{minerals} = 9.8\ Fe_d - 64$; $r^2 = 0.97$; $P < 0.001$). This correlation across the fractions is a hint that iron oxides are important contributors to the SA in these samples.

For the relationship between $Fe_d$ and OC contents, an analogous picture resulted as for the SSA-OC relation at the various sites. In Bad Lauchstädt, OC varied independently of iron oxides ($Fe_d$ and $Fe_o$) in both the fertilized plot and the bare fallow (Table 6). As seen before, the correlation approach including total OC contents (without differentiation on the basis of density) does not clarify the role of mineral surfaces/iron oxides in preserving OC in this soil. For Thyrow and Skierniewice, OC increased linearly with increasing total iron oxides ($Fe_d$) in both treatments (Fig. 4). OC contents were also significantly correlated with the two subfractions of iron oxides, i.e. the contents of poorly crystalline ($Fe_o$) and crystalline oxides ($Fe_d - Fe_o$) in both the fertilized plots ($OC = 1.5\ Fe_o + 39$; $r^2 = 0.88$; $P < 0.01$; $OC = 2.9\ (Fe_d - Fe_o) + 45$; $r^2 = 0.75$; $P < 0.05$) and the depleted plots ($OC = 1.4\ Fe_o + 18$; $r^2 = 0.91$; $P < 0.01$; $OC = 2.9\ (Fe_d - Fe_o) + 24$; $r^2 = 0.87$; $P < 0.01$). The similar slopes in the two treatments in Fig. 4 indicate that the dependence of OC concentrations on $Fe_d$ contents was similar in the depleted and fertilized plots. OC depletion, represented by the distance between the two regression lines, was similar across the fractions and approximated the decrease in the y-intercept values (21 mg C $g^{-1}$ fraction) (Fig. 4). One interpretation for these findings, analogous to the SSA-OC relationship, is that the amount of *refractory* C is proportional to the content of oxides in a separate, whereas the amount of *labile* C is independent of this content. Based on the correlation between refractory OC and both SSA and iron oxides, the association of OC with mineral surfaces, including the surface of iron oxides, may be a relevant mechanism for stabilizing OC in these sandy soils.

Positive correlations between iron oxides and OC in physical fractions have been reported for various soils, indicating that iron oxides may be involved in preserving SOC, for example for separates < 5 µm of a red-brown earth and a chernozemic soil (OC – $Fe_d$) (Turchenek and Oades, 1979), for the silt (OC – $Fe_o$) and for magnetic subfractions of clay separates (OC-$Fe_d$) in a tropical Alfisol and Oxisol (Shang and Tiessen, 1998), as well as for the clay in B horizons of Spodosols (OC – $Fe_o$) (Adams and Kassim, 1984).

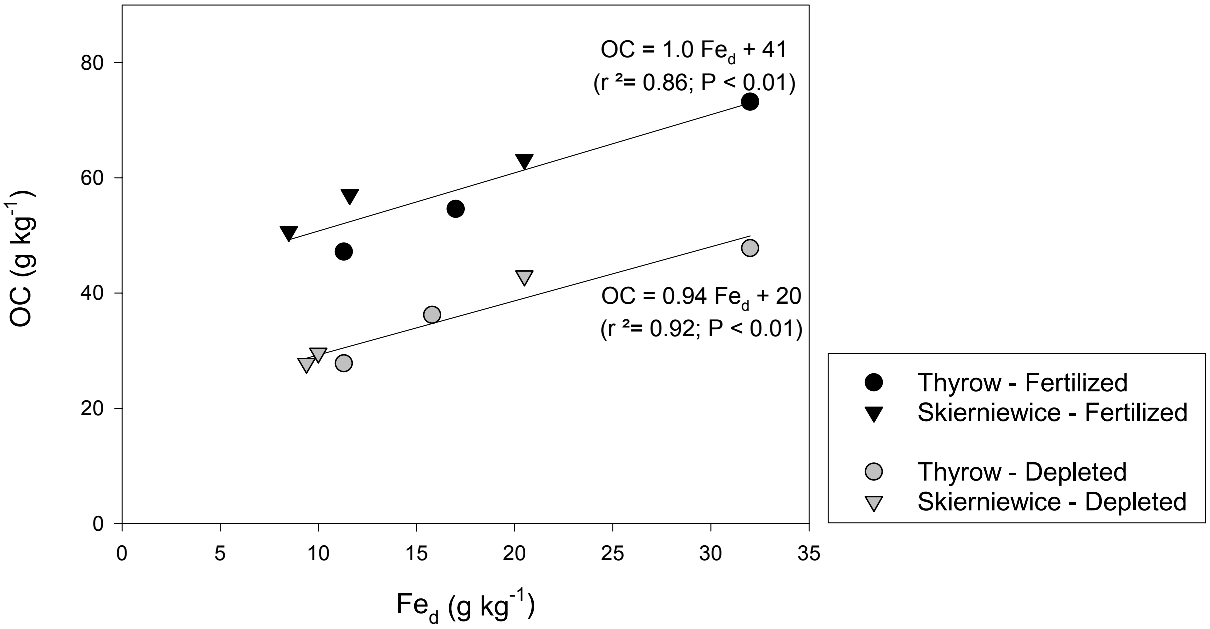


**Fig. 4:** OC contents versus contents of dithionite extractable iron (Fe$_d$) in fractions < 6 µm for the sandy soils (Thyrow, Skierniewice).

### *3.3.3.2 Contribution of oxides to the mineral surface area (sandy soils)*

The following section presents the results of the fertilized plots from Thyrow and Skierniewice. As the samples from the different treatments of a site had a similar mineralogy (total SSA, content and composition of iron oxides), they can also be expected to be similar regarding the influence of oxides/silicates on surface area. Thus, the results from the fertilized plots may also be valid for the depleted plots. Table 7 shows that the absolute SA contribution from oxides was always higher than that from the silicates, with one exception (coarse clay from Skierniewice) (calculations according to equations 2 and 3). The largest gap in these contributions occurred in the fine clay. In relative terms, oxides contributed 75 to 82% to the total mineral surface area for Thyrow and 52 to 79% for Skierniewice. The relevant contribution in terms of SA contrasts with the minor contribution in terms of mass, with DCB-extractable compounds accounting for 3% (fine silt), 5% (coarse clay) and 9% (fine clay) of the total mineral matter (means for the two sites).

Relating the SSA contribution of oxides to their content in a separate (equation 4), the resulting component-specific surface area values ranged from 260 to 460 m$^2$ g$^{-1}$ in the fractions 6-0.2 µm, and amounted to 800 m$^2$ g$^{-1}$ in the fraction < 0.2 µm. As mentioned before, the iron oxides in these soils are presumably dominated by ferrihydrite. For ferrihydrite in natural samples, a N$_2$-BET surface area between 200 and 400 m$^2$ g$^{-1}$ has been reported (Cornell and Schwertmann, 1996). The surface area of crystalline oxides may be considerably lower. Borggaard (1982) and Fontes and Weed (1996) observed surface areas between 45 and 200 $m^2$ $g^{-1}$ for crystalline oxides in soil samples. The SSA values for the oxides at Thyrow and Skierniewice are consistent with a high proportion of ferrihydrite. The exceptionally high value in the fine clay may reflect the presence of very fine oxide particles. Assuming spherical shape and a density of 4 g $cm^{-3}$, the surface area of 800 $m^2$ $g^{-1}$ would correspond to a particle diameter of 2 nm, which is near the lower size limit for ferrihydrite (Borggaard, 1982).

By contrast, silicates exhibited a specific surface area of 6 to 8 $m^2$ $g^{-1}$ silicate mass in the fractions 6-0.2 µm, and 20 $m^2$ $g^{-1}$ in the separates < 0.2 µm (equation 4), whereby the values for the two soils were nearly identical. This is considerably lower than the $N_2$-BET SA of different species of clay minerals (e.g. Theng et al., 1999), which are common constituents of fine particle-size fractions. To better interpret our findings, we investigated the mineralogical composition of the coarse clay at both sites. XRD analysis revealed a resemblance in the gross composition of the silicate-fraction. Analysis of the random powder samples showed that quartz and feldspars were abundant within the coarse clay. Regarding the relative composition of the layer silicates, there was a minor contribution of kaolinite (16% and 12%), and illite accounted for a large proportion of total layer silicate mass in Thyrow (44%) and Skierniewice (35%). Smectite was not detected in Thyrow but made up 43% of the layer silicates in Skierniewice. The corresponding values for the sum of chlorite and vermiculite were 41% (Thyrow) and 9% (Skierniewice). Based on the XRD data, the low SSA of silicates may be primarily explained by an abundance of quartz and feldspars, which presumably have a much lower surface area than most layer silicates. Among the factors which determine the SSA of minerals are i) particle-shape, whereby flat plates (layer silicates) have a higher SA-to-volume ratio than more spherical minerals (quartz), and ii) the presence of internal surfaces in expansible layer silicates (smectites); the $N_2$-BET method measures both internal and external surfaces (Keil et al., 1994; Ransom et al., 1998). Apart from a probably high contribution of quartz/feldspars, especially in Thyrow non-expansible minerals seem to dominate among the layer silicates. This may also have contributed to low SA values.

According to these data, oxides and silicates differed by one or two orders of magnitude in their component-specific SSA. Thus, these two compounds differ considerably in their potential SA contribution. Under the conditions of an overall low SA developed by the silicates in these sandy soils, the contribution from oxides determines the magnitude of the total SA in the fine fractions. Fig. 5 shows that the SA contribution from oxides across the fractions was directly proportional to the of iron oxide content, as given by the dithionite extractable iron ($Fe_d$). Thus, the oxide-related SA increase with decreasing particle-size (Table 7) resulted from the increase of $Fe_d$ contents (Table 5). The correlation between $Fe_d$ and SA, attributable to oxides/DCB-extractable components, corroborates the assumption that DCB-treatment removed mostly iron oxides (see 3.2.5.3). In terms of mass, oxides generally contributed little to total mineral matter in the three fractions (from 3 to 9% versus 91 to 97% attributable to silicates). Owing to the much higher SSA of oxides compared with that of the silicates, an increase in oxide content from e.g. 3% (fine silt) to 9% (fine clay) – a minor change in the gross composition of the mineral matrix – had a large effect on the (total) SA (Table 7). As shown in Table 7, the SA developed by the silicates also increased in the order fine silt < coarse clay < fine clay, but the range of values was much smaller than for the oxides. Comparing the two sites again reveals that a relatively small variation in oxide content has a considerable effect in terms of SA under these conditions (Table 7). Thyrow and Skierniewice had similar values of silicate-SA in the various separates. The higher total SA values in Thyrow versus Skierniewice were therefore due to oxides, and this higher oxide-SA can in turn be explained by higher $Fe_d$ contents (see Table 5).

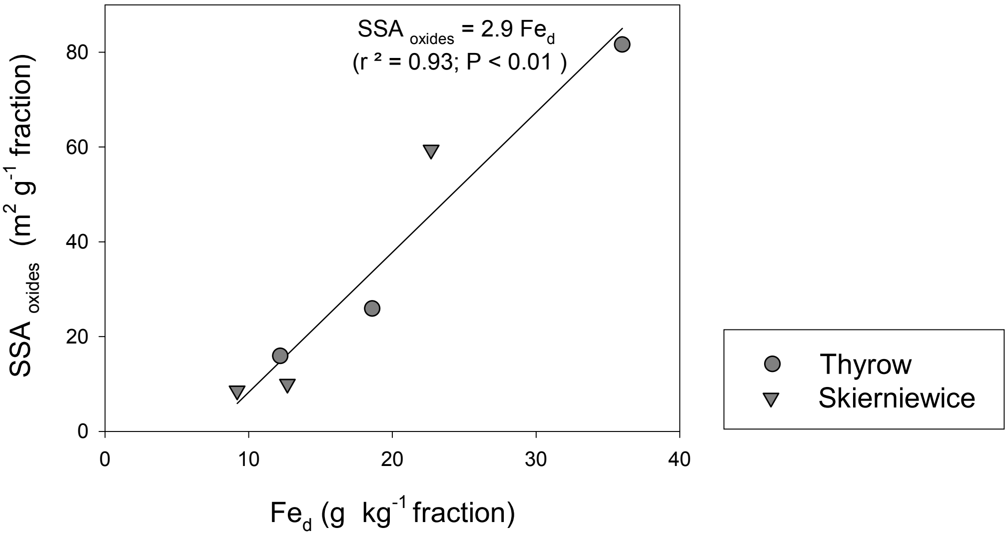


**Fig. 5:** SSA attributable to oxides ($SSA_{oxides}$) versus contents of dithionite extractable iron ($Fe_d$) in fractions < 6 µm of the fertilized plots from Thyrow and Skierniewice (y-intercept in the linear regression equation not significant at the 0.05 probability level).

Table 8 shows the SSA assigned to the two mineral fractions, silicates and oxides, together with the total OC contents in the fractions < 6 µm (two sites pooled). OC contents were significantly correlated with the SA contributed by both mineral fractions. Therefore, organic compounds probably interact with the surface of both silicates and oxides. However, given the dominant SA contribution from oxides in these soils, OC should to a major part be associated with the surface of oxides.

In summary, the interaction of organic compounds with (iron) oxides is probably the major mechanism responsible for stabilizing OC in the Thyrow and Skierniewice soils. The previous section (2.3.2) showed that the fine separates encompassed a large part of the refractory OC in these soils. Consequently, long-term storage of SOC in these soils may be controlled by the proportion of (iron) oxides. A relatively small increase in their content relative to the total silicates had a large effect on both the SA and the OC level, when comparing the different fractions within a soil sample or the soils from the two study sites. This strong effect can be explained by the high SSA of oxide crystals versus silicates. The dominance of ferrihydrite, showing a particularly small particle-size, presumably contributed to the high SSA in the bulk oxide fraction at both sites .

**Table 8:** Surface areas contributed by oxides and silicates and OC contents in fractions < 6 µm from Thyrow and Skierniewice (fertilized plots).

| **Particle-size fractions** (µm) | **SSA $_{oxides}$** [a] ($m^2\ g^{-1}$) | **SSA $_{silicates}$** [b] ($m^2\ g^{-1}$) | **OC** ($g\ kg^{-1}$) |
|---|---|---|---|
| Thyrow | | | |
| 6 - 2 | 16 | 5 | 47 |
| 2 – 0.2 | 26 | 7 | 55 |
| < 0.2 | 82 | 18 | 73 |
| Skierniewice | | | |
| 6 - 2 | 9 | 5 | 51 |
| 2 – 0.2 | 10 | 9 | 57 |
| < 0.2 | 60 | 16 | 63 |

[a] OC versus SSA $_{oxides}$ ($n = 6$; $r = 0.91$, $P < 0.05$)

[b] OC versus SSA $_{silicates}$ ($n = 6$; $r = 0.95$, $P < 0.01$)

## 3.4 Conclusions

- This study revealed different patterns for the relationship between OC contents and specific mineral surface area as well as iron oxide contents in different soil types. In the sandy soils from Thyrow and Skierniewice (Luvisols), OC was linearly related to both mineral surface area and the content of iron oxides ($Fe_d$ and $Fe_o$) under both management regimes. This indicates that the interaction of organic compounds with mineral surfaces represents a major stabilization mechanism in fine fractions of these sandy soils. In contrast, in the loamy soil from Bad Lauchstädt (Chernozem), OC contents were not related to surface area or iron oxide contents. For this soil, the correlation approach does not clarify the role of mineral surfaces in stabilizing OC.
- For the sandy soils, correlation analysis indicated that the amount of refractory C is correlated with both surface area and iron oxide contents across the fractions, while the labile C is independent of the mineralogical properties. For the sandy soils as well as the loamy soil, density fractionation confirmed that the refractory part of OC mainly consists of C which is associated with mineral surfaces, while the labile fraction of SOC shows no such close association. Mineral surfaces seem to play an important role for the long-term stabilization of OC in the different soils examined in this study.
- For the sandy soils, a dominant contribution from oxides (versus silicates) to the total surface area in fractions $< 6$ µm was noted. This high SA contribution may be related to the predominance of ferrihydrite within the iron oxides. The higher surface area contribution from oxides relative to silicates led us to conclude that the oxides are mostly responsible for the surface area-controlled stabilization of OC. Finally, the long-term stabilization of OC in these sandy soils is probably controlled by the content of (iron) oxides.

## 3.5 References

Adams, W.A., Kassim, J.K., 1984. Iron oxyhydroxides in soils developed from Lower Paleozoic sedimentary rocks in mid-Wales and implications for some pedogenetic processes. Journal of Soil Science 35, 117-126.

Amelung, W., Zech, W., 1999. Minimisation of organic matter disruption during particle-size fractionation of grassland epipedons. Geoderma 92, 73-85.

Amelung, W., Zech, W., Zhang, X., Follett, R.F., Tiessen, H., Knox, E., Flach, K.-W., 1998. Carbon, nitrogen, and sulfur pools in particle-size fractions as influenced by climate. Soil Science Society of America Journal 62, 172-181.

Anderson, B.J., Jenne, E.A., 1970. Free-iron and -manganese oxide content of reference clays. Soil Science 109, 163-169.

Baldock, J.A., Skjemstad, J.O., 2000. Role of the mineral matrix and minerals in protecting natural organic materials against decomposition. Organic Geochemistry 31, 697-710.

Bigham, J.M., Golden, D.C., Buol, S.W., Weed, S.B., Bowen, L.H., 1978. Iron oxide mineralogy of well-drained Ultisols and Oxisols: II. Influence on color, surface area, and phosphate retention. Soil Science Society of America Journal 42, 825-830.

Borggaard, O.K., 1982. The influence of iron oxides on the surface area of soil. Journal of Soil Science 33, 443-449.

Broersma, K., Lavkulich, L.M., 1980. Organic matter distribution with particle-size in surface horizons of some sombric soils in Vancouver Island. Canadian Journal of Soil Science 60, 583-586.

Brunauer, S., Emmett, P., Teller, E., 1938. Adsorption of gases in multimolecular layers. Journal of the American Chemical Society 60, 309-319.

Burford, J.R., Deshpande, T.L., Greenland, D.J., Quirk, J.P., 1964. Influence of organic materials on the determination of the specific surface areas of soils. Journal of Soil Science 15, 192-201.

Chiou, C.T., 1990. The surface area of soil organic matter. Environmental Science & Technology 24, 1164-1166.

Christensen, B.T., 1992. Physical fractionation of soil and organic matter in primary particle size and density separates. In: Advances in Soil Science, Volume 20. Springer-Verlag, New York, pp. 1-90.

Christensen, B.T., 1996. Carbon in primary and secondary organomineral complexes. In: Carter, M.R., Stewart, B.A. (Eds.), Structure and Organic Matter Storage in Agricultural Soils. Advances in Soil Science. CRC Press, Boca Raton, pp. 97-165.

Cornejo, J., Hermosin, M.C., 1996. Interaction of humic substances and soil clays. In: Piccolo, A. (Ed.), Humic Substances in Terrestrial Ecosystems. Elsevier Science, pp. 595-624.

Cornell, R.M., Schwertmann, U., 1996. The Iron Oxides - Structure, Properties, Reactions, Occurrence and Uses. VCH, Weinheim.

De Jonge, H., Mittelmeijer-Hazeleger, M.C., 1996. Adsorption of $CO_2$ and $N_2$ on soil organic matter: nature of porosity, surface area, and diffusion mechanisms. Environmental Science & Technology 30, 408-413.

FAO (Ed.), 1998. World Reference Base For Soil Resources, Rome.

Feller, C., Schouller, E., Thomas, F., Rouiller, J., Herbillon, A.J., 1992. $N_2$-BET specific surface areas of some low activity clay soils and their relationships with secondary constituents and organic matter contents. Soil Science 153, 293-299.

Fontes, M.P.F., Weed, S.B., 1996. Phosphate adsorption by clays from Brazilian Oxisols: relationship with specific surface area and mineralogy. Geoderma 72, 37-51.

Fontes, M.R., Weed, S.B., Bowen, L.H., 1992. Association of microcrystalline goethite and humic acid in some Oxisols from Brazil. Soil Science Society of America Journal 56, 982-990.

Gee, G.W., Bauder, J.W., 1986. Particle-size analysis. In: American Society of Agronomy, Soil Science Society of America (Eds.), Methods of Soil Analysis, Part 1. Physical and Mineralogical Methods - Agronomy Monographs no. 9. Madison, USA, pp. 383-411.

Homgren, G.G.S., 1967. A rapid citrate-dithionite extractable iron procedure. Soil Science Society of America Proceedings 31, 210-211.

Hsieh, Y.-P., 1994. Radiocarbon methods for dynamics of soil organic matter. In: Senesi, N., Miana, T.M. (Eds.), Humic Substances in the Global Environment and Implications on Human Health. Elsevier Science B.V., pp. 305-310.

Jenkinson, D.S., 1977. Studies on the decomposition of plant material in soil. V. The effects of plant cover and soil type on the loss of carbon $^{14}C$ labelled ryegrass decomposing under field conditions. Journal of Soil Science 28, 424-434.

Kaiser, K., Guggenberger, G., 2000. The role of DOM sorption to mineral surfaces in the preservation of organic matter in soils. Organic Geochemistry 31, 711-725.

Keil, R.G., Tsamakis, E., Fuh, C.B., Giddings, J.C., Hedges, J.I., 1994. Mineralogical and textural controls on the organic composition of coastal marine sediments: Hydrodynamic separation using SPLITT-fractionation. Geochimica et Cosmochimica Acta 58, 879-893.

Körschens, M., 1998. Effect of different management systems on carbon and nitrogen dynamics of various soils. In: Lal, R., Kimble, J.M., Follett, R.F., Stewart, B.A. (Eds.), Management of Carbon Sequestration in Soil, Advances in Soil Science. CRC Press Boca Raton, pp. 297-304.

Ladd, J.N., Amato, M., Oades, J.M., 1985. Decomposition of plant materials in Australian soils. III. Residual organic and microbial biomass C and N from isotope - labelled legume material and soil organic matter decomposing under field conditions. Australian Journal of Soil Research 23, 603-611.

Mayer, L.M., 1994a. Relationship between mineral surfaces and organic carbon concentrations in soils and sediments. Chemical Geology 114, 347-363.

Mayer, L.M., 1994b. Surface area control of organic carbon accumulation in continental shelf sediments. Geochimica et Cosmochimica Acta 58, 1271-1284.

Mehra, O.P., Jackson, M.L., 1960. Iron oxide removal from soils and clays by dithionite-citrate system bufferd with sodium bicarbonate. Clays and Clay Minerals Proceedings of the 7th National Conference, 317-327.

Meier, L.P., Menegatti, A.P., 1997. A new, efficient, one-step method for the removal of organic matter form clay-containing sediments. Clay Minerals 32, 557-563.

Oades, J.M., 1989. An introduction to organic matter in mineral soils. In: Dixon, J.B., Weed, S.B. (Eds.), Minerals in Soil Environments. Soil Science of America Book Series. Soil Science Society of America, Madison, pp. 89-159.

Pennell, K.D., Boyd, S.A., Abriola, L.M., 1995. Surface area of soil organic matter reexamined. Soil Science Society of America Journal 59, 1012-1018.

Ransom, B., Kim, D., Kastner, M., Wainwright, S., 1998. Organic matter preservation on continental slopes: Importance of mineralogy and surface area. Geochimica et Cosmochimica Acta 62, 1329-1345.

Rühlmann, J., 1999. A new approach to estimating the pool of stable organic matter in soil using data from long-term field experiments. Plant and Soil 213, 149-160.

Saggar, S., Parshotam, A., Hedley, C., Salt, G., 1999. $^{14}$C-labelled glucose turnover in New Zealand soils. Soil Biology & Biochemistry 31, 2025-2037.

Saggar, S., Parshotam, A., Sparling, G.P., Feltham, C.W., Hart, P.B.S., 1996. $^{14}$C - labelled ryegrass turnover and residence times in soils varying in clay content and mineralogy. Soil Biology & Biochemistry 28, 1677-1686.

Schlichting, E., Blume, H.-P., Stahr, K., 1995. Bodenkundliches Praktikum. Blackwell Wissenschafts-Verlag, Berlin.

Schwertmann, U., 1964. Differenzierung der Eisenoxide des Bodens durch Extraktion mit Ammoniumoxalat-Lösung. Zeitschrift für Pflanzenernährung und Bodenkunde 105, 194-202.

Shang, C., Tiessen, H., 1998. Organic matter stabilization in two semiarid tropical soils: size, density and magnetic separation. Soil Science of America Journal 62, 1247-1257.

Sollins, P., Homann, P., Caldwell, B.A., 1996. Stabilization and destabilization of soil organic matter: mechanisms and controls. Geoderma 74, 65-105.

Stanjek, H., Niederbudde, E.A., Häusler, W., 1992. Improved evaluation of layer charge of n-alkylammonium-treated fine soil clays by Lorentz- and polarization-correction and curve-fitting. Clay Minerals 27, 3-19.

Theng, B.K.G., Ristori, G.G., Santi, C.A., Percival, H.J., 1999. An improved method for determining the specific surface areas of topsoils with varied organic matter content, texture and clay mineral composition. European Journal of Soil Science 50, 309-316.

Torres Sanchez, R.M., Falasca, S., 1997. Specific surface area and surface charges of some Argentinian soils. Zeitschrift für Pflanzenernährung und Bodenkunde 160, 223-226.

Trumbore, S.E., 1997. Potential response of soil organic carbon to global environmental change. Proceedings of the National Academy of Science 94, 8284-8291.

Turchenek, L.W., Oades, J.M., 1979. Fractionation of organo-mineral complexes by sedimentation and density techniques. Geoderma 21, 311-343.

# 4 Structural characterization of refractory soil organic carbon by means of $^{13}$C NMR spectroscopy and polysaccharide analysis

## Abstract

This part aims at characterizing the chemical composition of the refractory SOC pool by means of $^{13}$C NMR spectroscopy and polysaccharide analysis. Bulk soil samples from eight long-term agroecosystem experiments were investigated. In the C-depleted plots from most sites, lower proportions of O/N-alkyl C and higher contributions of aromatic C compared with the fertilized counterparts are observed. Ratios of alkyl-to-O/N-alkyl C are higher in the depleted plots. The amount of all C-species declined in the course of the overall SOC-depletion. However, on average, the extent of decline is highest for O/N-alkyl C and smallest for aromatic C. Contents of chemolytically determined polysaccharides in the C-depleted plots are similar to the fertilized plots. The refractory C pool of the investigated arable soils appears to be relatively enriched in aromatic structures, but depleted in O/N-alkyl C compared with more labile OC fractions. Despite the depletion in O/N-alkyl C, the refractory C appears to have a similar contribution of polysaccharides as labile C pools.

## 4.1 Introduction

SOM is thought to comprise refractory compounds which reside in soil for hundreds to thousands of years. A compartment of refractory / passive OM, which is turned over very slowly, is included in most currently used SOM model approaches, apart from compartments of rapid turnover („active / labile“ OM) (e.g. Cambardella, 1998; Van Veen and Paul, 1981; Parton et al., 1987). According to Sollins et al. (1996), stability of OM in soil results from three sets of characteristics, namely *chemical recalcitrance* of organic molecules against microbial attack, *interactions* between organic and mineral compounds, and *accessibility* of organics to microbes / enzymes within the soil structure. Among the chemically most resistant compounds are aromatic and paraffinic structures (Oades, 1995). With regard to interactions and accessibility, the stabilization of organic compounds in soils is influenced by the clay content, and those factors that control the aggregation status (Oades, 1995).

Long-term agroecosystem studies have been used many times as a means to investigate the effect of management practices on C sequestration in soils. In this study, we will investigate SOC structure in two contrasting treatments from such long-term experiments, namely *fertilized* plots and plots *depleted* in OC (unmanured plots, bare fallows) (Fig. 1). The fertilized plots selected for this study receive organic and mineral fertilizers in order to guarantee high crop yields. Thus, high amounts of organic materials enter the soil, as crop residues and farmyard manure. In the plots where no inorganic or organic nutrients are added, crop productivity is reduced. In the unmanured plots, the total OM-input to the soil is much lower than in the fertilized counterparts. Bare fallows are kept free of any vegetation cover. Thus, these soils do not receive any (or negligible quantities of) organic matter input. The pronounced differences in organic input over the long-term result in different SOC levels in the fertilized and „C-depleted“ plots (Fig. 1; see Körschens et al., 1998). The type of soil management is expected to mainly affect labile SOC fractions, whereas the refractory pool should remain unaffected at a time-scale of decades (Elliott et al., 1996). The plots with low / missing organic input (C-depleted plots) are essentially depleted in labile compounds (with a residence time of years to decades); consequently, in these plots the refractory pool makes up a higher proportion of total SOC than in the fertilized plots (Fig. 1).

In agroecosystem experiments usually a particular treatment is established on several plots, arranged randomly within the experimental station. According to this design, factors which may influence the actual SOC level (e.g. variation in geological substrate, soil texture, an atmospheric input of mineral dust / or organic pollutants) should randomly affect the various treatments. Therefore, differences in SOC contents observable between the treatments should be mainly the result of the specific type of soil management.

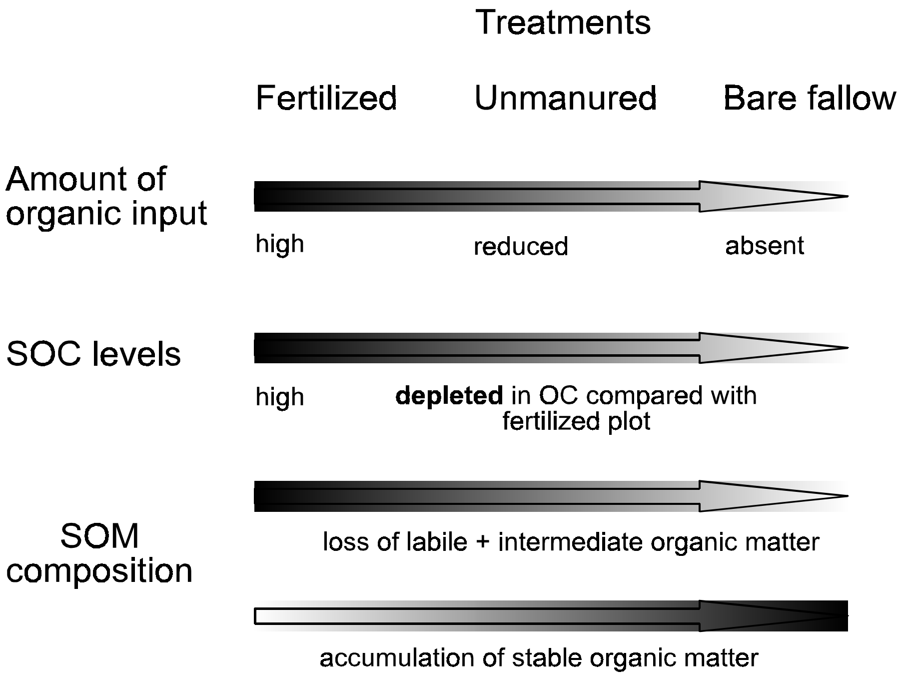


**Fig. 1:** Differences in organic input, contents and composition of SOC between the differently managed plots from the long-term agroecosystem experiments.

The objective of this study is to assess the chemical composition of refractory OC in arable soils, by comparing C-depleted treatments with fertilized plots from long-term field experiments. This includes the analysis of bulk soil samples with respect to the gross composition of OC (by means of $^{13}$C NMR spectroscopy), and the content of polysaccharides as a compound class which is quantitatively important for SOM (Lowe, 1978). We will use solid-state NMR spectroscopy since this is a non-invasive technique which allows to examine the composition of SOC as a whole in solid samples (Kögel-Knabner, 1997).

## 4.2 Materials and methods

### 4.2.1 Study sites and soil sampling

We obtained soils from eight European long-term agroecosystem experiments (Table 1). The study sites are located in various regions of central and eastern Europe, covering a range of climatic features, of geological substrates and of soil types. Samples were taken from different treatments of the experiments: plots with the combined addition of mineral and organic fertilizers („fertilized plots“), unmanured plots with the same crop rotation as in the fertilized treatments (but without fertilization), and bare fallows (only for Bad Lauchstädt and Prague) (unmanured plots and bare fallows are denoted as „C-depleted plots“).

Except for the bare fallows in Prague and Bad Lauchstädt, all treatments are replicated at least three-fold on separate plots arranged randomly within the experimental fields (three to five replications depending on the experiment). The bare fallow plots of Prague and Experiment A (Bad Lauchstädt) are 2 to 3 m $^2$ in size and have no replications. The bare fallow treatment of Experiment B (Bad Lauchstädt) is replicated twice.

Soil samples were collected from a depth 0-20 cm. Soil material collected at 10 sampling points on each of the replication plots of a particular treatment was mixed. Subsequently, samples were air-dried and components > 2 mm were removed by dry sieving. For elemental analysis and hydrolysis of polysaccharides, aliquots of the samples were ground by a ball mill.

### 4.2.2 Elemental analysis and pH

C and N contents were determined by dry combustion using a Elementar Vario EL Analyzer. Inorganic and organic C was differentiated by determining the amount of C before (total C) and after ignition of the samples at 550°C for 3 h (inorganic C). Inorganic C was not detected in any sample.

**Table 1:** Site characteristics of the long-term agroecosystem experiments and treatments considered in the present study.

| **Location** | **Start of experiment** | **Soil classification** (FAO) | **Clay** (%) | **MAT** (°C) | **MAP** (mm) | **Treatments** | | **Literature** |
|---|---|---|---|---|---|---|---|---|
| Thyrow (Germany) | 1937 | Albic Luvisol | 2.7 | 8.6 | 520 | Fertilized | NPK + lime + farmyard manure (15 t ha$^{-1}$ y $^{-1}$) | Schnieder (1990) |
| | | | | | | Unmanured | No fertilization | |
| Groß Kreutz (Germany) | 1967 | Albic Luvisol | 4.0 | 8.9 | 537 | Fertilized | N + farmyard manure (3 t ha$^{-1}$ y $^{-1}$) | Asmus (1990) |
| | | | | | | Unmanured | No fertilization | |
| Skierniewice (Poland) | 1923 | Luvisol | 6.0 | 7.9 | 527 | Fertilized | NPK + lime + farmyard manure (6 t ha$^{-1}$ y $^{-1}$) | Mercik et al. (1997) |
| | | | | | | Unmanured | No fertilization | |
| Puch (Germany) | 1983 [a] | Orthic Luvisol | 18 | 7.9 | 927 | Fertilized | NPK + lime + farmyard manure (10 t ha$^{-1}$ y $^{-1}$) | Krauss et al. (1997)<br>Diez et al. (1997) |
| | 1953 [b] | | | | | Bare fallow | Without crops and fertilization | |
| Lauterbach (Germany) | 1966 | Dystric Cambisol | 18 | 6.3 | 900 | Fertilized | NPK + lime + farmyard manure | Reichelt (1990) |
| | | | | | | Unmanured | No fertilization (but liming) | |
| Bad Lauchstädt (Germany) | | Haplic Chernozem | 23 | 8.6 | 490 | | | |
| *Experiment A* | 1902 [c] | | | | | Fertilized | NPK + farmyard manure (15 t ha$^{-1}$ y $^{-1}$) | Körschens and Eich (1990) |
| | 1956 [d] | | | | | Unmanured | No fertilization | |
| | | | | | | Bare fallow | Without crops and fertilization | |
| *Experiment B* | 1984 | | | | | Fertilized | Farmyard manure (200 t ha$^{-1}$ y $^{-1}$) | Körschens et al. (1998) |
| | | | | | | Bare fallow | Without crops and fertilization | |
| Prague (Czech Republic) | 1958 | Luvi-haplic Chernozem | 29 | 8.1 | 450 | Bare fallow + fertilization | Farmyard manure (80 t ha$^{-1}$ y $^{-1}$) | Kubát and Novák (1992) |
| | | | | | | Bare fallow (1) | Without crops and fertilization | |
| | | | | | | Bare fallow with soil tillage (0-20 cm) (2) | | |

[a] Fertilized treatment since 1983

[b] Bare fallow since 1953

[c] Fertilized and unmanured plots since 1902

[d] Bare fallow since 1956

pH values were measured in the supernatant of a soil suspension in 0.01 M $CaCl_2$ by using a glass electrode. The ratio of soil material (w) to $CaCl_2$-solution (v) was 1:2.5 (Schlichting et al., 1995).

### 4.2.3 $^{13}$C CPMAS NMR spectroscopy

Sieved samples were treated with 10% hydrofluoric acid (HF) to remove paramagnetic species and mineral matter, resulting in a concentration of organic material (Schmidt et al., 1997). 50 ml of 10% (v/v) HF were added to 10 g of soil in a polyethylene bottle. The suspension was shaken horizontally for 16 h. After centrifugation, the supernatant was removed. The HF treatment was repeated four times. Finally, the residue was washed with deionized water and freeze-dried.

The solid-state $^{13}$C nuclear magnetic resonance (NMR) spectra were obtained on a Bruker DSX 200 spectrometer operating at a $^{13}$C resonance frequency of 50.3 MHz by using the cross polarization magic-angle spinning (CP MAS) technique (Schaefer and Stejskal, 1976). Samples were packed into a rotor of zirconium dioxide with a diameter of 7 mm, and spun at a frequency of 6.8 kHz. A pulse delay of 400 ms and a contact time of 1 ms were used. Due to low sensitivity for the highly sandy soils from Thyrow, Groß Kreutz, and Skierniewice, the spectra of these samples were obtained by using a pulse delay of 250 ms. A ramped $^{1}$H-pulse was used during contact time in order to circumvent inexact Hartmann-Hahn conditions (Peersen et al., 1993). After accumulation of 23,000 to 350,000 scans and prior to Fourier transformation, a line broadening of 100 to 150 Hz was applied.

The chemical shift scale is referenced to tetramethylsilane (= 0 ppm). The spectra are divided into four major chemical shift regions, assignable to alkyl C (0 to 45 ppm), O/N-alkyl C (45 to 110 ppm), aromatic C (110 to 160 ppm) and carboxyl/carbonyl C (160 to 220 ppm). A detailed scheme of tentative assignment of chemical shift regions to organic compounds is given in Table 2. Signal intensities for aromatic and carboxyl C were corrected for spinning side bands: i) For aromatic C, the intensities of the ranges 276 to 220 ppm and 0 to -50 ppm were added to the region 110 to 140 ppm. ii) One side band of carboxyl C is found in the range 323 to 276 ppm. Assuming that the second side band for carboxyl C, between 0 and 45 ppm, is of equal size, the integral of the first side band was doubled and added to the central band (160 to 220 ppm); then, the intensity of the first side band (326 to 276 ppm) was subtracted from the alkyl C region (0 to 45 ppm).

The two contrasting treatments are compared with regard to relative signal intensities for the various C-species (equivalent to relative contributions to the total OC in a particular soil sample). Furthermore, to assess the extent of C decrease in the course of SOC-depletion, the relative contributions of the species in the C-depleted plot are normalized to the total SOC of the fertilized plot:

$$\frac{\text{C-species (\% of OC}_{\text{Depleted}}\text{)} \times \text{OC}_{\text{Depleted}}}{\text{OC}_{\text{Fertilized}}} = \text{C-species (\% of OC}_{\text{Fertilized}}\text{)} \qquad (1)$$

| | |
|---|---|
| $OC_{\text{Depleted}}$ | SOC content of the depleted plot (g C $kg^{-1}$ soil) |
| $OC_{\text{Fertilized}}$ | SOC content of the fertilized plot (g C $kg^{-1}$ soil) |

For a particular C-species, this calculated „residual“ contribution (C-depleted plot) will be compared with the „original“ contribution in the fertilized plot (relative intensity in NMR spectrum of fertilized plot).

**Table 2:** Tentative assignment of signals in the $^{13}$C NMR spectra (from Almendros et al., 1992; Knicker and Lüdemann, 1995).

| **Chemical shift range** (ppm) | **Assignment** |
|---|---|
| 0-45 | Terminal $CH_3$ groups (0-25); $CH_2$ groups in chains (30) (lipids, proteins) |
| 45-60 | $OCH_3$ in aromatic structures (lignin) and in polysaccharides (hemicelluloses); $\alpha$-amino C (amino acids); C-6 of some polysaccharides |
| 60-90 | Higher alcohols, C-2 to C-5 of hexoses; $\alpha$-, $\beta$-, $\gamma$-C in $\beta$-O-4 linked units (lignin) |
| 90-110 | (103-105) C-1 in polymeric carbohydrates (anomeric C) and C-2 and C-6 in syringyl units (lignin) |
| 110-140 | Protonated and C-substituted aromatics; olefinic carbons |
| 140-160 | Aromatic COR and CNR groups |
| 160-220 | Carboxyl-C in aliphatic and aromatic acids, in esters, in amides; Carbonyl-C (aldehydes, ketones) |

### 4.2.4 Polysaccharide analysis

Analysis of polysaccharides was carried out according to Kögel-Knabner (1995). The method includes acid hydrolysis of polysaccharides followed by colorimetric determination of sugar monomers by the MBTH (3-methyl-2-benzothiazolinone hydrazone hydrochloride) procedure. In this procedure, monosaccharides are reduced to alditols, followed by an oxidation of the terminal glycol ($-CH_2OH$) groups of the alditols, yielding two moles of formaldehyde per mole of original monosaccharide. The formaldehyde concentration is determined photometrically at 635 nm after reaction with MBTH (Pakulski and Benner, 1992).

For the hydrolysis of non-cellulosic polysaccharides, soil samples were incubated with 1 M HCl at 105°C for 5 h. For the hydrolysis of total polysaccharides (cellulosic and non-cellulosic), the samples were incubated with 12 M $H_2SO_4$ at room temperature for 16 h, and subsequently with 1 M $H_2SO_4$ at 105°C for 5 h. The cellulosic fraction of polysaccharides was determined by calculating the difference of monosaccharides released by $H_2SO_4$ (total polysaccharides) and HCl hydrolysis (non-cellulosic polysaccharides). Yields of monosaccharides were expressed relative to a calibration curve of glucose.

All analyses were run in triplicate. The coefficient of variation ranged between 2 and 25% for non-cellulosic polysaccharides, and between 3 and 23% for total polysaccharides.

To assess the net decrease of polysaccharide-C with SOC-depletion, the content of total polysaccharide-C in the C-depleted plot is normalized to the SOC of the fertilized plot at a given site:

$$\frac{\text{Polysaccharide-C (\% of OC}_{\text{Depleted}}) \times \text{OC}_{\text{Depleted}}}{\text{OC}_{\text{Fertilized}}} = \text{Polysaccharide-C (\% of OC}_{\text{Fertilized}}) \quad (2)$$

| | |
|---|---|
| $OC_{Depleted}$ | SOC content of the depleted plot (g C $kg^{-1}$ soil) |
| $OC_{Fertilized}$ | SOC content of the fertilized plot (g C $kg^{-1}$ soil) |

## 4.3 Results and discussion

### 4.3.1 OC and N contents, pH values

The contrasting management practices led to considerable differences in OC and N contents (Table 3). Unmanured plots and bare fallows contain between 40 and 67% of the OC, and

**Table 3:** Contents of organic carbon and total nitrogen, C/N-ratios and pH values of the different treatments from the long-term agroecosystem experiments.

| | | **Organic carbon** | | | **Total nitrogen** | | | **C/N-ratio** | | **pH** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Fertilized | Depleted | Depleted / Fertilized [a] | Fertilized | Depleted | Depleted / Fertilized | Fertilized | Depleted | Fertilized | Depleted |
| **Experimental site** | | ($g\ kg^{-1}$) | | (%) | ($g\ kg^{-1}$) | | (%) | | | | |
| Thyrow | | 6.8 | 3.2 | 47 | 0.61 | 0.27 | 44 | 11 | 12 | 6.1 | 4.1 |
| Groß Kreutz | | 10.3 | 4.1 | 40 | 1.00 | 0.43 | 43 | 10 | 10 | 5.8 | 6.6 |
| Skierniewice | | 8.8 | 4.4 | 51 | 0.76 | 0.39 | 51 | 11 | 11 | 6.1 | 4.5 |
| Puch | | 12.0 | 7.0 | 58 | 1.44 | 0.91 | 63 | 8 | 8 | 6.9 | 5.2 |
| Lauterbach | | 48.3 | 30.1 | 62 | 4.39 | 2.74 | 62 | 11 | 11 | 6.0 | 6.6 |
| Bad Lauchstädt | | | | | | | | | | | |
| *Experiment A* | | | | | | | | | | | |
| | Unmanured plot | 24.0 | 16.0 | 67 | 1.97 | 1.32 | 67 | 12 | 12 | 7.2 | 7.5 |
| | Bare fallow | | 14.9 | 62 | | 1.12 | 57 | | 13 | | N.D. |
| *Experiment B* | | 41.3 | 19.7 | 48 | 3.68 | 1.64 | 44 | 11 | 12 | 7.1 | 7.1 |
| Prague | Bare fallow | 29.1 | 14.5 | 50 | 2.62 | 1.22 | 47 | 11 | 12 | 7.1 | 6.4 |
| | Bare fallow + tillage | | 12.8 | 44 | | 1.07 | 41 | | 12 | | 6.9 |

[a] $$\frac{\text{OC / N content}_{\text{Depleted}} \times 100}{\text{OC / N content}_{\text{Fertilized}}}$$

N.D. Not determined

between 41 and 67% of the N of the fertilized plots. As the relative decrease is similar for OC and total N, C/N-ratios in the depleted plots do not essentially differ from the fertilized plots. Only in four experiments, three of which include bare fallows, C-depleted plots have slightly higher C/N-ratios than the fertilized counterparts (Table 3). The bare fallow of Experiment A (Bad Lauchstädt) with no OM-input is more depleted in C and N than the corresponding unmanured soil. In the bare fallow experiment at Prague, soil tillage resulted in a further loss of organic matter compared with the plot with no tillage. This enhanced OM decline may be explained by the breakdown of aggregates due to tillage, leading to an exposure of OM previously sequestered within the aggregate (Gregorich et al., 1989).

For three sites (Thyrow, Skierniewice, Puch), the lack of lime application in the depleted plots - in contrast to the fertilized ones - resulted in lower pH values (Table 3). In contrast, at Groß Kreutz and Lauterbach, the fertilized plots show lower pH values than the C-depleted (=unmanured) plots. One possible explanation for this decrease in pH may be the nitrification of ammonium-N present in organic and inorganic fertilizers, resulting in a net release of protons (Paul and Clark, 1989). For the chernozemic soils at Bad Lauchstädt and Prague, a similar pH is found in the differently managed plots, although no lime is applied at all.

### 4.3.2 Structural composition of SOC ($^{13}$C NMR)

$^{13}$C nuclear magnetic resonance (NMR) spectra of the soils are given in Figs. 2 and 3, relative signal intensities are presented in Table 4. In the spectra, the resonance line around 30 ppm, in the region of alkyl carbon, can probably be assigned to methylene structures. The peak around 56 ppm may originate from methoxyl groups and N-substituted alkyl carbons. The peaks around 72 and 104 ppm are most probably assignable to carbohydrates. The signal between 110 and 140 ppm peaking at 130 ppm may originate from protonated and C-substituted aryl carbon, as well as from unsaturated alkyl structures ($sp^2$ –hybridized carbons). The signal around 175 ppm is attributable to carboxyl and amide functional groups. (compare with Table 2)

Comparing the relative signal distributions between the contrasting treatments, divergences are evident, which are most pronounced for O/N-alkyl and aromatic C (Table 4). Five of the study sites (Thyrow, Skierniewice, Groß Kreutz, Bad Lauchstädt, Prague) show a decrease of the O/N-alkyl C proportions, and an increase of the aromatic C contributions in the C-depleted versus the fertilized plots. For these sites, the percentage of aromaticity – given as the ratio of aromatic C to the sum of aromatic and total aliphatic C (Table 4) - increases by a factor 1.2 on average with SOC-depletion. Highest proportions for aromatic C among all the

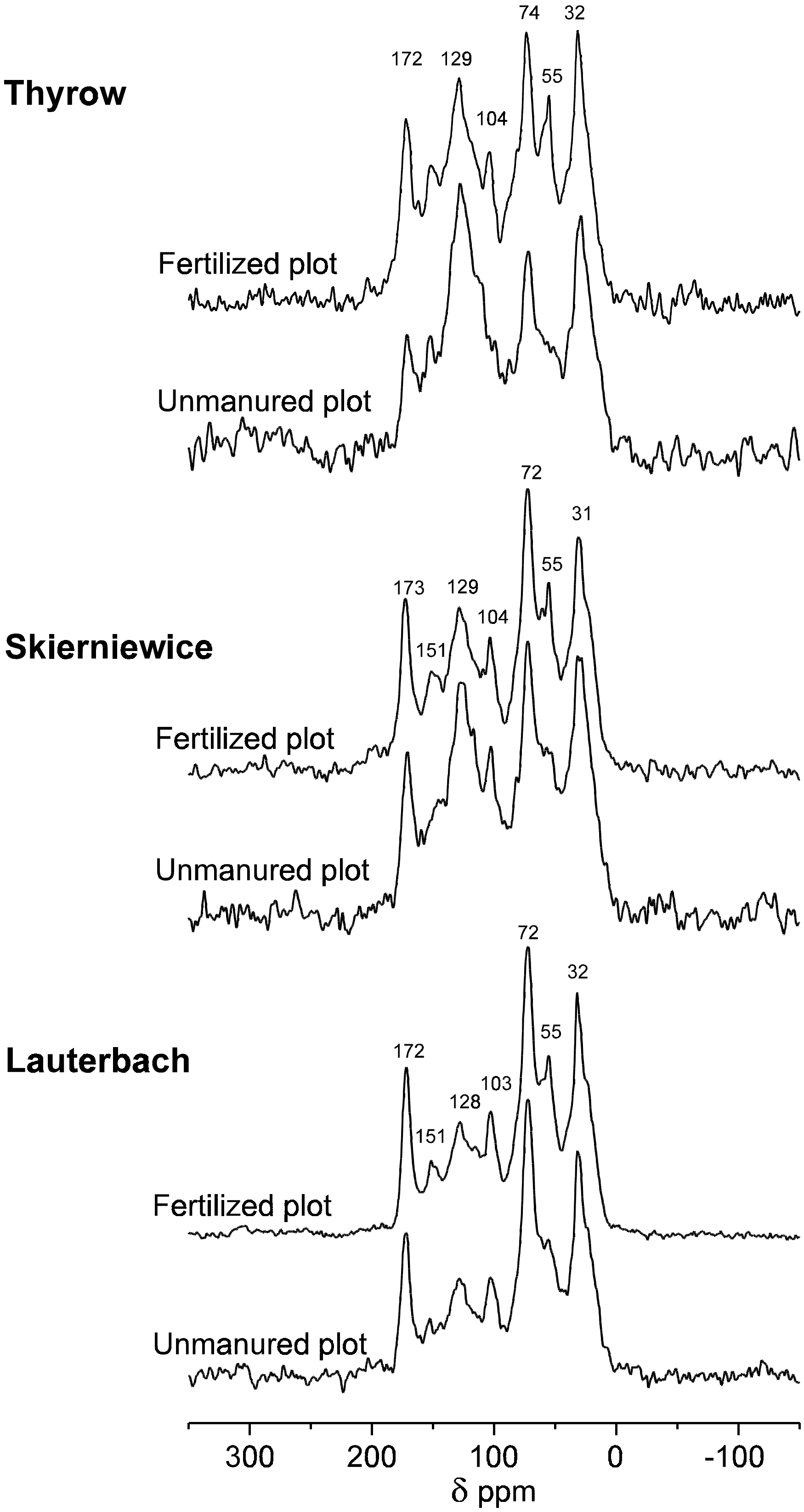


**Fig. 2:** $^{13}$C CPMAS NMR spectra of the different treatments from three experimental sites.

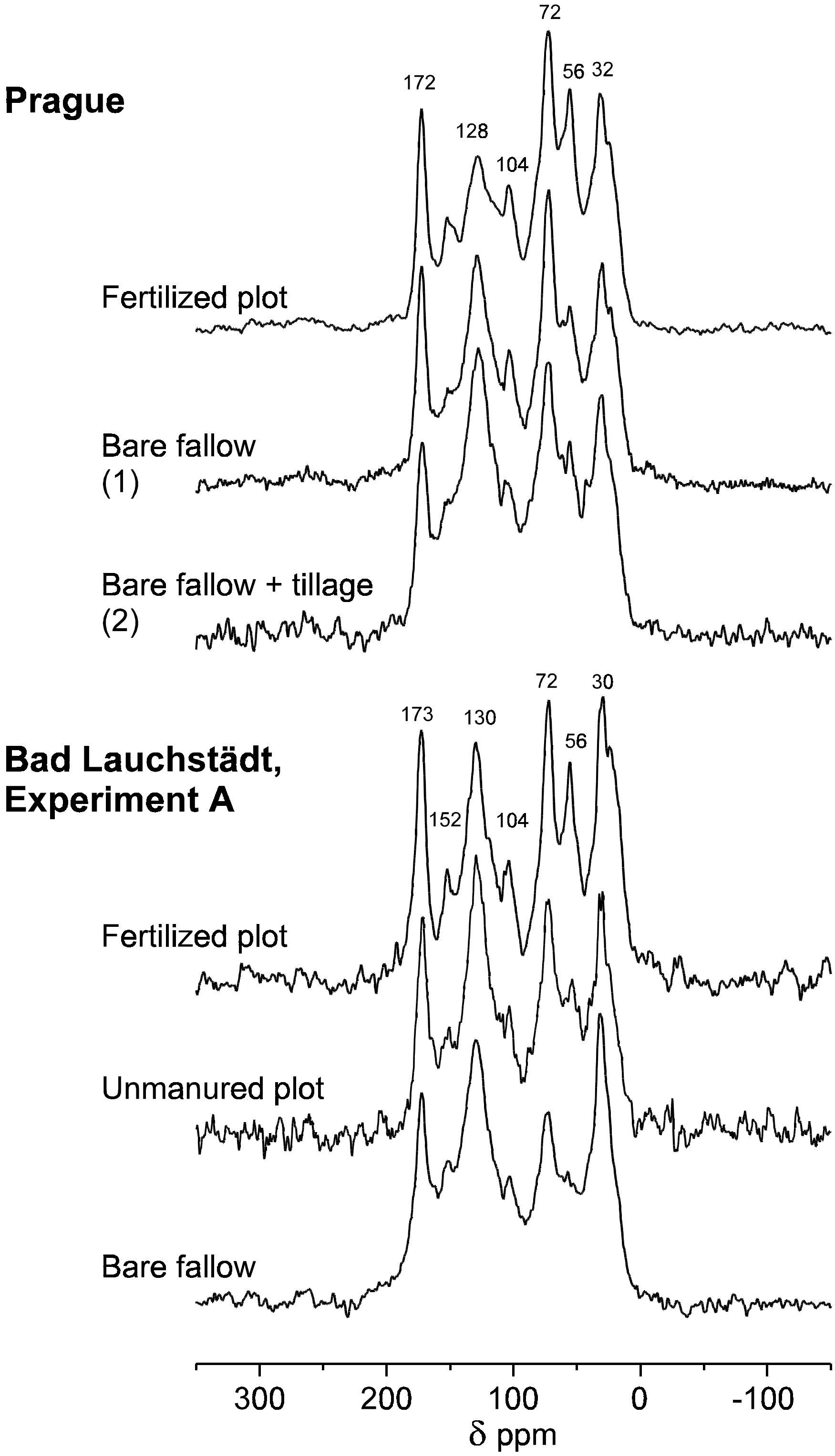


**Fig. 3:** $^{13}$C CPMAS NMR spectra of the different treatments from two experimental sites.

**Table 4:** Relative contributions of C-species to the total signal intensity in $^{13}$C NMR spectra for samples from contrasting treatments in the long-term field experiments.

| | **Alkyl C** (0-45 ppm) | | **O/N-Alkyl C** (45-110 ppm) | | **Aromatic C** (110-160 ppm) | | **Carboxyl C** (160-220 ppm) | | **Aromaticity** [a] | | $\frac{\textbf{Alkyl C}}{\textbf{O/N-Alkyl C}}$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Fertilized | Depleted | Fertilized | Depleted | Fertilized | Depleted | Fertilized | Depleted | Fertilized | Depleted | Fertilized | Depleted |
| **Experimental site** | (% of signal intensity) | | | | | | | | (%) | | | |
| Thyrow | 19 | 17 | 35 | 28 | 30 | 36 | 15 | 17 | 36 | 44 | 0.55 | 0.62 |
| Groß Kreutz | 22 | 20 | 41 | 38 | 25 | 28 | 11 | 14 | 28 | 33 | 0.55 | 0.53 |
| Skierniewice | 21 | 22 | 38 | 36 | 26 | 30 | 15 | 11 | 31 | 34 | 0.55 | 0.66 |
| Puch | 19 | 22 | 43 | 41 | 27 | 25 | 11 | 12 | 30 | 28 | 0.43 | 0.54 |
| Lauterbach | 22 | 25 | 43 | 43 | 22 | 20 | 13 | 12 | 25 | 22 | 0.51 | 0.57 |
| Bad Lauchstädt | | | | | | | | | | | | |
| *Experiment A* | | | | | | | | | | | | |
| Unmanured plot | 27 | 20 | 33 | 33 | 26 | 33 | 14 | 13 | 30 | 39 | 0.81 | 0.60 |
| Bare fallow | | 20 | | 28 | | 34 | | 17 | | 41 | | 0.72 |
| *Experiment B* | 21 | 19 | 39 | 35 | 28 | 32 | 13 | 14 | 32 | 37 | 0.53 | 0.55 |
| Prague Bare fallow | 20 | 19 | 41 | 35 | 26 | 30 | 13 | 15 | 30 | 36 | 0.50 | 0.54 |
| Bare fallow + tillage | | 18 | | 35 | | 34 | | 12 | | 39 | | 0.51 |

[a] $\frac{\text{Aromatic C (110-160)} \times 100}{\text{Aromatic C (110-160)} + \text{Aliphatic C (0-110)}}$ (Fründ et al., 1994)

soil samples are found in the bare fallows from Bad Lauchstädt and Prague, and in the depleted plot from Thyrow (up to 36% of total signal intensity). In these samples, aromatic C is the most abundant C-species. In all fertilized plots, O/N-alkyl C is the dominant type of carbon, as it is reported for most arable soils investigated so far (Mahieu et al., 1999). At the five sites, alkyl C intensities are slightly lower in the C-depleted versus the fertilized plots.

Two of the sites (Puch, Lauterbach) do not agree with the mentioned trend. Deviations in signal intensity between the two treatments are rather small, and in contrast to the other sites, alkyl C proportions tend to be higher in the C-depleted versus the fertilized plots. For all sites, the proportion of carboxyl C either increases slightly or remains unchanged in the depleted plots. Knicker (1993) showed that the variability associated with phase- and baseline correction of Fourier-transformed spectra is highest for the shift ranges 160 to 220, 45 to 60 and 0 to 45 ppm (relative standard deviation up to 13%). Consequently, differences in relative signal intensity observed between the treatments may be more sound for the O/N-alkyl and aromatic C region than for alkyl and carboxyl C.

The total range of aromatic C can be further divided into a region of O-aryl C (140 to 160 ppm) and aryl C (110 to 140 ppm). The signal in the O-aryl C region, around 150 ppm, is mainly assignable to lignin. A prominent signal in this region is usually found in plant materials in first stages of decomposition (Lüdemann and Nimz, 1974; Kögel et al., 1988). In our soils, the contribution of O-aryl C ranges from 6 to 10% of total signal intensity (Fig. 4). This indicates that lignin from plant residues is not a major component within the bulk SOC. Many of our soils show a broad signal in the aryl C range with a peak around 130 ppm (see Figs. 2 and 3). The increase of aromaticity in the C-depleted plots (except for Puch and Lauterbach) is mainly attributable to an increase of this aryl C signal (Fig. 4). Components which give rise to a broad signal at 130 ppm are, for example, charred plant materials and coal materials, which are supposed to contain condensed aromatic rings (Haumaier and Zech, 1995; Skjemstad et al., 1996; Rumpel et al., 1998; Schmidt et al., 1999). Charred plant residues could be identified in a number of soils so far, for example in chernozemic soils of Australia and Germany (Skjemstad et al., 1996; Schmidt et al., 1999). The atmospheric deposition of dust particles, emitted by the coal processing industry, is a further source of highly aromatic compounds in SOM (Schmidt et al., 1996). As some of the soils are located in the surroundings of industrialized areas, the possibility of dust imissions into the soils should be considered.

Baldock et al. (1997) proposed the ratio of alkyl-to-O-alkyl C intensities as an indicator for the degree of decomposition of organic materials / plant residues, mainly based on data from decomposition in the absence of a mineral matrix. According to the authors, this ratio can also be applied to mineral soils, although caution is needed in the interpretation, since the mineral matrix interferes with decomposition processes. In five of our experiments, the ratio of alkyl-to-O/N-alkyl C is higher in the C-depleted than in the fertilized plots (Table 4). According to Baldock et al. (1997), this would suggest that the organic matter in the depleted plots is on average more processed compared with the fertilized plots.

The present results are in line with the trend noted for various long-term experiments at Rothamsted (comparison of land-uses and farming practices), namely a decrease of O-alkyl C contributions with decreasing SOC contents (Kinchesh et al., 1995). Also for various German agroecosystem experiments Fründ et al. (1994) found that plots with lower SOC levels show more aryl C (110 to 140 ppm) and less CO/CN-compounds (60 to 80 ppm) compared with fields which are enriched in SOC. However, in the long-term experiments at Askov (Danmark) and Rothamsted which were investigated by Randall et al. (1995), long-continued contrasting treatment (comparable to the treatments of this work) did not result in significantly different intensity distributions.

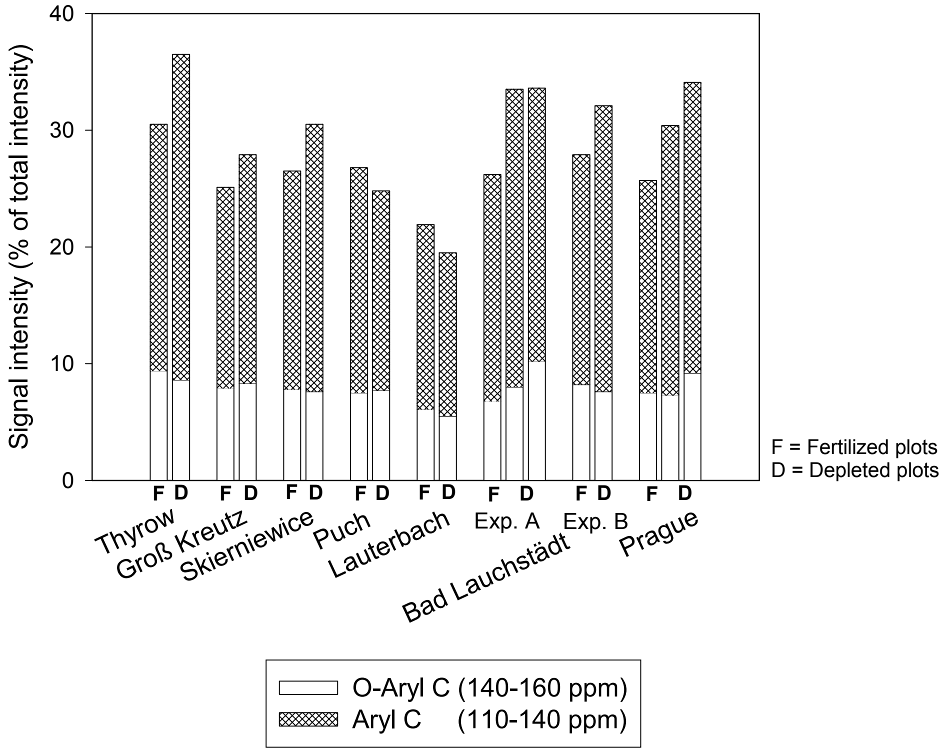


**Fig. 4:** Relative intensities of aryl C (110-140 ppm) and O-aryl C (140-160 ppm) in fertilized and C-depleted plots.

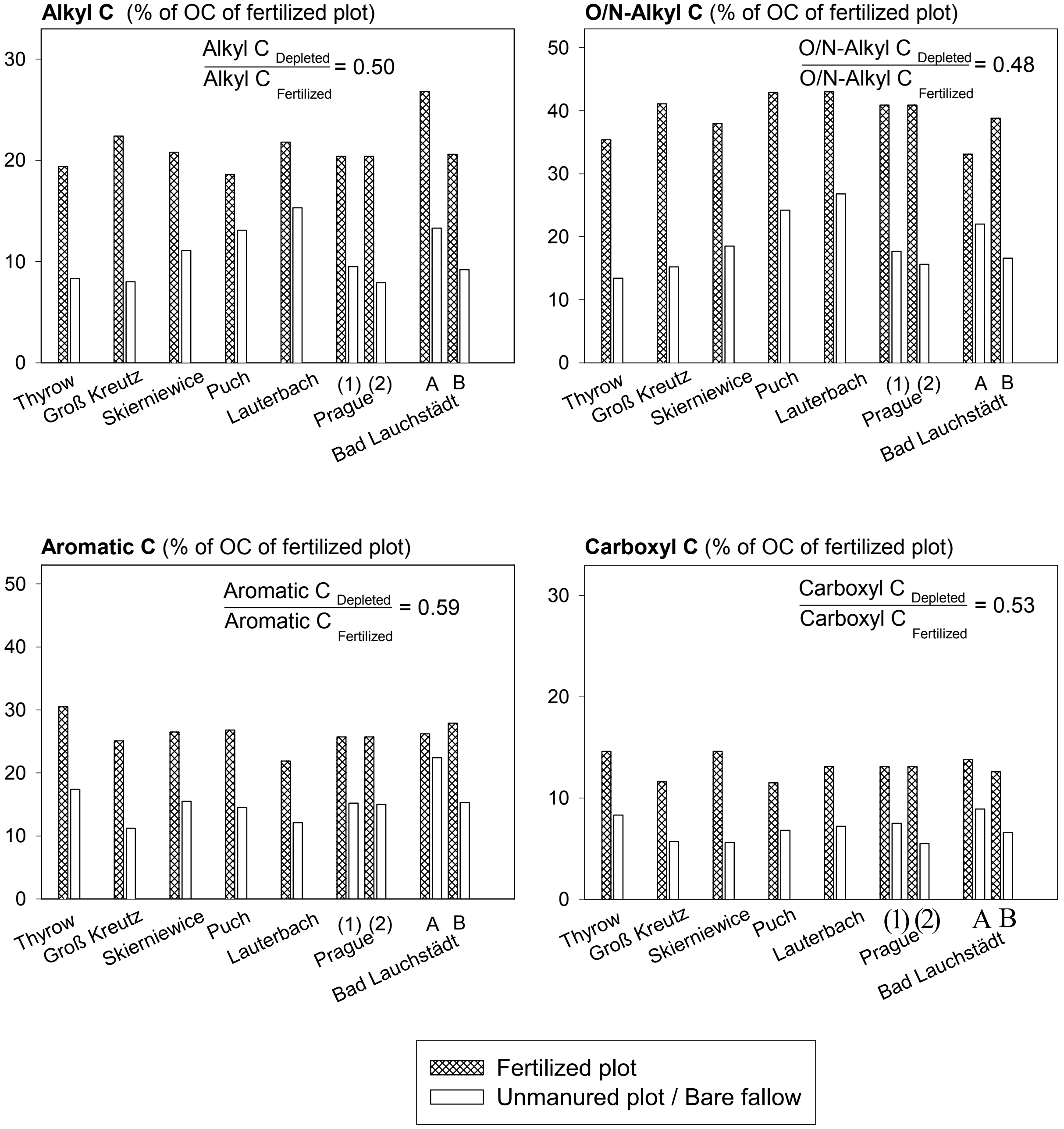


**Fig. 5:** Contributions of the C-species expressed as a percentage of OC of the fertilized plots. (the ratios are the mean values for the eight experiments)

### 4.3.3 C-depletion associated with the various species ($^{13}$C NMR)

Applying equation 1, the contributions of the various C-species in the depleted plots were normalized to the SOC of the fertilized plots. Fig. 5 plots the contributions of the C-species in the two contrasting treatments (expressed uniformly as: % of SOC in the fertilized plots). Ratios relating the contributions in the depleted plots to those in the fertilized treatments are also given (mean values for all experiments in Fig. 5). In this way, net changes in the course of C-depletion become evident. The ratios show that the residual contribution in the C-depleted plots is highest for aromatic C (0.59) and lowest for O/N-alkyl C (0.48). According to these ratios, the relative extent of C decrease on average follows the order: O/N-alkyl C > alkyl C > carboxyl C > aromatic C. When the total decrease of SOC is set 100%, O/N-alkyl C accounts for the greatest part (42% of the C loss); alkyl and aromatic C account for 22% each, and carboxyl C for 14% (mean values for the experiments).

In the bare fallows at Prague, soil tillage led to a further decrease of all C-species except the aromatic C (Fig. 5). In Experiment A of Bad Lauchstädt, a gradient of decreasing SOC contents exists, following the order: fertilized plot > unmanured plot > bare fallow (see Table 3). Fig. 6 illustrates that the decrease of C in the two types of C-depleted plots is smallest for the aromatic C. When comparing the bare fallow with the unmanured plot, further depletion in SOC mainly affects O/N-alkyl C compounds.

Given the different extent of C-depletion, there are gradual differences between the C-species with regard to their stability, with aromatic C being most stable and O/N-alkyl C being least persistent. Since the decline is smallest for aromatic C, this carbon type accumulates in the C-depleted plots relative to the other species, as seen in the previous section. A high stability for aromatic C is particularly evident in the soils from Bad Lauchstädt and Prague, which both represent chernozemic soils.

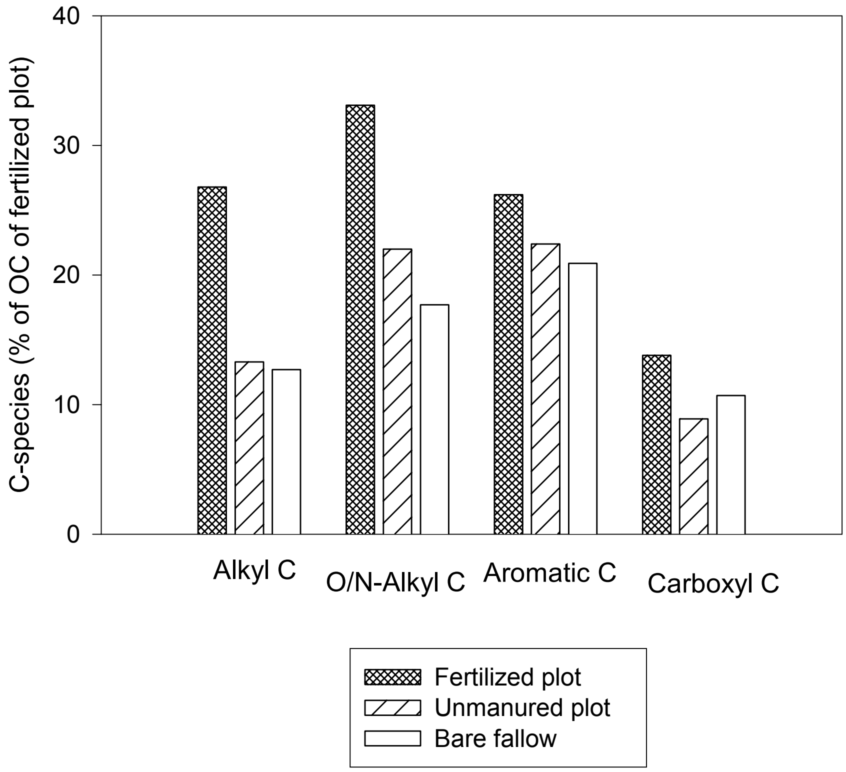


**Fig. 6:** Contributions of the C-species in Experiment A (Bad Lauchstädt), expressed as a percentage of OC of the fertilized plot.

**Table 5:** Contents of non-cellulosic and cellulosic polysaccharide-C in the contrasting treatments, and total polysaccharide-C normalized to OC of the fertilized plots for the various long-term experiments.

| | **Non-cellulosic C** | | **Cellulosic C** | | **Total polysaccharide-C** | | **Total polysaccharide-C** | | |
|---|---|---|---|---|---|---|---|---|---|
| | Fertilized | Depleted | Fertilized | Depleted | Fertilized | Depleted | Fertilized | Depleted | $\frac{\text{Polysacch.-C}_{\text{Depleted}}}{\text{Polysacch.-C}_{\text{Fertilized}}}$ |
| **Experimental site** | | | (% of OC) | | | | (% of OC of fertilized plot) | | |
| Thyrow | 12.4 | 15.0 | 1.5 | 0.4 | 13.9 | 15.4 | 13.9 | 7.3 | 0.52 |
| Groß Kreutz | 12.7 | 15.7 | 1.9 | n.d. | 14.6 | 15.7 | 14.6 | 6.3 | 0.43 |
| Skierniewice | 13.4 | 13.5 | 2.1 | n.d. | 15.5 | 13.5 | 15.5 | 6.8 | 0.44 |
| Puch | 13.7 | 17.2 | 2.0 | 1.3 | 15.7 | 18.5 | 15.7 | 10.8 | 0.69 |
| Lauterbach | 13.0 | 13.6 | 1.8 | n.d. | 14.8 | 13.6 | 14.8 | 8.5 | 0.57 |
| Bad Lauchstädt | | | | | | | | | |
| *Experiment A* | | | | | | | | | |
| Unmanured plot | 10.2 | 11.5 | 1.0 | 0.5 | 11.2 | 12.0 | 11.2 | 8.0 | 0.72 |
| Bare fallow | | 8.2 | | | | 8.2 | | 5.1 | 0.46 |
| *Experiment B* | 10.4 | 8.9 | 2.1 | 1.3 | 12.5 | 10.2 | 12.5 | 4.9 | 0.39 |
| Prague Bare fallow | 10.5 | 10.9 | n.d. | 0.6 | 10.5 | 11.5 | 10.5 | 5.8 | 0.55 |
| Bare fallow + tillage | | 10.1 | | 0.1 | | 10.2 | | 4.5 | 0.43 |

n.d. not detectable

### 4.3.4 Polysaccharides

Table 5 shows the contents of cellulose and non-cellulosic polysaccharides for the contrasting treatments, expressed as a percentage of SOC in the respective plots. In both treatments, the polysaccharide pool is dominated by the non-cellulosic fraction. Contents of non-cellulosic polysaccharides are similar in the two treatments. Cellulose contents are generally low compared with the non-cellulosic fraction; they further decline with C-depletion, so that cellulose is not detectable any more in the depleted plots of some experiments. The overall low contents of cellulose and the decrease together with reduced organic input seem to reflect that cellulose is turned over rapidly in soils (Haider, 1992).

Analogous to the C-species, we assessed the extent of decrease of polysaccharide-C along with C-depletion. Table 5 gives total polysaccharide contents of the depleted plots normalized to the SOC of the fertilized plots (see equation 2). Residual contents in the depleted plots range from 0.39 to 0.72 of the total polysaccharide-C in the fertilized plots (mean value 0.52). Comparing the mean ratio for polysaccharide-C with that for O/N-alkyl C (0.48), it is evident that the sum of O/N-alkyl C compounds declined to a higher extent than polysaccharides.

### 4.3.5 Polysaccharides *versus* O-alkyl C ($^{13}$C NMR)

The contents of polysaccharide-C significantly correlate with the relative intensities of O-alkyl C (60 to 110 ppm) in the soils (Fig. 7). Nevertheless, as can be seen from the high y-intercept in the regression equation in Fig. 7, O-alkyl C intensities are consistently higher than the yields of polysaccharides obtained by the chemolytic method. For the 18 soil samples, polysaccharide-C accounts for 47% of the signal intensity in the O-alkyl C range on average. Lower contents of polysaccharides by the chemolytic approach have been reported in several studies, for example for forest floors (Kögel-Knabner et al., 1988), bulk mineral soils and their fractions (Cheshire et al., 1992; Leifeld, 1998). The discrepancy between carbohydrate yields obtained by hydrolysis and O-alkyl C intensities may have several reasons (see Kögel-Knabner, 1997): First, it may be due to methodological problems associated with the hydrolysis procedure. An underestimation of colorimetrically determined polysaccharides may result from the fact that sugars are involved in secondary reactions (Allard et al., 1997), or that they are destroyed during hydrolysis, or from incomplete breakdown of polysaccharides to sugar monomers, which is necessary for their determination by the MBTH procedure. Second, the discrepancy may be due to the fact that other compounds (for example lignin side chains, proteinaceous materials, other alcohols) contribute to the O-alkyl C signal in $^{13}C$ NMR spectra. Third, Chesire et al. (1992) proposed the existence of „pseudo polysaccharides“ in SOM, which have an O-alkyl structure but - unlike „primary“ polysaccharides of plant / microbial origin - can not be hydrolyzed or identified as sugars by standard analytical procedures.

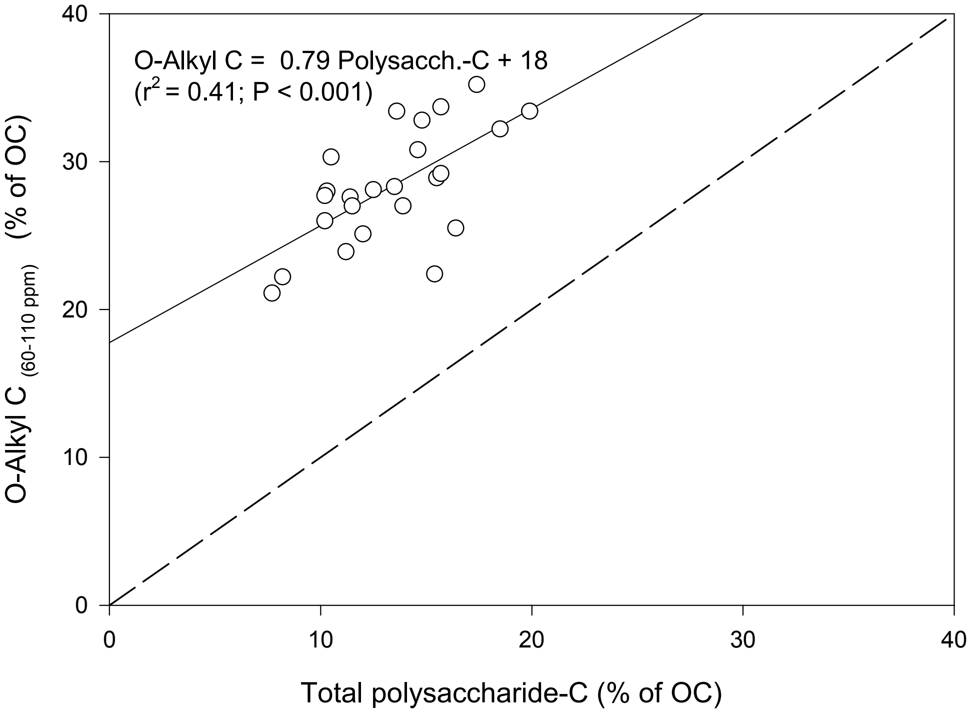


**Fig. 7:** Correlation between O-alkyl C signal intensities and yields of total polysaccharide-C in the samples of the long-term experiments.

To assess the contribution of both carbohydrates and non-hydrolyzable structures to the O-alkyl C region, $^{13}C$ NMR spectra were obtained from the residues after $H_2SO_4$ hydrolysis (Fig. 8). The spectra show that most of the polysaccharides (with resonances around 72 and 105 ppm) are hydrolyzed. Between 21 and 31% of the O-alkyl C signal of the untreated soils remain in the spectra of the hydrolysis residues (Table 6). This residual intensity may be mainly derived from lignin side chains, other non-specified alcohol and ether structures, and from overlapping signals of alkyl or aromatic carbons. According to Table 6, between 25 and 40% of the signal intensity is represented by hydrolyzable, non-identified O-alkyl C compounds. The existence of this „fraction“ may have several reasons, as was mentioned above: the hydrolyzate may contain oligomeric carbohydrates, or „pseudo polysaccharides“; moreover, the fact that O-alkyl compounds could not be identified may be due to the loss of

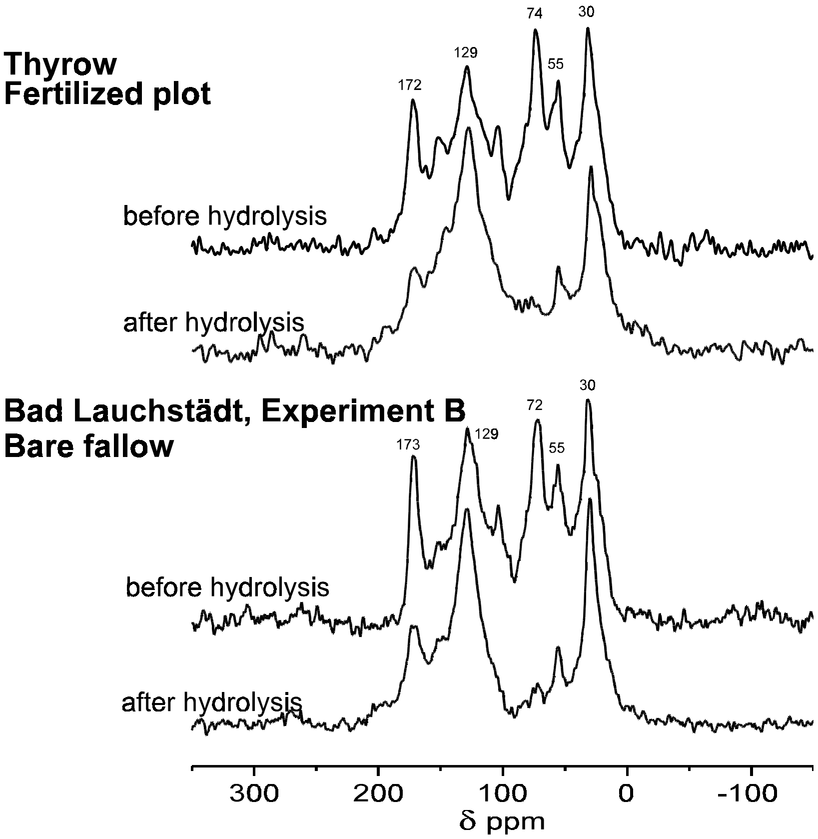


**Fig. 8:** $^{13}$C CPMAS NMR spectra of soil samples before and after hydrolysis with $H_2SO_4$.

**Table 6:** Distribution of signal intensity in the shift range 60 to 110 ppm ($^{13}$C NMR) for soil samples from the long-term experiments.

| | **O-Alkyl C (60-110 ppm) = 100%** | | |
|---|---|---|---|
| | **Hydrolyzable C, identified as polysaccharides** [a] | **Hydrolyzable C, not identified** [b] | **Non hydrolyzable C** [c] |
| **Soil samples** | | (%) | |
| Thyrow<br>fertilized plot | 51 | 25 | 24 |
| Bad Lauchstädt, *Experiment A*<br>fertilized plot | 47 | 25 | 28 |
| Bad Lauchstädt, *Experiment B*<br>bare fallow | 39 | 40 | 21 |
| Bad Lauchstädt, *Experiment B*<br>fertilized plot | 44 | 25 | 31 |

[a] total polysaccharide-C determined by $H_2SO_4$ hydrolysis + MBTH method

[b] calculated values by taking into account the polysaccharide-C and the non hydrolyzable O-alkyl C

[c] determined by $^{13}$C NMR analysis of the hydrolysis residue

monosaccharides during hydrolysis. Summarizing, for the examined soils, the discrepancy between polysaccharide yields and O-alkyl C in $^{13}$C NMR has two major reasons, i.e. overlapping signals from compounds other than carbohydrates in the O-alkyl C range, and the fact that a considerable portion of hydrolyzable O-alkyl compounds could not be identified.

## 4.4 Conclusions

- The refractory SOC for most of the study sites is characterized by
  i) a higher contribution of aromatic C, mainly of protonated / C-substituted aryl compounds (130 ppm resonance),
  ii) a lower contribution of O/N-alkyl C compounds,
  iii) a rather similar proportion of alkyl C and of carboxyl functional groups as compared with more labile SOC fractions,
  iv) a higher ratio of alkyl-to-O/N-alkyl C compounds compared with labile C fractions, suggesting a higher degree of decomposition of the OM within the refractory pool.
- From the extent of decline of the various C-species it follows that aromatic C is the most stable carbon type over the long-term.
- The refractory C has a similar contribution of non-cellulosic polysaccharides as labile C fractions. The extent of C decline indicates that polysaccharides are more stable than the sum of O/N-alkyl C compounds determined by NMR spectroscopy. This work shows that the behavior of the compound class of polysaccharides is not completely identical to that of O/N-alkyl C from $^{13}$C NMR spectroscopy. Furthermore, comparing the results from the two methods reveals that polysaccharides can explain only about half of the intensity in the O-alkyl C range.

## 4.5 References

Allard, B., Templier, J., Largeau, C., 1997. Artifactual origin of myobacterial bacteran. Formation of melanoidin-like artifact macromolecular material during the isolation process. Organic Geochemistry 26, 691-703.

Almendros, G., Martinez, A.T., Gonzalez, A.E., Gonzales-Vila, F.J., Fründ, R., Lüdemann, H.-D., 1992. CPMAS $^{13}$C NMR study of lignin preparations from wheat straw transformed by five lignocellulose-degrading fungi. Journal of Agricultural and Food Chemistry 40, 1297-1302.

Asmus, F., 1990. Versuch M4 Groß Kreutz - Wirkung organischer und mineralischer Düngung und ihrer Kombination auf Pflanzenertrag und Düngung. In: Dauerfeldversuche - Übersicht, Entwicklung und Ergebnisse von Feldversuchen mit mehr als 20 Jahren Versuchsdauer. Akademie der Landwirtschaftswissenschaften, Berlin. pp. 245-250.

Baldock, J.A., Oades, J.M., Nelson, P.N., Skene, T.M., Golchin, A., Clarke, P., 1997. Assessing the extent of decomposition of natural organic materials using solid-state $^{13}$C NMR spectroscopy. Australian Journal of Soil Research 35, 1061-1083.

Cambardella, C.A., 1998. Experimental verification of simulated soil organic matter pools. In Lal, R., Kimble, J.M., Follett, R.F., Stewart, B.A. (Eds.) Soil Processes and the Carbon Cycle. Advances in Soil Science. CRC Press, Boca Raton. pp. 519-526.

Cheshire, M.V., Russell, J.D., Fraser, A.R., Bracewell, J.M., Robertson, G.W., Benzing-Purdie, L.M., Ratcliffe, C.I., Ripmeester, J.A., Goodman, B.A., 1992. Nature of soil carbohydrate and its association with soil humic substances. Journal of Soil Science 43, 359-373.

Diez, T., Beck, T., Brandhuber, R., Capriel, P., Krauss, M., 1997. Veränderungen der Bodenparameter im Internationalen Organischen Stickstoffdauerdüngungsversuch (IOSDV) Puch nach 12 Versuchsjahren. Archiv für Acker- und Pflanzenbau und Bodenkunde 41, 113-121.

Elliott, E.T., Paustian, K., Frey, S.D., 1996. Modeling the measurable or measuring the modelable: a hierarchical approach to isolating meaningful soil organic matter fractionations. In Powlson, D.S., Smith, P., Smith, J.U. (Eds.) Evaluation of Soil Organic Matter Models Using Existing Long-term Datasets. NATO ASI Series, vol. I 38. Springer Verlag, Berlin. pp. 161-179.

Fründ, R., Haider, K., Lüdemann, H.-D., 1994. Impact of soil management practices on the organic matter structure - investigations by CPMAS $^{13}$C NMR-spectroscopy. Zeitschrift für Pflanzenernährung und Bodenkunde 157, 29-35.

Gregorich, E.G., Kachanoski, R.G., Voroney, R.P., 1989. Carbon mineralization in soil size fractions after various amounts of aggregate disruption. Journal of Soil Science 40, 649-659.

Haider, K., 1992. Problems related to the humification processes in soils of temperate climates. In Stotzky, G., Bollag, J.-M. (Eds.) Soil Biochemistry, vol. 7. Marcel Dekker, Inc. pp. 55-94.

Haumaier, L., Zech, W., 1995. Black carbon - possible source of highly aromatic components of soil humic acids. Organic Geochemistry 23, 191 - 196.

Kinchesh, P., Powlson, D.S., Randall, E.W., 1995. $^{13}$C NMR studies of organic matter in whole soils: II. A case study of some Rothamsted soils. European Journal of Soil Science 46, 139-146.

Knicker, H., 1993. Quantitative $^{15}$N- und $^{13}$C-CPMAS-Festkörper- und $^{15}$N-Flüssigkeits-NMR-Spektroskopie an Pflanzenkomposten und natürlichen Böden. Ph.D.-Thesis, Universität Regensburg.

Knicker, H., Lüdemann, H.-D., 1995. N-15 and C-13 CPMAS and solution NMR studies of N-15 enriched plant material during 600 days of microbial degradation. Organic Geochemistry 23, 329-341.

Kögel, I., Hempfling, R., Zech, W., Hatcher, P.G., Schulten, H.-R., 1988. Chemical composition of the organic matter in forest soils: 1. Forest litter. Soil Science 146, 124-136.

Kögel-Knabner, I., 1995. Composition of soil organic matter. In Nannipieri, P., Alef, K. (Eds.) Methods in Applied Soil Microbiology and Biochemistry. Academic Press. pp. 66-78.

Kögel-Knabner, I., 1997. $^{13}$C and $^{15}$N NMR spectroscopy as a tool in soil organic matter studies. Geoderma 80, 243-270.

Kögel-Knabner, I., Zech, W., Hatcher, P.G., 1988. Chemical composition of the organic matter in forest soils: The humus layer. Zeitschrift für Pflanzenernährung und Bodenkunde 151, 331-340.

Körschens, M., Eich, D., 1990. Der Statische Versuch Lauchstädt. In: Dauerfeldversuche - Übersicht, Entwicklung und Ergebnisse von Feldversuchen mit mehr als 20 Jahren Versuchsdauer. Akademie der Landwirtschaftswissenschaften, Berlin. pp. 7-23.

Körschens, M., Weigel, A., Schulz, E., 1998. Turnover of soil organic matter (SOM) and long-term balances - tools for evaluating sustainable productivity of soils. Zeitschrift für Pflanzenernährung und Bodenkunde 161, 409-424.

Krauss, M., Pommer, G., Beck, R., Brandhuber, R., Capriel, P., 1997. Dauerversuche mit Fruchtfolgen und Düngung am Staatsgut Puch, Oberbayern. Auswirkungen auf Bodenmerkmale. Archiv für Acker- und Pflanzenbau und Bodenkunde 42, 193-199.

Kubát, J., Novák, B., 1992. C- und N-Dynamik im Boden unter Schwarzbrache im Modellversuch in Prag-Ruzyné. In: Tagungsbericht zum Symposium Dauerfeldversuche und Nährstoffdynamik. pp. 70-72.

Leifeld, J., 1998. Einfluß von Kompostanwendung auf den Umsatz der organischen Substanz in Böden - Ergebnisse aus Modell- und Feldversuchen. Ph.D.-Thesis, Ruhr-Universität Bochum. Shaker Verlag, Reihe Umweltwissenschaft.

Lowe, L.E., 1978. Carbohydrates in soil. In Schnitzer, M., Khan, S.U. (Eds.) Soil Organic Matter. Elsevier, Amsterdam. pp. 65-93.

Lüdemann, H.-D., Nimz, H., 1974. $^{13}$C-Kernresonanzspektren von Ligninen, 2: Buchen- und Fichten-Björkmann-Lignin. Die Makromolekulare Chemie 175, 2409-2422.

Mahieu, N., Powlson, D.S., Randall, E.W., 1999. Statistical analysis of published carbon-13 CPMAS NMR spectra of soil organic matter. Soil Science Society of America Journal 63, 307-319.

Mercik, S., Stepien, W., Gebski, M., 1997. Results of 75 years of continuous fertilization experiments in Skierniewice as a contribution to modification of fertilization. Archiv für Acker- und Pflanzenbau und Bodenkunde 42, 201-210.

Oades, J.M., 1995. An overview of processes affecting the cycling of organic carbon in soils. In Zepp, R.G., Sonntag, C. (Eds.) Role of Nonliving Organic Matter in the Earth's Carbon Cycle. John Wiley & Sons Ltd. pp. 293-303.

Pakulski, J.D., Benner, R., 1992. An improved method for the hydrolysis and MBTH analysis of dissolved and particulate carbohydrates in seawater. Marine Chemistry 40, 143-160.

Parton, W.J., Schimel, D.S., Cole, C.V., Ojima, D.S., 1987. Analysis of factors controlling soil organic matter levels in Great Plains grasslands. Soil Science Society of America Journal 51, 1173-1179.

Paul, E.A., Clark, F.E., 1989. Soil Microbiology and Biochemistry. Academic Press, San Diego.

Peersen, O.B., Wu, X., Kustanovich, I., Smith, S.O., 1993. Variable-Amplitude Cross-Polarization MAS NMR. Journal of Magnetic Resonance 104, 334-339.

Randall, E.W., Mahieu, N., Powlson, D.S., Christensen, B.T., 1995. Fertilization effects on organic matter in physically fractionated soils as studied by $^{13}$C NMR: results from two long-term field experiments. European Journal of Soil Science 46, 557-565.

Reichelt, H., 1990. Dauerversuche auf Berglehm-Braunerde im Erzgebirge. In: Dauerfeldversuche - Übersicht, Entwicklung und Ergebnisse von Feldversuchen mit mehr als 20 Jahren Versuchsdauer. Akademie der Landwirtschaftswissenschaften, Berlin. pp. 275-318.

Rumpel, C., Knicker, H., Kögel-Knabner, I., Skjemstad, J.O., Hüttl, R.F., 1998. Types and chemical composition of organic matter in reforested lignite-rich mine soils. Geoderma 86, 123-142.

Schaefer, J., Stejskal, E.O., 1976. Carbon-13 nuclear magnetic resonance of polymers spinning at magic angle. Journal of the American Chemical Society 98, 1031-1032.

Schlichting, E., Blume, H.-P., Stahr, K., 1995. Bodenkundliches Praktikum. Blackwell Wissenschafts-Verlag, Berlin.

Schmidt, M.W.I., Knicker, H., Hatcher, P.G., Kögel-Knabner, I., 1996. Impact of brown coal dust on the organic matter in particle-size fraction of a Mollisol. Organic Geochemistry 25, 29-39.

Schmidt, M.W.I., Knicker, H., Hatcher, P.G., Kögel-Knabner, I., 1997. Improvement of $^{13}$C and $^{15}$N CPMAS NMR spectra of bulk soils, particle size fractions and organic material by treatment with 10% hydrofluoric acid. European Journal of Soil Science 48, 319-328.

Schmidt, M.W.I., Skjemstad, J.O., Gehrt, E., Kögel-Knabner, I., 1999. Charred organic carbon in German chernozemic soils. European Journal of Soil Science 50, 351-365.

Schnieder, E., 1990. Die Dauerversuche in Thyrow. In: Dauerfeldversuche - Übersicht, Entwicklung und Ergebnisse von Feldversuchen mit mehr als 20 Jahren Versuchsdauer. Akademie der Landwirtschaftswissenschaften, Berlin. pp. 205-229.

Skjemstad, J.O., Clarke, P., Taylor, J.A., Oades, J.M., McClure, S.G., 1996. The chemistry and nature of protected carbon in soil. Australian Journal of Soil Science 34, 251-271.

Sollins, P., Homann, P., Caldwell, B.A., 1996. Stabilization and destabilization of soil organic matter: mechanisms and controls. Geoderma 74, 65-105.

Van Veen, J.A., Paul, E.A., 1981. Organic carbon dynamics in grassland soils. 1. Background information and computer simulation. Canadian Journal of Soil Science 61, 185-201.

# 5 Contribution of airborne contaminants to the refractory organic carbon of arable soils in highly industrialized areas

## Abstract

This section assesses the contribution of airborne contaminants to the refractory C pool of three long-term experimental sites that are located in industrialized regions. The methodological approach included i) particle-size fractionation of the soils, ii) analysis of SOC structure by $^{13}$C NMR spectroscopy (conventional CPMAS experiments for the whole range of separates, dipolar dephasing experiments for fine separates), and iii) morphological characterization of OM (organic matter) composition by techniques of coal petrography (for coarse-intermediate separates). Comparing the contrasting treatments revealed higher proportions of aromatic C in nearly all separates of the C-depleted plots, most pronounced in coarse-intermediate separates. Coarse-intermediate separates showed relatively high proportions of aromatic C as compared with published data of other soils, especially in the C-depleted plots. Coal petrographic analysis showed that high aromaticity in separates 2000-20 µm can be explained by the presence of contaminants, i.e. coal, coke, combustion residues from fossil fuels. For the fine separates (< 6 µm), the sites differed with regard to aromaticity and the protonation degree of aryl C (110 to 140 ppm): For the two sandy sites, aromatic C values were similar to other soils and dipolar dephasing experiments revealed a dominance of protonated aryl C compounds; for the loamy soil, aromatic C intensities were comparatively high, and about half of the aryl signal was attributable to non-protonated/C-substituted carbons. Our results indicate a contribution of airborne contaminants to the refractory C pool for the investigated sites. But whereas for the two sandy soils this contribution appears to be limited to coarse-intermediate separates, in the loamy soil a contamination may also occur in fine separates. From the OC balance of the C-depleted plots it was roughly assessed that the contribution from contaminants to the refractory C pool is smaller than that from native OM which is stabilized mainly within fine particle-size separates. Nevertheless, the contribution of contaminants to the refractory C will - to a certain extent - affect the (long-term) turnover of SOC at these sites.

## 5.1 Introduction

Long-term agroecosystem experiments have been used as a basis for investigating quantitative and qualitative aspects of soil organic matter (SOM) in relation to management practices. Questions that have been addressed in previous studies include e.g. the SOM storage potential (Körschens, 1998), the dynamics of SOM fractions, e.g. light fraction OM (Janzen et al., 1997) and the structural composition of SOM (Randall et al., 1995, Beavis and Mott, 1996; Capriel, 1997) in relation to management practices, the turnover of C derived from various organic amendments (Gerzabek et al., 1997). Furthermore, data from long-term field experiments provide a unique possibility for the evaluation of model approaches for the turnover of SOM. According to Falloon and Smith (2000), an accurate estimation of the refractory SOM compartment is of major importance in these models.

In agroecosystem experiments various treatments with regard to soil fertilization are established, including plots which do not receive any fertilization (unmanured / „control“ plots). At some experimental stations long-term bare fallows have been set up. Unmanured plots and bare fallows represent treatments that are considerably depleted in the content of soil organic carbon (SOC) in comparison with fertilized plots, provided that the duration of the experiment is at least several decades (Körschens et al., 1998). This results from the reduced (unmanured plots) or completely missing OM-supply (bare fallow) as compared with plots under fertilization over the long-term. To characterize the refractory pool of SOC, we selected „C-depleted plots“ (unmanured plots and bare fallows) and for comparative purposes „fertilized plots“ (with the combined application of mineral and organic fertilizers) from long-term field experiments (Fig. 1). We expect that the C-depleted plots are primarily depleted in labile SOC compounds (with a turnover time of years to decades). Compounds assignable to the refractory C pool (with a turnover time from hundreds to thousands of years) should not be affected by C-depletion at a time-scale of decades, as corresponds to the duration of our experiments (from 40 to nearly 100 years) (Fig. 1). Consequently, the SOC of the C-depleted plots should to a large part consist of refractory compounds. Such C-depleted plots have been the basis for quantifying the refractory pool in arable soils (Rühlmann, 1999), and for studying the chemical composition of refractory compounds (Kiem et al., 2000).

Examining the soil samples from the long-term experiments by solid-state $^{13}$C NMR spectroscopy, we noted unusually high contributions of aromatic C, particularly in the C-depleted plots. The experimental sites are located either within highly industrialized regions or in the extended surroundings of industrial centers. This led to the suspicion that particles of airborne contaminants such as coal or combustion residues might have accumulated in the soils. Great amounts of carbonaceous particles have been emitted since the beginning of industrialization and dispersed ubiquitously in the environment (Schmidt and Noack, 2000). Coal and charred OM are considered to be rather resistant against degradation, and therefore, these compounds would mainly accumulate in the refractory SOC compartment, affecting the estimation of the amount and composition of the refractory C pool.

In the present study, we make use of the experimental design of long-term field experiments to evaluate the contribution of airborne contaminants to the refractory OC of arable soils in industrialized areas. By comparing C-depleted plots, enriched in refractory C, with fertilized plots, information on specific structural properties of the refractory C pool can be obtained. We will use $^{13}$C NMR spectroscopy for the study of SOC in the whole range of particle-size fractions and coal petrographic analysis for selected coarse-intermediate separates.

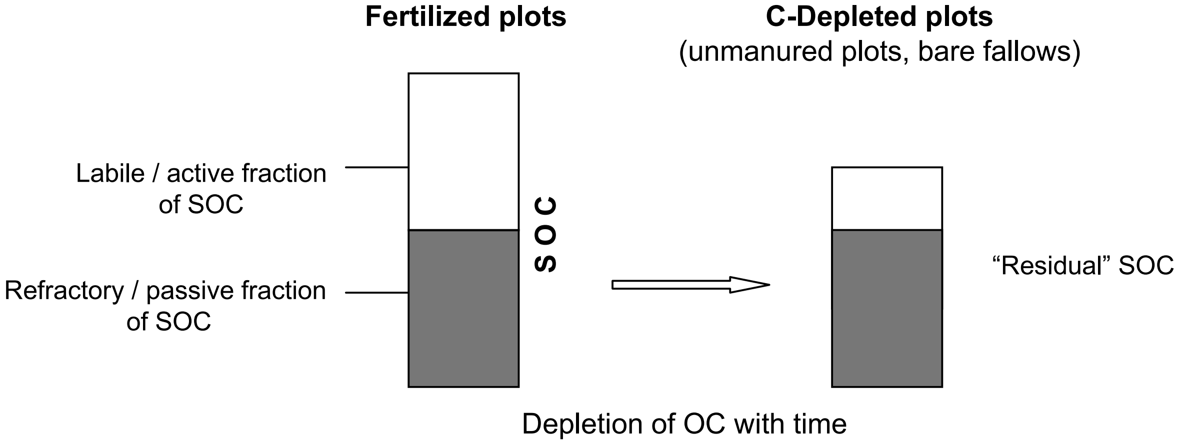


**Fig. 1:** Comparison of C-depleted with fertilized plots from long-term agroecosystem experiments: behavior of labile and refractory pools of SOC in the course of C-depletion.

## 5.2 Materials and methods

### 5.2.1 Study sites

#### *5.2.1.1 Arable soils from industrialized areas (Thyrow, Skierniewice, Bad Lauchstädt)*

We investigated soils from long-term agroecosystem experiments situated in eastern Germany (Thyrow, Bad Lauchstädt) and Poland (Skierniewice) (Table 1). The soils at Thyrow and Skierniewice have a sandy texture and are classified as Luvisols. The soil at the experimental station Bad Lauchstädt represents a Chernozem, and has a high silt content (silt loam according to USDA, 1998). Samples were taken in summer 1998 from the following experimental plots at the three stations (to a depth of 20 cm):

**Table 1:** Characteristics of the study sites and overview of treatments in the long-term agroecosystem experiments.

| **Site** | **Soil type** (FAO)[a] | **Texture**[b] (%) | | | **Start of experiment** | **Treatments** | **Organic carbon** (g kg$^{-1}$) Plots | |
|---|---|---|---|---|---|---|---|---|
| | | Sand | Silt | Clay | | | **Fertilized** | **C-Depleted** |
| Thyrow (Germany)[c] | Albic Luvisol | 82 | 15 | 2.7 | 1937 | I) Fertilized (NPK + organic manure) | 6.8 | |
| | | | | | | II) Unmanured | | 3.2 |
| Skierniewice (Poland)[d] | Luvisol | 72 | 21 | 6.2 | 1923 | I) Fertilized (NPK + organic manure) | 8.8 | |
| | | | | | | II) Unmanured | | 4.4 |
| Bad Lauchstädt (Germany)[e] | Haplic Chernozem | 7 | 70 | 23 | 1902 | I) Fertilized (NPK + organic manure) | 24 | |
| | | | | | 1902 | II) Unmanured | | 16 |
| | | | | | 1956 | III) Bare fallow | | 15 |

[a] FAO (1998)

[b] sand (2000-63 µm), silt (63-20 µm), clay (< 2 µm); mean value of the various plots at a site

[c] Ellmer et al. (2000)

[d] Mercik et al. (2000)

[e] Körschens et al. (1998)

I) plots with a four-to five-field crop rotation, receiving mineral fertilizers and farmyard manure (fertilized plots),

II) plots with the same crop rotation as the fertilized plots, but kept permanently unmanured (unmanured plots),

III) a bare fallow (established at Bad Lauchstädt).

The unmanured plots (II) and the bare fallow (III) are depleted in total SOC compared with the fertilized counterparts (Table 1), and are thus designated as “C-depleted plots”.

Bad Lauchstädt is located approximately 15 km south west of Halle (federal state Saxony-Anhalt). The region around Halle and Leipzig is highly industrialized and has been an important brown coal mining district for centuries (Pätz et al., 1989). Thyrow lies approximately 20 km south of Berlin (federal state Brandenburg), while Skierniewice is located approximately 75 km in the south west of Warsaw (Poland). Even if the experimental stations at Thyrow and Skierniewice are not in close proximity to industrial plants, they are situated in the (extended) surroundings of big cities which are industrial centers. In contrast to the Halle-Leipzig area, mining for coal does not play a role in the regions around Berlin and Warsaw.

#### *5.2.1.2 Arable soil from a rural region (Scheyern)*

Samples were also obtained from the research station Scheyern located in a rural area in southern Germany (federal state Bavaria) (von Lützow et al., 2001). Samples were taken in spring 1999 to a depth of 3 cm at plots which are managed according to the guidelines for organic farming (seven-field crop rotation, farmyard manure as sole fertilization). The soil at the sampled plots is classified as Cambisol (FAO, 1998), showing 56% sand, 33% silt and 11% clay in the topsoil (sandy loam; USDA, 1998). The soil from Scheyern was expected to show no substantial contamination from industrial emissions. Therefore, this soil was taken as a reference comparing it with the soils from the long-term experiments which were suspected to contain airborne contaminants.

All the soil samples were air-dried and components > 2 mm were removed by dry sieving.

### 5.2.2 Particle-size fractionation

The procedure used for the long-term experiments (Thyrow, Skierniewice, Bad Lauchstädt) involved ultrasonic dispersion of the soil samples by the two-step method proposed by Amelung et al. (1998), and isolation of the particle-size fractions by a combination of wet sieving (fractions 2000-20 µm), SPLITT-fractionation (Keil et al., 1994) (fractions 20-0.2 µm) and centrifugation (< 0.2 µm).

A suspension of air-dried soil < 2 mm (soil-to-water ratio 1:3 for Thyrow and Skierniewice, and 1:5 for Bad Lauchstädt) was ultrasonicated at 60 J $ml^{-1}$ using a probe-type ultrasonic disintegrator (Branson 250, Branson). Thereafter, particles 2000-250 µm (coarse and medium sand), released by the first dispersion step, were removed by wet sieving. For the Thyrow and Bad Lauchstädt samples, organic compounds in this fraction were shown to be plant residues as well as organic contaminants (coal, coke, char) (see section 5.3.1.3). Amelung and Zech (1999) recommended the removal of particulate plant residues (2000-250 µm) after weak dispersion, in order to avoid mechanical disruption during the subsequent high-energy ultrasonification. Analogously, Schmidt et al. (1999a) for soils containing sand-sized particles of coal / combustion residues proposed a first step of limited dispersion, followed by sieving to isolate the very coarse fraction. This is to circumvent a possible fragmentation and redistribution of coal and combusted particles by high-enery ultrasonification necessary for complete dispersion. The authors found lignite particles to be particularly fragile.

In a second step, the suspension < 250 µm (soil-to-water ratio 1:5 for Thyrow and Skierniewice, and 1:10 for Bad Lauchstädt) was ultrasonicated at 440 J $ml^{-1}$ for complete dispersion. The fractions 250-63 µm (fine sand) and 63-20 µm (coarse silt) were gained by wet sieving. In two successive SPLITT runs, employing the cutoffs of 6 µm (1st run) and 2 µm (2nd run), the fractions 20-6 µm (medium silt), 6-2 µm (fine silt) and 2-0.2 µm (coarse clay) were obtained. The fine clay (< 0.2 µm) was isolated by repeated centrifugation. The 250 µm limit was adopted from Amelung and Zech (1999), while the other size limits follow the German classification system (AG Boden, 1994).

For the soil from Scheyern, two fractions of particulate organic matter (POM) (2000-20 µm, density ≤ 1.8 g $cm^{-3}$) were isolated which are denoted as “free POM” and “occluded POM”. The fractionation procedure is described into detail by von Lützow et al. (2001). Briefly, free POM was obtained from undispersed soil samples (prior to ultrasonification); the POM occluded within aggregates was gained after low-energy sonification of the samples (22 J $ml^{-1}$), resulting in a partial breakdown of aggregates and thus in a release of the POM.

All particle-size fractions and the particulate organic matter from Scheyern were freeze-dried. For elemental analysis of the size fractions, aliquots were ground in a ball mill. Prior to NMR analysis, the particulate OM was ground by a mortar.

### 5.2.3 Elemental analysis

C and N contents in particle-size fractions were determined by dry combustion in a Vario EL elemental analyzer (Elementar Analysen-Systeme, Hanau, Germany). To determine the proportion of inorganic carbon, aliquots of the samples were analyzed after ignition at 550°C for 3 h (removal of organic matter). Inorganic carbon was not detected in any of the samples.

### 5.2.4 $^{13}$C CPMAS NMR spectroscopy

#### *5.2.4.1 Sample pretreatment*

Prior to nuclear magnetic resonance (NMR) spectroscopic analysis, fractions < 63 µm from Thyrow, Skierniewice and Bad Lauchstädt were treated with 10% hydrofluoric acid (HF) (Schmidt et al., 1997). This was done to to enrich the organic material, and to remove paramagnetic materials such as $Fe^{3+}$-minerals which are concentrated in fine particle-size fractions. The removal of paramagnetic species together with the enrichment in organic matter result in a higher resolution of the $^{13}$C NMR spectra (Schmidt et al., 1997). 20 ml of 10% (v/v) HF solution were added to 1 g of sample material in a polyethylene bottle. The suspension was shaken horizontally overnight. After centrifugation, the supernatant was removed. The HF treatment was repeated twice. The residue was washed extensively with deionized water and freeze-dried. The recovery of C following the HF treatment ranged from 75 to 100% in the various fractions.

In fractions 2000-63 µm of the sandy soils (Thyrow, Skierniewice - both treatments) and the C-depleted plots of the loamy soil (Bad Lauchstädt), OC contents are very low (see Table 7). These separates were subjected to density fractionation using a sodium polytungstate solution solution [$Na_6(H_2W_{12}O_{40}) \times H_2O$, Sometu, Berlin, Germany] of a density 1.8 g cm$^{-3}$. This allowed the OM to be concentrated within the light fraction (≤ 1.8 g cm$^{-3}$). The recovery of OC in the light fraction (relative to OC in the total separates) was between 80 and 90%. NMR analyses were carried out on the light subfractions of these separates. In fractions 2000-63 µm of the fertilized plot from Bad Lauchstädt a satisfactory sensitivity in the NMR experiments was obtained without any pretreatment.

#### *5.2.4.2 Parameters used in CPMAS NMR experiments*

The solid-state $^{13}$C NMR spectra were obtained on a Bruker DSX 200 spectrometer operating at a $^{13}$C resonance frequency of 50.3 MHz. The cross polarization magic-angle spinning (CP MAS) technique was applied (Schaefer and Stejskal, 1976), using a contact time of 1 ms and spinning the samples at 6.8 kHz. A ramped $^1$H-pulse was used during contact time in order to circumvent inexact Hartmann-Hahn conditions (Peersen et al., 1993). Between 30,000 and 600,000 scans were accumulated. The pulse delay used was 400 ms for the separates of the long-term experiments and the POM from Scheyern, and 2 s for plant materials (rye straw, potatio foliage) (Knicker, 1993). The plant materials were air-dried and ground by a mortar prior to NMR measurements. Prior to Fourier transformation, a line broadening of 50 to 200 Hz was applied. A contact time of 1 ms and a pulse delay of 400 ms should be optimal for recording CP spectra of native SOM (Knicker, 1993). According to Hatcher (1988) and Rumpel et al. (1998b), these parameters should also be adequate for obtaining representative spectra of coal samples for those carbons which are basically detectable by the cross polarization technique. For carbons isolated from protons (e.g. in highly condensed aromatic structures) there is no effective cross polarization, and in materials which contain such structures part of the carbons is not „seen“ in CP NMR experiments (Skjemstad et al., 1997) (see section 5.3.1.4).

The chemical shift scale was referenced to tetramethylsilane (= 0 ppm). For quantification, NMR signals were integrated by using an integration routine supplied by the instrument software. Relative signal intensities were calculated for four chemical shift regions, which can be assigned to alkyl C (0 to 45 ppm), O/N-alkyl C (45 to 110 ppm), aromatic C (110 to 160 ppm) and carboxyl/carbonyl C (160 to 220 ppm). Signal intensities for aromatic and carboxyl C were corrected for spinning side bands: i) Side bands of the peak at 128 ppm are found at 220 to 276 ppm and -50 to 0 ppm; the side band intensities were added to the central band (110 to 140 ppm). ii) Side bands of the carboxyl C are found in the range 276 to 323 ppm and 0 to 45 ppm. Assuming similar size for the two side band, twice the integral in the range 276 and 323 ppm was added to the central band (160 to 220 ppm). Then, the integral 276 to 323 ppm was subtracted from the alkyl C region (0 to 45 ppm).

The recovery rates for signal intensities in particle-size separates (relative to the intensity determined for the bulk sample) were 98 ± 13% (alkyl C), 84 ± 8% (O/N-alkyl C), 76 ± 14% (aromatic C), and 99 ± 25% (carboxyl C) (mean value ± standard deviation for six soils from the long-term experiments).

#### *5.2.4.3 Dipolar dephasing experiments*

The aromatic C region can be further divided into a range 110 to 140 ppm, with the (overlapping) signals of protonated and C-substituted aryl species (denoted as „aryl C“ in the following), and 140 to 160 ppm, with the resonances of mainly O-substituted aryl carbons (O-aryl C). To differentiate between the signals of protonated and non-protonated/C-substituted aryl carbons in the range 110 to 140 ppm, we performed dipolar dephasing (DD) experiments (fraction 2-0.2 µm from Thyrow and Bad Lauchstädt). Applying the variable amplitude cross polarization technique (VA CP), as described above, a delay ($t_{dd}$) was inserted during which the $^1$H decoupler was turned off between the cross polarization and signal acquisition. A 180° refocusing pulse was inserted at ½ $t_{dd}$ to assist phasing (Wilson, 1987). A series of spectra was acquired for each sample varying the dephasing delay ($t_{dd}$) between 2 and 150 µsec.

For the range 110 to 140 ppm, the signal decay with increasing dephasing delay can be modelled by combining the Gaussian equation, describing the decay of the protonated carbons, with the Lorentzian equation, describing the signal loss of the non-protonated carbons (Wilson, 1987).

Carbons with directly attached protons exhibit a strong dipolar $^1$H-$^{13}$C interaction, and consequently show a fast signal decay during interrupted decoupling. The signal decay of protonated carbons can be described as an exponential function of $t_{dd}{}^2$ (Gaussian equation):

$$I_A\,(t_{dd}) = I_A\,(0) \times \exp\,(-t_{dd}^2/2\,T_A{}^2) \qquad (1)$$

where $I_A$ ($t_{dd}$) is the signal intensity at the dephasing delay $t_{dd}$, $I_A$ (0) is the initial signal intensity at $t_{dd} = 0$, and $T_A$ is the DD time constant for the protonated aryl C.

For non-protonated carbons the dipolar $^1$H-$^{13}$C interaction is weaker, as compared with protonated C. Non-protonated carbons dephase at a slower rate, the signal decay being a simple exponential function of $t_{dd}$ (Lorentzian equation):

$$I_B\,(t_{dd}) = I_B\,(0) \times \exp\,(-t_{dd}/T_B\,) \qquad (2)$$

where $I_B$ ($t_{dd}$) is the signal intensity at the dephasing time $t_{dd}$, $I_B$ (0) is the intensity at $t_{dd} = 0$, and $T_B$ is the DD time constant for the non-protonated aryl C.

The total signal intensity in the range 110 to 140 ppm at the dephasing time $t_{dd}$ (I ($t_{dd}$)) can be calculated as the sum of the intensity of protonated aryl C ($I_A$ ($t_{dd}$)) and non-protonated aryl C ($I_B$ ($t_{dd}$)):

$$I\,(t_{dd}) = I_A\,(t_{dd}) + I_B\,(t_{dd}) \qquad (3)$$

Using equations 1 and 2, results in:

$$I\ (t_{dd}) = I_A\ (0) \times \exp\ (-t_{dd}^2/2\ T_A^{\ 2}) + I_B\ (0) \times \exp\ (-t_{dd}/T_B\ ) \qquad (4)$$

Equation 4 was fitted to the datapoints of aryl C (110 to 140 ppm) by means of the software Sigmaplot for Windows (SPSS Corp.). For each sample two replicate series of DD experiments were performed; the data of the two series were combined and subjected to the fitting procedure. Two kinds of parameter were obtained from fitting: i) the dephasing time constants for protonated and non-protonated aryl C ($T_A$, $T_B$), and ii) the relative contributions of protonated and non-protonated C to the total signal intensity of aryl C. These contributions were calculated according to the following equations (Wilson, 1987):

$$\text{Aryl } C_p = I_A\ (0)\ /\ (I_A\ (0) + I_B\ (0)) \qquad (5)$$

$$\text{Aryl } C_{np} = I_B\ (0)\ /\ (I_A\ (0) + I_B\ (0)) \qquad (6)$$

where Aryl $C_p$ is the fraction of protonated aryl C, and Aryl $C_{np}$ the fraction of non-protonated aryl C, $I_{A/B}$ (0) are the initial intensities of protonated/non-protonated aryl C at a dephasing delay of zero (obtained from equation 4).

In addition, the two fractions were expressed as a percentage of total signal intensity in the NMR spectrum:

$$\text{Aryl } C_p{}^* = \text{Aryl } C_p \times \text{Aryl } C_{tot} \qquad (7)$$

$$\text{Aryl } C_{np}{}^* = \text{Aryl } C_{np} \times \text{Aryl } C_{tot} \qquad (8)$$

where Aryl $C_{p\ /\ np}{}^*$ is the proportion of protonated/non-protonated aryl C (% of total signal intensity), Aryl $C_{p\ /\ np}$ is the fractional contribution of protonated/non-protonated aryl C (to total aryl C, see equations 5 and 6), Aryl $C_{tot}$ is the relative intensity in the range 110 to 140 ppm (% of total signal intensity).

### 5.2.5 Coal petrography (Thyrow and Bad Lauchstädt)

Coal petrographic analysis was carried out for the sand (2000-63 µm) and coarse silt (63-20 µm) fractions of the C-depleted plots from Thyrow (unmanured plot) and Bad Lauchstädt (unmanured plot and bare fallow). We focused on the C-depleted plots, since we expected the contaminants to accumulate in these plots. The sand fractions 2000-250 µm and 250-63 µm obtained by the fractionation procedure (section 5.2.2) were combined for this analysis. To get rid of the minerals, the combined sand and the coarse silt fractions were subjected to density fractionation using sodium polytungstate solution [$Na_6(H_2W_{12}O_{40})$ x $H_2O$, Sometu, Berlin, Germany] of a density 1.9 g cm$^{-3}$. Previous investigations had shown that a density 1.9 g cm$^{-3}$ is adequate to isolate all types of organic materials (not associated with minerals) within the light fraction, including coke. Coke particles may show a high content of mineral components and thus exhibit a density of up to 2 g cm$^{-1}$, although they are highly porous. For the sand separates, 90% of the total OC were recovered in the light subfraction (≤ 1.9 g cm$^{-3}$), while in the coarse silt separates between 55 and 85% of the OC could be isolated by this procedure. The lower recovery of OC within the light fraction in the coarse silt may be a hint that more of the OM is physically associated with minerals compared with the sand. Subsequently, the light fraction material was embedded in epoxy resin (Struers, Germany), and the mount was polished parallel to the base. Microscopic analysis was done with a Leitz DMRX microscope photometre in reflected white light and under blue light irradiation (fluorescence mode). The petrographic composition of the samples was quantified by a standard point counting method (Taylor et al., 1998). The contribution of the various constituents is expressed relative to the total volume of OM (volume %). The terminology used for the various groups of organic matter follows the classification systems published in Tyson (1995) and Taylor et al. (1998).

## 5.3 Results and discussion

### 5.3.1 Fractions 2000-20 µm

#### *5.3.1.1 C-to-N ratios*

In separates 2000-20 µm, C/N-ratios are higher in the C-depleted plots compared with the fertilized counterparts in all of the long-term experiments (Table 2). For the sandy soils (Thyrow, Skierniewice), the increase is most pronounced in the sand fractions (increase by 11 to 15 units). For the loamy soil (Bad Lauchstädt), C/N-ratios are considerably higher in fractions 250-20 µm of the bare fallow.

#### *5.3.1.2 $^{13}$C NMR spectroscopy*

$^{13}$C NMR spectra of particle-size fractions from the contrasting treatments are given in Figs. 2 through 4. The band from 0 to 45 ppm may comprise methyl C (around 18 ppm) and

**Table 2:** . C-to-N-ratios in particle-size fractions of the contrasting treatments from the three study sites.

| | **C-to-N-ratios** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Thyrow** | | **Skierniewice** | | **Bad Lauchstädt** | | |
| **Particle-size fractions** (µm) | **Fertilized** | **C-Depleted** | **Fertilized** | **C-Depleted** | **Fertilized** | **C-Depleted** | |
| | | | | | | **Unmanured** | **Bare fallow** |
| 2000 - 250 | 16 | 27 | 20 | 35 | 22 | 22 | 24 |
| 250 - 63 | 17 | 33 | 22 | 36 | 20 | 27 | 50 |
| 63 - 20 | 14 | 19 | 19 | 25 | 14 | 16 | 87 |
| 20 - 6 | 13 | 16 | 15 | 18 | 13 | 11 | 18 |
| 6 - 2 | 11 | 13 | 12 | 11 | 12 | 12 | 14 |
| 2 – 0.2 | 11 | 12 | 11 | 11 | 12 | 11 | 13 |
| < 0.2 | 8 | 9 | 8 | 8 | 9 | 10 | 11 |

methylene C (around 30 ppm). The peak around 56 ppm is probably assignable to methoxyl C (e.g. in lignin, hemicellulose) and the alpha-C of amino acids. The signal in the region 60 to 110 ppm may be mainly due to carbohydrate-carbons, with distinct peaks at 72 ppm and 104 ppm (anomeric $C_1$ in polysaccharides); in some of the spectra a peak around 65 ppm can be seen ($C_6$ of sugars). The O-alkyl C range may also comprise signals from lignin (60 to 90 ppm propyl side-chains, 90 to 110 ppm carbons from syringyl units). The signal in the range 110 to 140 ppm may originate from protonated and C-substituted aromatic structures, but also from olefinic C. Protonated aryl C resonates mainly on the lower chemical shift side (around 120 ppm), while C-substituted aryl C is found around 130 ppm. The range 140 to 160 ppm may be attributed to O-substituted aryl C (e.g. in lignin). The signal around 172 ppm may arise from free carboxyl groups, ester and amide groups.

For the C-depleted plots from all sites, a strong decrease of the signals at 56, 72 and 104 ppm is evident in comparison with the fertilized plots (see fractions 250-63 and 63-20 µm, Figs. 2 through 4). A distinct peak at 150 ppm is discernable only in the fertilized plots. By contrast, the spectra of the depleted plots are dominated by a broad band in the aromatic C range (110 to 160 ppm), centred at 128 ppm, possibly overlaying the signal of O-aryl compounds. These features of the NMR spectra indicate that the C-depleted plots are relatively depleted in polysaccharides and presumably lignin. Plant litter shows prominent signals attributable to polysaccharides (at 72 and 104 ppm) and a clear signal pattern indicative of lignin (at 56 and around 150 ppm) (Kögel et al., 1988; Knicker and Lüdemann, 1995). The present results therefore suggest a lower contribution of plant residues to OC in coarse-intermediate separates of the C-depleted versus the fertilized plots.

Table 3 shows the relative signal distribution for fractions 2000-20 µm from the long-term experiments and the particulate OM from Scheyern. In the fertilized plots of the sandy soils (Thyrow, Skierniewice), in most fractions the highest relative intensity is found in the region of O/N-alkyl C (32 to 52%), and aromatic C being the second most abundant C-species (24 to 34%). By contrast, the fertilized plot of the loamy soil (Bad Lauchstädt) shows similar proportions of O/N-alkyl C and aromatic C (each around 30%). Management practices of the soil from Scheyern are comparable with the fertilized plots of the long-term field experiments. In the particulate OM from Scheyern, O/N-alkyl C accounts for 54 to 62% of total signal intensity and aromatic C for 17 to 20%, whereby the POM occluded within aggregates is depleted in O/N-alkyl C relative to the free POM, but shows slighty higher alkyl and aromatic C intensities. Alkyl C is in all cases the third most abundant C-species, with relative intensities around 20% in the long-term experiments. OM in separates 2000-20 µm mostly consists of plant residues in first stages of decomposition (e.g. Amelung et al., 1998). Thus, we also evaluated the composition of rye straw and potato foliage, as examples for plant litter compounds entering the soil under arable land. In these materials, O/N-alkyl C makes up 63 to 75% of total signal intensity, followed by 13 to 21% of alkyl C, and low proportions of aromatic and carboxyl C (each < 10%). The magnitude of O/N-alkyl C proportions is in line with the data on plant materials in the studies of Knicker (1993) and Mahieu et al. (1999). The most apparent difference between OM from Scheyern and the long-term experiments (fertilized plots) is the lower O/N-alkyl C and the higher aromatic C proportion in the long-term experiments. The composition of POM from Scheyern is closer to that of plant materials than OM in separates 2000-20 µm of the long-term experiments.

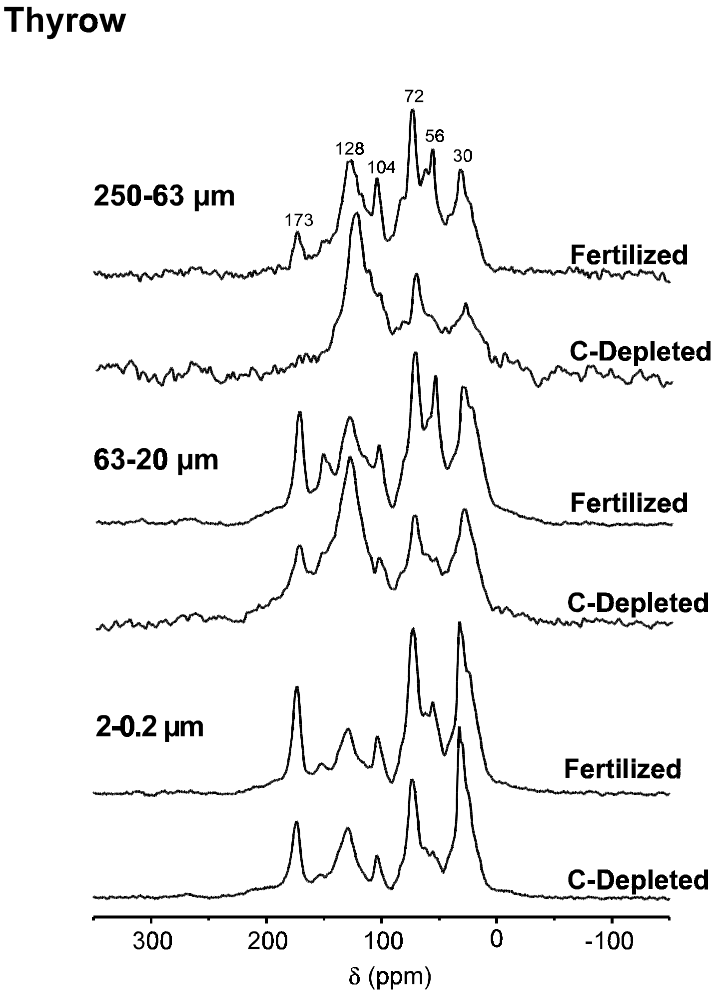


**Fig. 2:** $^{13}$C NMR spectra of selected particle-size fractions from the contrasting treatments of the Thyrow soil.

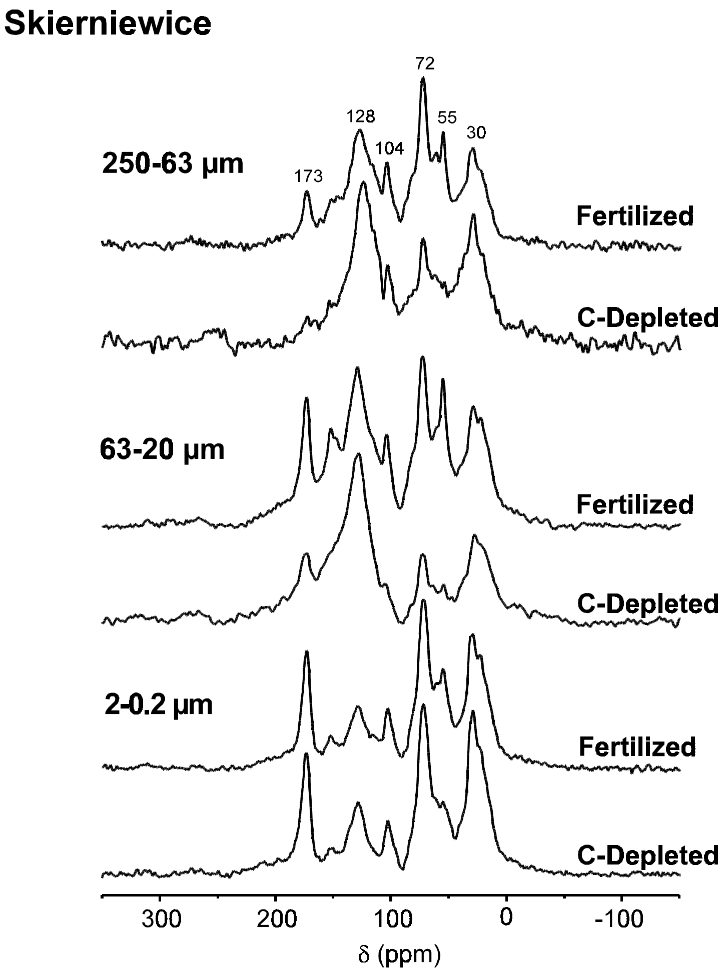
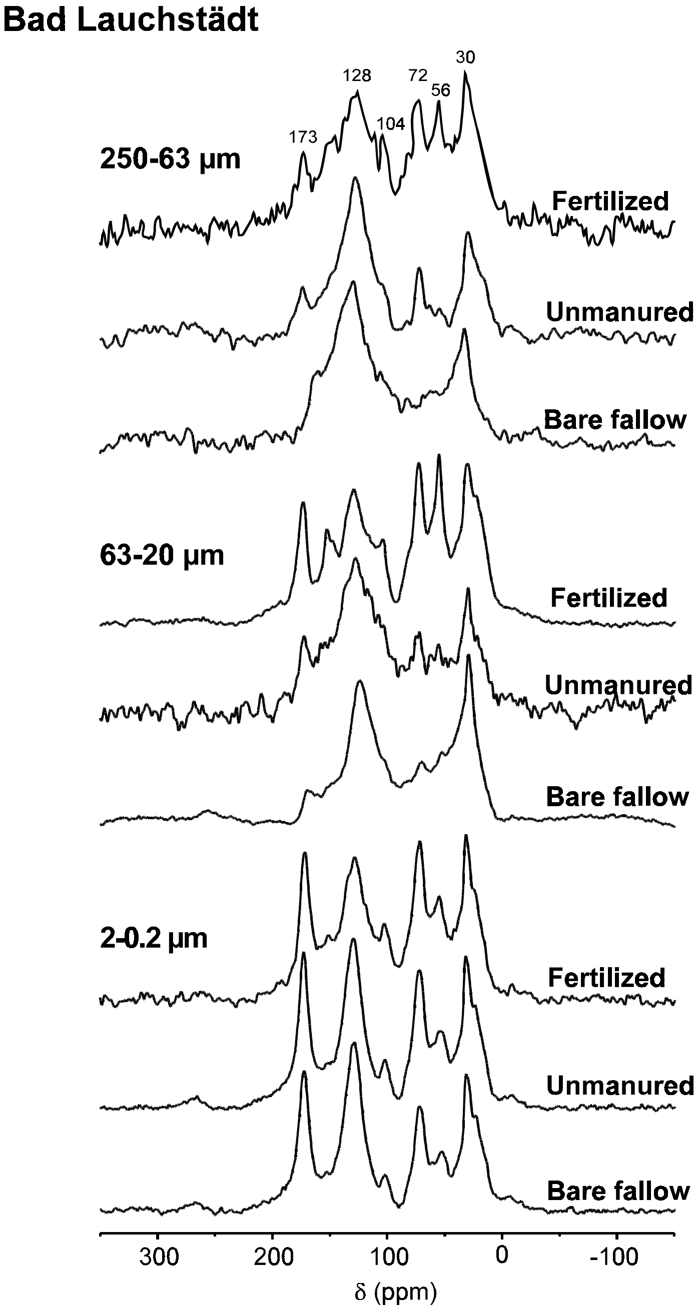


**Fig. 3:** $^{13}$C NMR spectra of selected particle-size fractions from the contrasting treatments of the Skierniewice soil.

Comparing the fertilized with the C-depleted plots in the long-term experiments, the most consistent trend is the higher abundance of aromatic C in the depleted plots (Table 3). The increase of aromatic C is in most cases associated with a lower proportion of O/N-alkyl C in the depleted plots. Aromatic C is the dominant C-species in the depleted plots, except for the coarsest fraction (2000-250 µm) at Thyrow and Bad Lauchstädt. Changes in the intensity of alkyl and carboxyl C along with C-depletion are smaller than for O/N-alkyl and aromatic C, and do not show a clear trend.

Mahieu et al. (1999) reported an average contribution of 48% O-alkyl C and 21% aromatic C for the sand-sized fractions of 52 soils. The aromatic C proportions in fertilized and depleted plots of our long-tem experiments are above the value of 21%. Compared with the average of 48% O-alkyl C, the loamy soil (Bad Lauchstädt) generally shows a lower contribution, while the fertilized plots of the sandy soils are close to this value. Mahieu et al. (1999) also showed



| | | Relative signal intensity (%) | | | |
|---|---|---|---|---|---|
| | | Carboxyl C | Aromatic C | O/N-Alkyl C | Alkyl C |
| 250-63 µm | Fertilized | 11 | 32 | 33 | 24 |
| | Unmanured | 19 | 47 | 20 | 14 |
| | Bare fallow | 9 | 48 | 26 | 17 |
| 63-20 µm | Fertilized | 14 | 31 | 33 | 22 |
| | Unmanured | 11 | 43 | 28 | 19 |
| | Bare fallow | 4 | 47 | 21 | 28 |
| 2-0.2 µm | Fertilized | 16 | 30 | 33 | 21 |
| | Unmanured | 20 | 33 | 27 | 20 |
| | Bare fallow | 20 | 36 | 22 | 21 |

**Fig. 4:** $^{13}$C NMR spectra of selected particle-size fractions from the different treatments of the Bad Lauchstädt soil.

that the contributions of the four C-species in sand-sized fractions of the 52 soils (from various land-uses) cover a wide range of values. This variation is probably due to differences in the composition of the input material and the degree to which OM degradation has progressed. Increasing extent of decomposition of plant materials was shown to result in a decrease of O-alkyl C and a concomitant increase of alkyl and also aromatic C signals (Baldock et al., 1997; Knicker and Lüdemann, 1995). Although the composition of particulate

**Table 3:** Relative signal distribution in $^{13}$C NMR spectra (% of total signal intensity) for particle-size fractions 2000-20 µm from the contrasting treatments of the long-term experiments as well as the particulate organic matter from Scheyern.

| | **Fertilized** | | | | **C-Depleted** | | | | **Δ (C-Depleted – Fertilized)** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Particle-size fractions** (µm) | **Alkyl C** | **O/N-Alkyl C** | **Aromatic C** | **Carboxyl C** | **Alkyl C** | **O/N-Alkyl C** | **Aromatic C** | **Carboxyl C** | **Alkyl C** | **O/N-Alkyl C** | **Aromatic C** | **Carboxyl C** |
| Thyrow | | | | | | | | | | | | |
| 2000-250 | 21 | 46 | 24 | 9 | 13 | 58 | 23 | 6 | - 8 | +12 | - 1 | - 3 |
| 250-63 | 19 | 45 | 30 | 6 | 20 | 40 | 37 | 3 | + 1 | - 5 | + 7 | - 3 |
| 63-20 | 22 | 36 | 28 | 14 | 18 | 27 | 40 | 16 | - 4 | - 9 | +12 | + 2 |
| Skierniewice | | | | | | | | | | | | |
| 2000-250 | 19 | 52 | 24 | 5 | 14 | 30 | 42 | 13 | - 5 | -22 | +18 | + 8 |
| 250-63 | 18 | 40 | 32 | 10 | 25 | 30 | 41 | 3 | + 7 | -10 | + 9 | - 7 |
| 63-20 | 18 | 32 | 34 | 16 | 16 | 15 | 48 | 20 | - 2 | -17 | +14 | + 4 |
| Bad Lauchstädt [a] | | | | | | | | | | | | |
| 2000-250 | 20 | 37 | 30 | 12 | 14 | 50 | 28 | 8 | - 6 | +13 | - 2 | - 4 |
| 250-63 | 24 | 33 | 32 | 11 | 14 | 20 | 47 | 19 | -10 | -13 | +15 | + 8 |
| 63-20 | 22 | 33 | 31 | 14 | 19 | 28 | 43 | 11 | - 3 | - 5 | +12 | - 3 |
| Scheyern | | | | | | | | | | | | |
| Free POM [b] | 13 | 62 | 17 | 8 | | | | | | | | |
| Occluded POM | 16 | 54 | 20 | 10 | | | | | | | | |

[a] Data for the C-depleted plot refer to the unmanured plot

[b] POM (particulate organic matter): 2000-20 µm

OM may considerably vary, the relatively high presence of aromatic C in the long-term experiments - compared with a soil from a rural environment and the mean value of numerous soils - can be a hint for the presence of highly aromatic compounds at these sites. Within the long-term experiments, the NMR data indicate a relative accumulation of compounds rich in aromatic C (mainly protonated/C-substituted aryl C) in the depleted plots at the expense of plant residues.

#### *5.3.1.3 Coal petrographic analysis (Thyrow and Bad Lauchstädt)*

Table 4 presents the various groups of organic materials identified by coal petrographic analysis in the C-depleted plots from Thyrow and Bad Lauchstädt. At both sites, the major groups of OM in fractions 2000-20 µm are represented by recent (or native) OM, coal (raw brown coal and hard coal), and coke. There are minor contributions of char (3 to 8%), charcoal (1 to 4%, except for the 2000-63 µm fraction of Thyrow with 13%), and traces of asphalt (< 1%). Comparing the sites, raw brown coal is more abundant at Bad Lauchstädt than at Thyrow, while hard coal is present at similar proportions. Comparing the two types of depleted plots at Bad Lauchstädt (unmanured plot, bare fallow) shows a relative accumulation of contaminants (coal and coke) under the conditions of the bare fallow with virtually no recent OM-input (fraction 2000-63 µm). Recent OM in the bare fallow can be mainly identified as pollen and spores, which are distributed ubiquitously.

To selectively record recent/native OM, fluorescence microscopy was applied. In Fig. 5b, components of recent OM are characterized by a strong, yellow-green fluorescence, whereas the other components, i.e. particles of char, coke and charcoal appear as dark spots (compare Fig. 5a with Fig. 5b). Chemical transformations of plant tissues in the course of coalification (condensation, increase in aromaticity) result in a decreasing fluorescence of the OM until complete cessation (Stach et al., 1982; Kleineidam et al., 1999).

A characteristic feature of high-rank coal, as well as coke, charcoal and charred OM (from fossil fuel combustion) is their high reflectance when examined under incident white light (Figs. 5c and 5d). These compounds show a grey-white appearance in the reflected white light mode. Recent OM and raw brown coal, which represents an early stage of coalification, show weak reflectance and appear in a brown-grey color (Fig. 5c). Reflectance of OM increases with increasing coalification degree (Stach et al., 1982).

**Table 4:** Composition of OM in fractions 2000-20 µm of the C-depleted plots from Thyrow and Bad Lauchstädt as revealed by coal petrographic analysis.

| | **Recent OM** [a] | **Raw brown coal** | **Hard coal** | **Coke** | | | **Charcoal** | **Char** | | | **Asphalt** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Brown-coal coke | Hard-coal coke | Total | | Spheres | Solid | Total | |
| **Treatments/Particle -size fractions** (µm) | (% of OM volume) | | | | | | | | | | |
| Thyrow<br>Unmanured plot | | | | | | | | | | | |
| 2000-63 | 52 | 6 | 7 | – | 20 | 20 | 13 | 2 | 1 | 3 | – |
| 63-20 | 56 | 10 | 12 | – | 13 | 13 | 4 | 3 | 3 | 6 | 0.2 |
| Bad Lauchstädt<br>Unmanured plot | | | | | | | | | | | |
| 2000-63 | 61 | 11 | 6 | 2 | 14 | 16 | 1 | 1 | 4 | 5 | – |
| 63-20 | 36 | 33 | 11 | 4 | 9 | 13 | 1 | 1 | 4 | 5 | 0.4 |
| Bad Lauchstädt<br>Bare fallow | | | | | | | | | | | |
| 2000-63 | 38 | 19 | 12 | 18 | 9 | 27 | – | – | 4 | 4 | 0.4 |
| 63-20 | 36 | 34 | 6 | 5 | 9 | 14 | 1 | 1 | 7 | 8 | 0.4 |

[a] „Recent (or native) OM“ includes plant debris, fungal biomass, pollen and spores

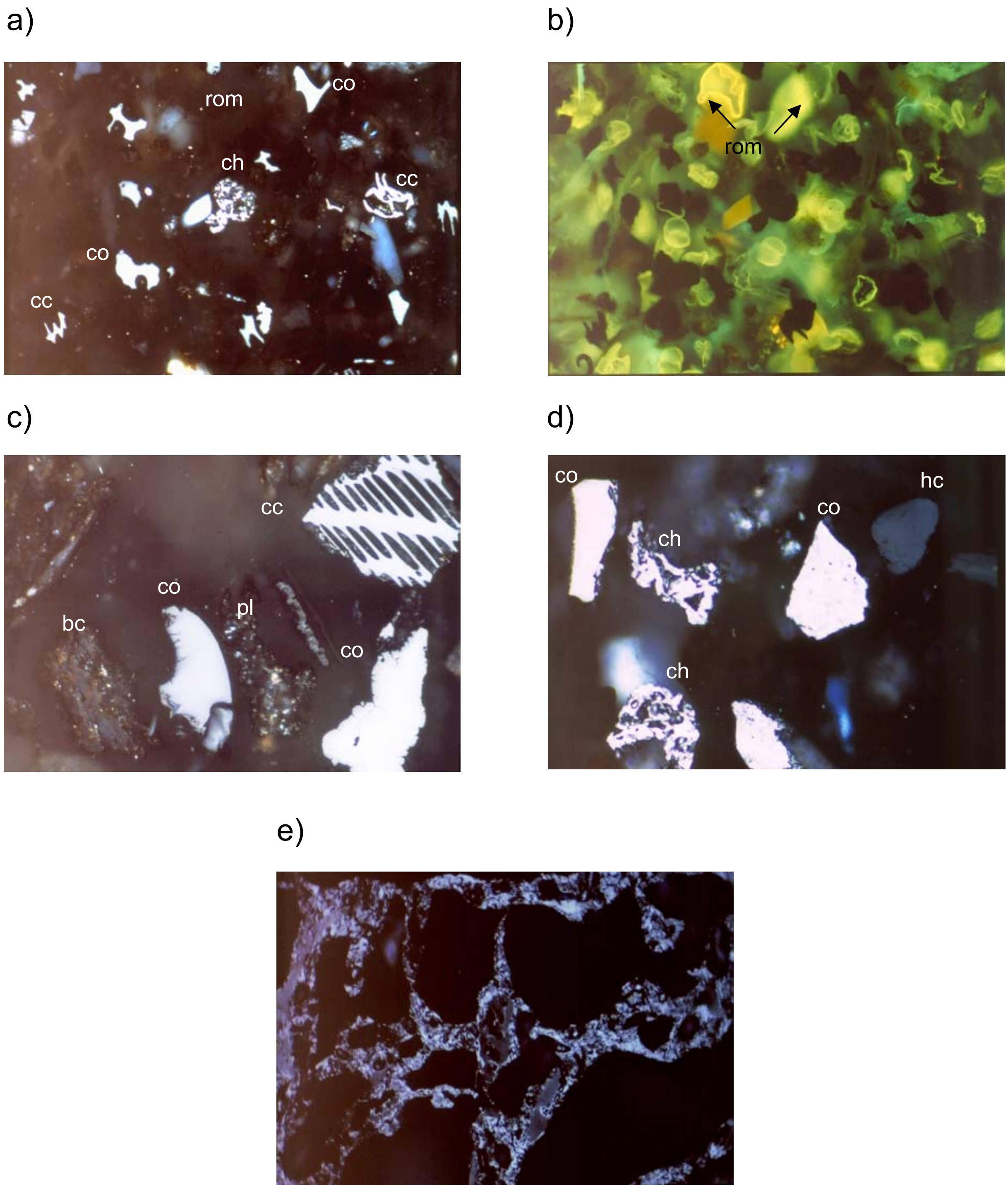


**Fig. 5:** Photomicrographs of organic compounds identified in the soils from Thyrow and Bad Lauchstädt. All photomicrographs taken on polished surfaces under oil immersion.

a) Charcoal (cc), hard-coal coke (co), char sphere (ch), and recent organic matter (rom) (no distinct structures visible). Reflected white light. Thyrow (unmanured plot), 63-20 µm. (actual length of the field of view = 524 µm)

b) Same field as Fig. 5a: Recent organic matter (pollen, spores, plant debris) with strong yellow-green fluorescence. Charcoal, coke and char are not fluorescing (dark fields). Fluorescence mode. Thyrow (unmanured plot), 63-20 µm.

c) Charcoal (cc), hard-coal coke (co), raw brown coal (bc), plant residue (pl). Reflected white light. Thyrow (unmanured plot), 2000-63 µm. (actual length of the field of view = 524 µm)

d) Hard-coal coke (co), char sphere (ch), hard coal (vitrite) (hc). Reflected light, one polarizer. Bad Lauchstädt (unmanured plot), 63-20 µm. (actual length of the field of view = 215 µm)

e) Char particle (network). Reflected light, crossed polarizers. Bad Lauchstädt (unmanured plot), 2000-63 µm. (actual length of the field of view = 215 µm)

“Char” denotes carbonaceous residues of coal/petroleum combustion. These residues were roughly classified based on their morphology and their optical properties (isotropy/anisotropy) into i) spheres and network structures (highly porous, with vesicles) and ii) solid particles (dense, without pores) (Taylor et al., 1998). Both types of char are present, in varying proportions, at Thyrow and Bad Lauchstädt (Fig. 5e). The contribution of char is less important compared with coal and coke. „Charcoal“ is the product of combustion of woody tissues; it can be easily recognized by its cellular structures which are apparent under reflected white light (Fig. 5c). The values for charcoal in Table 4 may encompass both recent and fossil charcoal, since the used microscopic procedure can not differentiate between the two groups. Fossil charcoal (pyrofusinite) can occur within coal seams (Jones and Chaloner, 1991), becoming exposed to the environment by coal mining. Its presence in soil would originate from airborne contamination, whereas recent charcoal would be indicative of vegetation fires at the sites during the Holocene.

Coal petrographic analysis demonstrates that airborne contaminants, mainly coal and coke, contribute substantially to SOM in coarse-intermediate fractions of our arable soils from industrialized areas. Pollution with coke may be due to coking plants, steel smelters, or gas manufactured plants. Coal dust is produced in large quantities in open-cast coal mines (Ghose and Majee, 2000), but is emitted also by the coal-processing industry (Rumpel et al., 1998a). In the eastern part of Germany, where the two study sites are located, burning of brown coal has been the major energy source over the last century. This can account for the occurrence of dust particles and combustion products of brown coal in soils of this area. The presence of asphalt in the soils suggests wind erosion of this material from pavings probably located in the vicinity of the experimental fields. Bad Lauchstädt is situated in a region with long tradition of brown-coal mining (open-cast mines) and high industrial activity. The observed soil contamination may reflect air pollution with carbonaceous particles in that region, especially with respect to brown coal. The experimental station of Thyrow is not closely surrounded by industrial plants or coal mines, and thus, the contaminants may have been transported over a distance of at least several dozens of km. According to Goldberg (1985) and Wik and Renberg (1987), movement of airborne particles of a size range 2000-20 µm occurs mainly over short to intermediate distances (up to hundreds of km). By contrast, finer particles, mainly those < 2 µm, are involved in long-range transport processes (Goldberg, 1985).

#### 5.3.1.4 Coal petrography versus $^{13}$C NMR

The high aromaticity seen in fractions 2000-20 µm from Thyrow and Bad Lauchstädt appears to reflect the presence of contaminants/charcoal at these sites. Coal, coal-derived products and charcoal are enriched in aromatic C compared with plant residues. Most of the signal intensity in spectra of coal samples is found in the alkyl and aromatic C range, whereby proportions of aromatic C increase with increasing rank (Van Krevelen, 1993; Haenel, 1992). Combustion of coal resulted in a decrease of the alkyl C signal, leaving a single peak of aromatic C in the spectrum of the char (Rumpel et al., 1998b). Coke, the solid residue of the pyrolysis of coal, is thought to consist of aromatic nuclei which are linked by aliphatic chains (Stach et al., 1982). Charred plant tissues show a pronounced aromatic C signal and are depleted in O-alkyl structures relative to the original plant materials (Knicker et al., 1996a; Skjemstad et al., 1996). The broad signal in the aromatic range (centred at 128-130 ppm) as seen in the C-depleted plots may be indicative of condensed and crosslinked aromatic structures. The NMR spectra thus seem to agree with the occurrence of contaminants and charcoal which are thought to contain such condensed structures, as described for coal (Haenel, 1992; Pugmire, 1996) and for charcoal (Skjemstad et al., 1996).

The increased aromatic C proportions in the C-depleted versus fertilized plots suggests a relative accumulation of the contaminants/charcoal in the former plots. This accumulation in turn indicates a contribution of these compounds to a refractory C compartment in separates 2000-20 µm (see Fig. 1). The identified contaminants/charcoal are generally considered to be recalcitrant. This is indicated e.g. by low mineralization rates for coal in incubation studies (Robertson and Morgan, 1995), no mineralization of charred plant materials during a 10-month incubation (Shindo, 1991), a high persistence of charcoal observed in a long-term field experiment (Skjemstad et al., 2001). There may have been a continuous deposition of atmospheric pollutants at the sites over extended periods of time. Chemical recalcitrance would support their accumulation within the refractory SOC pool. In contrast, native OM compounds/plant residues associated with the coarse separates are turned over within several years and are usually assigned to a labile pool of SOC (Christensen, 1996).

In the studies of Schmidt et al. (1996) and Rumpel et al. (1998a), airborne contamination of soils with coal and combusted particles caused alterations in SOC structure similar to this study, i.e. an increase of aromatic C together with a decrease of O-alkyl C. For a soil which was heavily contaminated with brown coal dust, Schmidt et al. (1996) also noted an increase of the alkyl C proportion. By contrast, the presence of contaminants in Thyrow and Bad

Lauchstädt is not reflected by exceptionally high alkyl C contributions in any of the treatments compared with other soils (Mahieu et al., 1999).

A problem often discussed in the literature is that CP spectra of coal (and other compounds with condensed structures) may not be quantitatively reliable, as the proportion of aromatic C may be relatively underestimated. The major reason for this is thought to be inefficient polarization transfer from $H^1$ to $^{13}C$ nuclei in highly condensed aromatic structures, where part of the carbons are isolated from protons. Efficient cross polarization occurs for $^{13}C$ nuclei which are in close proximity to protons (approximately within four to five bonds lengths). Carbon nuclei further removed from protons do not undergo cross polarization and are not detected in the CP experiment (Skjemstad et al., 1997). In single pulse excitation (Bloch decay) experiments, carbon nuclei are directly magnetized and theoretically all the carbons should be observed. Aromaticity in CP spectra was lower compared with the Bloch decay technique for lignite (Rumpel, 1999), various coal samples (Botto, 1996), an A-horizon contaminated with coal and combusted particles (Schmidt et al., 2000), and charcoal (Skjemstad et al., 1996). Accordingly, in the separates from Thyrow and Bad Lauchstädt, CP spectra may not be completely quantitative for coal / the highly aromatic compounds present, and thus the total contribution of aromatic C to signal intensity is possibly underestimated.

A relative accumulation of airborne contaminants in the C-depleted plots may also help explain the increase of C/N-ratios in the depleted versus the fertilized plots (see Table 2). C/N-ratios of coal samples can cover a wide range of values, from around 40 to > 150 (Hatcher, 1988; Knicker et al., 1996b); combustion residues of coal showed ratios > 100 (Rumpel et al., 1998b). For the bare fallow (Bad Lauchstädt), the identified contaminants may be mainly responsible for the high C/N-ratios. However, for the unmanured plots, the higher C/N-ratios may also arise from higher ratios in the recent OM-input as compared with the input of the fertilized plots. The fertilized plots, in addition to crop residues, receive high amounts of farmyard manure which has a narrower C/N-ratio than most crop residues (M. Körschens, oral communication).

### 5.3.2 Fractions < 20 µm

#### *5.3.2.1 C-to-N ratios*

In fractions < 6 µm, C/N-ratios are similar in the contrasting treatments of the long-term experiments, with values ranging from 8 to 14 (Table 2). Ratios in the medium silt fraction (20-6 µm) of the C-depleted plots are slightly higher than in the fertilized plots (by 3 to 5 units). But this increase is smaller compared with the coarse fractions (Table 2). Ratios around 10 for the clay (< 2 µm), and between 11 and 18 in fractions 20-2 µm are in accordance with other arable soils (Christensen, 1985; Catroux and Schnitzer, 1987; Guggenberger et al., 1994; Christensen and Johnston, 1997; Stemmer et al., 1998).

### *5.3.2.2 $^{13}$C NMR spectroscopy*

#### *5.3.2.2.1 Conventional CPMAS experiments*

The sandy soils (Thyrow, Skierniewice) are strikingly similar in their relative signal distribution in fractions < 20 µm when the same treatment type is considered (Table 5). A gradual increase of the alkyl C and O/N alkyl C proportion with decreasing particle-size is evident which is offset by a decrease of the aromatic C intensity from medium silt (20-6 µm) to fine clay (< 0.2 µm) in both treatments. The most abundant C-species in fractions < 2 µm of the sandy soils are O/N-alkyl C and alkyl C (see also Figs. 2 and 3). These figures show that in fine separates most of the intensity of aromatic C is found in the region 110 to 140 ppm; a weak signal is found around 150 ppm. Thus, O-aryl compounds are quantitatively not important within the aromatic fraction of these separates. Comparing the treatments at the two sites, an increased contribution of aromatic C and a lower contribution of O/N-alkyl C for the depleted plots can be observed, especially in the 20-6 µm fraction (Table 5). Deviations in alkyl and carboxyl C between the treatments are small for both sites.

Compared with the sandy soils, the loamy soil from Bad Lauchstädt is generally less abundant in alkyl and O/N-alkyl C but richer in aromatic compounds (Table 5, Fig. 4), especially in separates < 6 µm. The aromatic C is mainly present in structures which show a resonance in the region 110 to140 ppm (see Fig. 4). In the loamy soil, C-depletion caused an increase in the aromatic C intensity at the expense of the O/N-alkyl C percentage (Table 5). Fig. 4 illustrates a successive increase of aromatic C and a decrease of O/N-alkyl C with decreasing OC-level of the plots, from the fertilized to the unmanured plot to the bare fallow.

To summarize, compositional changes due to C-depletion in fractions < 20 µm are analogous for the three sites, as already found in coarser fractions (increase in aromatic C, decrease in O/N-alkyl C). Although there is a certain variation between the sites, changes in fine fractions (< 6 µm) appear to be smaller than in coarse separates (see values for delta (Δ) in Tables 5 and 3).

**Table 5:** Relative signal distribution in $^{13}$C NMR spectra (% of total signal intensity) for particle-size fractions < 20 µm from the contrasting treatments of the long-term experiments.

| | **Fertilized** | | | | **C-Depleted** | | | | **Δ (C-Depleted – Fertilized)** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Particle-size fractions** (µm) | **Alkyl C** | **O/N-Alkyl C** | **Aromatic C** | **Carboxyl C** | **Alkyl C** | **O/N-Alkyl C** | **Aromatic C** | **Carboxyl C** | **Alkyl C** | **O/N-Alkyl C** | **Aromatic C** | **Carboxyl C** |
| Thyrow | | | | | | | | | | | | |
| 20-6 | 24 | 30 | 29 | 18 | 19 | 23 | 39 | 20 | - 5 | - 7 | +10 | + 2 |
| 6-2 | 28 | 35 | 21 | 16 | 30 | 31 | 25 | 15 | + 2 | - 4 | + 4 | - 1 |
| 2-0.2 | 28 | 37 | 20 | 15 | 30 | 31 | 24 | 16 | + 2 | - 6 | + 4 | + 1 |
| < 0.2 | 30 | 41 | 13 | 16 | 32 | 34 | 16 | 18 | + 2 | - 7 | + 3 | + 2 |
| Skierniewice | | | | | | | | | | | | |
| 20-6 | 19 | 34 | 31 | 16 | 20 | 25 | 38 | 18 | + 1 | - 9 | + 7 | + 2 |
| 6-2 | 25 | 39 | 22 | 14 | 24 | 32 | 24 | 19 | - 1 | - 7 | + 2 | + 5 |
| 2-0.2 | 26 | 38 | 20 | 17 | 30 | 35 | 19 | 16 | + 4 | - 3 | - 1 | - 1 |
| < 0.2 | 30 | 42 | 13 | 15 | 29 | 40 | 15 | 16 | - 1 | - 2 | + 2 | + 1 |
| Bad Lauchstädt [a] | | | | | | | | | | | | |
| 20-6 | 22 | 30 | 31 | 16 | 19 | 26 | 41 | 14 | - 3 | - 4 | +10 | - 2 |
| 6-2 | 23 | 35 | 26 | 17 | 21 | 31 | 30 | 18 | - 2 | - 4 | + 4 | + 1 |
| 2-0.2 | 21 | 33 | 30 | 16 | 20 | 27 | 33 | 20 | -1 | - 6 | + 3 | + 4 |
| < 0.2 | 26 | 34 | 23 | 18 | 22 | 27 | 28 | 23 | - 4 | - 7 | + 5 | + 5 |

[a] Data for the C-depleted plot refer to the unmanured plot

In the sandy soils, changes in signal intensities across the separates follow a pattern similar to other soils: alkyl C proportions are highest in fine separates, and aromaticity reaches a maximum in intermediate separates (see Tables 3 and 5), as was observed by Oades et al. (1987), Baldock et al. (1992), Randall et al. (1995), Guggenberger et al. (1995), and Schmidt (1998). For the clay fractions of these soils, the contributions of the four C-species are close to the mean values determined for the clay of 25 soils, with O-alkyl C being the most abundant and alkyl C the second most abundant species (Mahieu et al., 1999). A pecularity of the loamy soil is the relatively high aromatic C contribution in the whole range of separates. Unusually high aromatic C contributions in the clay, compared with many other soils, were also noted e.g. in a German Mollisol (Schmidt et al., 1996; Schmidt et al., 1999b) and an Australian Black Earth (Skjemstad et al., 1996). In these soils, the pronounced aromatic C signal (around 130 ppm) recorded in both intermediate and fine separates was mainly ascribed to the presence of finely diveded charcoal.

**Table 6**: Time constants and contributions of protonated / non-protonated aryl carbons for the coarse clay fraction (2-0.2 μm) of the Thyrow and Bad Lauchstädt soil samples.

| | **$Aryl_p$** | **$Aryl_{np}$** | **$Aryl_p$*** | **$Aryl_{np}$ *** | **$T_A$** | **$T_B$** |
|---|---|---|---|---|---|---|
| **Samples** | (fraction of aryl C) [a] | | (% of total signal intensity) [b] | | (μsec) [c] | |
| Thyrow | | | | | | |
| Fertilized | 1.0 | n.s. | 15 | – | 20 | n.s. |
| Unmanured | 1.0 | n.s. | 19 | – | 23 | n.s. |
| Bad Lauchstädt | | | | | | |
| Fertilized | 1.0 | n.s. | 22 | – | 24 | n.s. |
| Unmanured | 0.4 | 0.6 | 10 | 15 | 24 | 173 |
| Bare fallow | 0.5 | 0.5 | 14 | 14 | 25 | 197 |

[a] see equations 5 and 6

[b] see equations 7 and 8

[c] DD time constants derived from equation 4

n.s. fit results for the Lorentzian component of equation 4 ($T_B$, $I_B$ (0)) statistically not significant

#### *5.3.2.2.2 DD experiments (Thyrow and Bad Lauchstädt)*

For the coarse clay, as a representative for the fine separates, we performed DD experiments to gain further structural information on the aryl C (110 to 140 ppm). Table 6 presents the parameters derived from DD experiments for one of the sandy soils (Thyrow) and the loamy soil (Bad Lauchstädt). For both treatments of Thyrow and the fertilized plot of Bad Lauchstädt, only the Gaussian component (of equation 4) can be significantly fitted to the data. This indicates a predominance of protonated C in aryl compounds for these plots. By contrast, for the C-depleted treatments at Bad Lauchstädt the combination of Gaussian and Lorentzian equation can be successfully fitted to the data, indicating that protonated and non-protonated carbons provide a significant contribution to the aryl C signal. The time constants for protonated aryl C ($T_A$) are similar for the two study sites, ranging from 20 to 25 µsec. $T_B$ values for non-protonated aryl C at Bad Lauchstädt vary between 173 and 197 µsec. These values for $T_A$ and $T_B$ are similar to those in model compounds and SOM reported by Hatcher (1988) and Wilson (1987).

For the unmanured plot and the bare fallow from Bad Lauchstädt, the relative contribution of protonated and non-protonated aryl C is around 0.5 on average (Table 6). In terms of total signal intensity, protonated aryl C makes up 22% in the fertilized plot, whereas it accounts for 12% on average in the two C-depleted plots of this site. In the latter, 15% of signal intensity is accounted for by non-protonated aryl C.

In soil samples with large charcoal contents, between 60 and 72% of the 130 ppm-resonance was attributable to non-protonated carbons (Smernik and Oades, 2001). These values appear to be in line with the idea of fused aromatic structures in charcoal. In coal samples, the contribution of non-protonated carbons to the aromatic signal can widely vary: For coal macerals, this contribution ranged from approximately 0.2 to 0.8, as can be deduced from Wilson (1987). In a series of samples from brown coal to anthracite the fraction of aryl C (110 to 140 ppm) that is not protonated was between 0.50 to 0.65 (Hatcher, 1988). According to Wilson (1987) and Hatcher (1988), the fraction of protonated aryl C decreases in early stages of coalification, again increases as coalification proceeds, and sharply decreases in advanced stages of coalification.

The native OM associated with the clay fraction is considered to be strongly humified material, showing a considerable contribution of secondary resources (microbial biomass and its metabolites) (Christensen, 1996). Baldock et al. (1990) showed that bacterial and fungal cultures isolated from soil differ in the DD behavior of their aryl carbon: Aryl C synthesized by bacteria was mainly protonated, whereas in fungi 70% of the aryl C signal showed slow signal decay during interrupted decoupling. The authors suggested that in fungi either 70% of aryl carbons were non-protonated or, alternatively, were contained in structures with high molecular motion. In the clay, a considerable proportion of total carbon can be present in peptide-structures (Knicker et al., 2000). The aromatic constituents of model proteins exhibited a rapid signal decay, indicating a high degree of protonation (Knicker, 2000). On the other hand, humification of OM in forest soils resulted in a relative accumulation of aromatic compounds in the range 110 to 140 ppm, encompassing a high proportion of non-protonated carbons (Kögel-Knabner et al., 1991). These examples demonstrate that both contaminants (coal/coal-derived products) and native SOM compounds vary in the fractional contribution of non-protonated/or protonated carbons to the aryl C signal. Nevertheless, we consider that relatively high values of non-protonated aryl C are indicative of the presence of condensed/crosslinked aromatic moieties, that may originate from contaminants/charcoal in the fine separates.

#### *5.3.2.2.3 Presence of contaminants in fractions < 6 µm*

For the fine separates, conventional and DD NMR spectra revealed differences in the overall composition of OC (aromaticity) as well as in structural features of aromatic C between the two sandy soils and the loamy soil.

For the sandy soils, the contribution of aromatic C is of similar extent as found in a number of soils. As shown for the Thyrow soil, aryl C is present mainly in protonated structures; possibly, part of this aryl C is contained in peptide/proteinaceous structures. This suggests no or only a small contribution of airborne contaminants / or pyrogenic C from plant tissues („charcoal“) in these soils.

For the loamy soil, aromaticity is found to be high (up to 33% signal intensity in fractions < 6 µm), and in the C-depleted plots on average half of the aryl C nuclei (110 to 140 ppm) are not protonated / show C-substitution. This results in 15% of total signal intensity being present in non-protonated aryl C compounds. These findings suggest that there is a substantial contribution of contaminants/pyrogenic C in the fine separates of this soil. The fact that non-protonated aryl C was detected only in the C-depleted plots but not in the fertilized one may arise from spatial heterogeneity in SOM composition within the experimental station. An additional explanation may be that non-protonated aryl C compounds are present in very low concentrations in the fertilized plot (so that they could not be detected by the methods used), and have accumulated within the C-depleted plots. In this case, non-protonated aryl carbon, possibly contained in contaminants/pyrogenic C, would be highly stable and accumulate within the refractory SOC of the fine separates. The tentative conclusion that contaminants and/or pyrogenic C may be present in the loamy soil should be evaluated by appropriate methods in future work. The traditional coal petrographic analysis (using light microscopy) is limited to coarse-intermediate size fractions, and therefore other approaches are needed for the identification of the mentioned compounds in fine separates.

Regardless of the differences in the composition of aromatic C, for all sites, aromatic C appears to be the most stable C-species in fractions < 20 µm. As seen before, aromatic C proportions increase along with C-depletion, at the expense of other C-species. For the loamy soil, the increase in aromaticity in the depleted plots again points to a possible accumulation of highly aromatic contaminants/charcoal within the refractory C pool.

### 5.3.3 OC balances (Thyrow and Bad Lauchstädt)

The previous results indicate that airborne contaminants (coal, coke, char) contribute to the refractory SOC compartment. To assess their contribution to the refractory C pool quantitatively, one may consider the C balance of the C-depleted plots. The OC pool of these plots should to a major part contain refractory compounds. Contaminants at Thyrow and Bad Lauchstädt may be mainly localized in separates 2000-20 µm.

Table 7 gives the OC contents as well as the C balances across the separates for fertilized and C-depleted plots of the two sites. OC contents among the separates are generally highest in fractions < 6 µm. The two sites, however, differ in the distribution of total SOC between the separates, which can be explained by the contrasting textures (see Table 1). In the sandy soil (Thyrow), the contribution from separates 2000-63 µm is more important for the C balance than in the Bad Lauchstädt soil with its higher silt and clay content. Conversely, in the loamy soil a higher proportion of total OC is associated with separates < 2 µm. Nevertheless, for both sites, the OC-distribution is shifted towards fine separates in the C-depleted plots compared with the fertilized ones (Table 7). In the depleted plots, 75% (sandy soil) and 90% (loamy soil) of SOC are associated with separates < 20 µm. Considering the content of contaminants in separates 2000-20 µm (see Table 4), their contribution to the C balance of the depleted plots may be estimated < 25% (Thyrow) and < 10% (Bad Lauchstädt).

This indicates that the refractory SOC is to a major part associated with separates < 20 µm, mainly those < 6 µm. The contribution from contaminants to the refractory SOC seems to be (much) smaller than that from native OM compounds, which are stabilized within fine particle-size separates. This conclusion seems to be valid also for the loamy soil, even if there may be some contribution of contaminants to the fine separates, too. Nevertheless, the data

**Table 7:** OC contents in the particle-size fractions and OC balances for the contrasting treatments from Thyrow and Bad Lauchstädt.

| | **OC (g $kg^{-1}$ fraction)** | | | | **OC (% of bulk OC)** | | | |
|---|---|---|---|---|---|---|---|---|
| | **Thyrow** | | **Bad Lauchstädt** | | **Thyrow** | | **Bad Lauchstädt** | |
| **Particle-size fractions** (µm) | **Fertilized** | **C-Depleted** | **Fertilized** | **C-Depleted** [a] | **Fertilized** | **C-Depleted** | **Fertilized** | **C-Depleted** [a] |
| 2000-63 | 2 | 0.5 | 20 | 7 | 27 | 16 | 6 | 4 |
| 63-20 | 12 | 4 | 7 | 2 | 14 | 9 | 12 | 5 |
| Sum 2000-20 | | | | | 41 | 25 | 18 | 10 |
| 20-6 | 15 | 8 | 7 | 4 | 8 | 9 | 7 | 7 |
| 6-2 | 47 | 28 | 63 | 31 | 21 | 25 | 24 | 22 |
| 2-0.2 | 55 | 36 | 62 | 38 | 22 | 30 | 31 | 35 |
| < 0.2 | 73 | 48 | 43 | 34 | 8 | 10 | 20 | 26 |
| Sum < 20 | | | | | 59 | 75 | 82 | 90 |

[a] Bare fallow

also suggest that the input of airborne contaminants (presumably mainly in coarse-intermediate separates) can not be neglected in the C budget of the sites. This is especially true for the sandy soil (Thyrow). In this soil, due to low contents of fine particles, OM in coarse separates generally plays an important role for the C balance. In sum, at both sites the fine separates appear to be crucial for the amount of refractory SOC, but contaminants lead to an increase of this C pool which may be quantitatively important especially in the sandy soil. Thus, the presence of contaminants should be considered for estimations of the refractory C pool in SOM models at such sites.

Rühlmann (1999) for non-contaminated arable soils showed that the size of the refractory C pool can be described/estimated as a function of the content of particles < 20 µm. Accordingly, the native refractory OC is mostly associated with separates < 20 µm, which seems to be corroborated by our findings. The role of fine particle-size fractions for the (long-term) stabilization of OC is recognized in several SOM models as reviewed by Falloon and Smith (2000). In these models, texture or the clay content is among those factors which have an effect on the size of the refractory C compartment. If SOM model approaches are used at sites with contaminants, it is necessary to quantify their contribution to SOC at a site. This can be achieved by radiocarbon dating (Schmidt et al., 1996; Rumpel et al., 1998b), or infrared spectroscopy in combination with multivariate data analysis, as has recently been proposed by Rumpel et al. (2001).

## 5.4 Conclusions

- For the separates 2000-20 µm, the combination of coal petrography and $^{13}$C NMR spectroscopy showed that the pronounced aromatic C signal in $^{13}$C NMR is attributable to the presence of airborne contaminants. Highest values of aromatic C were noted in the C-depleted plots of the long-term experiments.
- For the fine separates, the combination of conventional and dipolar dephasing NMR experiments allowed a first estimation of the presence of contaminants, based on aromaticity and the relative contribution of non-protonated carbons to the total aryl C signal.
- Different contamination patterns with respect to particle-size were found: For both a sandy (Thyrow) and a loamy soil (Bad Lauchstädt), contaminants (i.e. coal, coke, char from fossil fuel combustion) represent a considerable proportion of OM in separates 2000-20 µm. For the fine separates, however, it appeared that the two sandy soils (Thyrow, Skierniewice) are not or only slightly affected by contamination, whereas for the Bad

Lauchstädt soil NMR data suggested a presence of contaminants and/or charcoal in fine separates, too. In the Thyrow soil mainly pollutants of coarse-intermediate particle-size were deposited, whereas at Bad Lauchstädt contamination may have occurred in a wider size range, possibly including the input of fine dust particles.

- The increase of aromatic C intensities in the C-depleted versus fertilized plots was interpreted as a relative accumulation of the airborne contaminants within the SOC pool of the C-depleted plots (for separates 2000-20 µm, and possibly < 20 µm for Bad Lauchstädt). This provides evidence that the recorded contaminants contribute to the refractory SOC pool of the investigated sites.
- The contribution from contaminants to the total refractory OC seemed to be smaller than that from native OM stabilized within fine particle-size separates. Nevertheless, it may be suggested that any increase of the refractory OC pool by contaminants affects the turnover of total SOC and thus the long-term SOC dynamics at such sites.

## 5.5 References

AG Boden, 1994. Bodenkundliche Kartieranleitung, 4. Auflage. E. Schweizerbart`sche Verlagsbuchhandlung, Stuttgart.

Amelung, W., Zech, W., 1999. Minimisation of organic matter disruption during particle-size fractionation of grassland epipedons. Geoderma 92, 73-85.

Amelung, W., Zech, W., Zhang, X., Follett, R.F., Tiessen, H., Knox, E., Flach, K.-W., 1998. Carbon, nitrogen, and sulfur pools in particle-size fractions as influenced by climate. Soil Science Society of America Journal 62, 172-181.

Baldock, J.A., Oades, J.M., Vassallo, A.M., Wilson, M.A., 1989. Incorporation of uniformly labelled $^{13}$C-glucose carbon into the organic fraction of a soil. Carbon balance and CP/MAS $^{13}$C NMR measurements. Australian Journal of Soil Research 27, 725-746.

Baldock, J.A., Oades, J.M., Vassallo, A.M., Wilson, M.A., 1990. Solid-state CP/MAS $^{13}$C NMR analysis of bacterial and fungal cultures isolated from a soil incubated with glucose. Australian Journal of Soil Research 28, 213-225.

Baldock, J.A., Oades, J.M., Waters, A.G., Peng, X., Vassallo, A.M., Wilson, M.A., 1992. Aspects of the chemical structure of soil organic materials as revealed by solid-state $^{13}$C NMR spectroscopy. Biogeochemistry 16, 1-42.

Baldock, J.A., Oades, J.M., Nelson, P.N., Skene, T.M., Golchin, A., Clarke, P., 1997. Assessing the extent of decomposition of natural organic materials using solid-state $^{13}$ NMR spectroscopy. Australian Journal of Soil Research 35, 1061-1083.

Beavis, J., Mott, C.J.B., 1996. Effects of land use on the amino acid composition of soils: 1. Manured and unmanured soils from the Broadbalk continuous wheat experiment, Rothamsted, England. Geoderma 72, 259-270.

Botto, R.E., 1996. Fossil fuels. In: Encyclopedia of NMR. John Wiley & Sons, Chichester, pp. 2101-2118.

Capriel, P., 1997. Hydrophobicity of organic matter in arable soils: influence of management. European Journal of Soil Science 48, 457-462.

Catroux, G., Schnitzer, M., 1987. Chemical, spectroscopic, and biological characteristics of the organic matter in particle size fractions separated from an Aquoll. Soil Science Society of America Journal 51, 1200-1207.

Christensen, B.T., 1985. Carbon and nitrogen in particle size fractions isolated from Danish arable soils by ultrasonic dispersion and gravity-sedimentation. Acta Agriculturae Scandinavica 35, 175-187.

Christensen, B.T., 1996. Carbon in primary and secondary organomineral complexes. In: Carter, M.R., Stewart, B.A. (Eds.), Structure and Organic Matter Storage in Agricultural Soils. Advances in Soil Science. CRC Press, Boca Raton, pp. 97-165.

Christensen, B.T., Johnston, A.E., 1997. Soil organic matter and soil quality - lessons learned from long-term experiments at Askov and Rothamsted. In: Gregorich, E.G., Carter, M.R. (Eds.), Soil Quality for Crop Production and Ecosystem Health. Developments in Soil Science 25. Elsevier, pp. 399-430.

Ellmer, F., Peschke, H., Köhn, W., Chmielewski, F.-M., Baumecker, M., 2000. Tillage and fertilizing effects on sandy soils. Review and selected results of long-term experiments at Humboldt-University Berlin. Journal of Plant Nutrition and Soil Science 163, 167-272.

Falloon, P.D., Smith, P., 2000. Modelling refractory soil organic matter. Biology and Fertility of Soils 30, 388-398.

FAO, 1998. World Reference Base For Soil Resources, Rome.

Gerzabek, M.H., Pichlmayer, F., Kirchmann, H., Haberhauer, G., 1997. The response of soil organic matter to manure amendments in a long-term experiment at Ultuna, Sweden. European Journal of Soil Science 48, 273-282.

Ghose, M.K., Majee, S.R., 2000. Assessment of dust generation due to opencast coal mining - an Indian case study. Environmental Monitoring and Assessment 61, 255-263.

Goldberg, E.D., 1985. Black Carbon in the Environment. John Wiley & Sons.

Guggenberger, G., Christensen, B.T., Zech, W., 1994. Land-use effects on the composition of organic matter in particle-size separates of soils: I. Lignin and carbohydrate signature. European Journal of Soil Science 45, 449-458.

Guggenberger, G., Zech, W., Haumaier, L., Christensen, B.T., 1995. Land-use effects on the composition of organic matter in particle-size separates of soils: II. CPMAS and solution $^{13}$C NMR analysis. European Journal of Soil Science 46, 147-158.

Haenel, M.W., 1992. Recent progress in coal structure research. Fuel 71, 1211-1222.

Hatcher, P.G., 1988. Dipolar-dephasing $^{13}$C NMR studies of decomposed wood and coalified xylem tissue: evidence for chemical structural changes associated with defunctionalization of lignin structural units during coalification. Energy & Fuels 2, 40-58.

Janzen, H.H., Campbell, C.A., Ellert, B.H., Bremer, E., 1997. Soil organic matter dynamics and their relationship to soil quality. In: Gregorich, E.G., Carter, M.R. (Eds.), Soil Quality for Crop Production and Ecosystem Health. Developments in Soil Science 25. Elsevier, pp. 277-291.

Jones, T.P., Chaloner, W.G., 1991. Fossil charcoal, its recognition and paleoatmospheric significance. Paleogeography, Paleoclimatology, Paleoecology (Global and Planetary Change Section) 97, 39-50.

Keil, R.G., Tsamakis, E., Fuh, C.B., Giddings, J.C., Hedges, J.I., 1994. Mineralogical and textural controls on the organic composition of coastal marine sediments: Hydrodynamic separation using SPLITT-fractionation. Geochimica et Cosmochimica Acta 58, 879-893.

Kiem, R., Knicker, H., Körschens, M., Kögel-Knabner, I., 2000. Refractory organic carbon in C-depleted arable soils, as studied by $^{13}$C NMR spectroscopy and carbohydrate analysis. Organic Geochemistry 31, 655-668.

Kleineidam, S., Rügner, H., Ligouis, B., Grathwohl, P., 1999. Organic matter facies and equilibrium sorption of phenanthrene. Environmental Science & Technology 33, 1637-1644.

Knicker, H., 1993. Quantitative $^{15}$N- und $^{13}$C-CPMAS-Festkörper- und $^{15}$N-Flüssigkeits-NMR-Spektroskopie an Pflanzenkomposten und natürlichen Böden. Ph. D.-thesis, Universität Regensburg, 156 pp.

Knicker, H., 2000. Biogenic nitrogen in soils as revealed by solid-state carbon-13 and nitrogen-15 nuclear magnetic resonance spectroscopy. Journal of Environmental Quality 29, 715-723.

Knicker, H., Lüdemann, H.-D., 1995. $^{15}$N and $^{13}$C CPMAS and solution NMR studies of $^{15}$N enriched plant material during 600 days of microbial degradation. Organic Geochemistry 23, 329-341.

Knicker, H., Almendros, G., González-Vila, F.J., Martin, F., Lüdemann, H.-D., 1996a. $^{13}$C and $^{15}$N- NMR spectroscopic examination of the transformation of organic nitrogen in plant biomass during thermal treatment. Soil Biology & Biochemistry 28, 1053-1060.

Knicker, H., Hatcher, P.G., Scaroni, A.W., 1996b. A solid-state $^{15}$N NMR spectroscopic investigation of the origin of nitrogen structures in coal. International Journal of Coal Geology 32, 255-278.

Knicker, H., Schmidt, M.W.I., Kögel-Knabner, I., 2000. Nature of organic nitrogen in fine particle size separates of sandy soils of highly industrialized areas as revealed by NMR spectroscopy. Soil Biology & Biochemistry 32, 241-252.

Kögel-Knabner, I., Hempfling, R., Zech, W., Hatcher, P.G., Schulten, H.-R., 1988. Chemical composition of the organic matter in forest soils: 1. forest litter. Soil Science 146, 124-136.

Kögel-Knabner, I., Hatcher, P.G., Zech, W., 1991. Chemical structural studies of forest soil humic acids: aromatic carbon fraction. Soil Science Society of America Journal 55, 241-247.

Körschens, M., 1998. Effect of different management systems on carbon and nitrogen dynamics of various soils. In: Lal., R., Kimble, J.M., Follett, R.F., Stewart, B.A. (Eds), Management of Carbon Sequestration in Soil. CRC Press, Boca Raton, pp. 297-304.

Körschens, M., Weigel, A., Schulz, E., 1998. Turnover of soil organic matter (SOM) and long-term balances - tools for evaluating sustainable productivity of soils. Zeitschrift für Pflanzenernährung und Bodenkunde 161, 409 - 424.

Mahieu, N., Powlson, D.S., Randall, E.W., 1999. Statistical analysis of published carbon-13 CPMAS NMR spectra of soil organic matter. Soil Science Society of America Journal 63, 307-319.

Mercik, S., Stepien, W., Labetowicz, J., 2000. The fate of nitrogen, phosphorus and potassium in long-term experiments in Skierniewice. Journal of Plant Nutrition and Soil Science 163, 273-278.

Oades, J.M., Vassallo, A.M., Waters, A.G., Wilson, M.A., 1987. Characterization of organic matter in particle size and density fractions from a red-brown earth by solid-state $^{13}$C N.M.R. Australien Journal of Soil Research 25, 71-82.

Pätz, H., Rascher, J., Seifert, A., 1989. Kohle - ein Kapitel aus dem Tagebuch der Erde. Verlag Harri Deutsch, Thun - Frankfurt/Main.

Peersen, O.B., Wu, X., Kustanovich, I., Smith, S.O., 1993. Variable-amplitude cross-polarization MAS NMR. Journal of Magnetic Resonance 104, 334-339.

Pugmire, R.J., 1996. Coal structure from solid state NMR. In: Encyclopedia of NMR. John Wiley & Sons, Chichester, pp. 1355-1365.

Randall, E.W., Mahieu, N., Powlson, D.S., Christensen, B.T., 1995. Fertilization effects on organic matter in physically fractionated soils as studied by $^{13}$C NMR: results from two long-term field experiments. European Journal of Soil Science 46, 557-565.

Robertson, F.A., Morgan, W.C., 1995. Mineralization of C and N in organic materials as affected by duration of composting. Autralian Journal of Soil Research 33, 511-524.

Rühlmann, J., 1999. A new approach to estimating the pool of stable organic matter in soil using data from long-term field experiments. Plant and Soil 213, 149-160.

Rumpel, C., 1999. Differenzierung und Charakterisierung pedogener und geogener organischer Substanz in forstlich rekultivierten Kippböden. Cottbuser Schriften zu Bodenschutz und Rekultivierung, Band 5. Brandenburgische Technische Universität Cottbus, 125 pp.

Rumpel, C., Knicker, H., Kögel-Knabner, I., Hüttl, R.F., 1998a. Airborne contamination of immature soil (Lusatian mining district) by lignite-derived material: its detection and contribution to the soil organic matter budget. Water, Air and Soil Pollution 105, 481-492.

Rumpel, C., Knicker, H., Kögel-Knabner, I., Skjemstad, J.O., Hüttl, R.F., 1998b. Types and chemical composition of organic matter in reforested lignite-rich mine soils. Geoderma 86, 123-142.

Rumpel, C., Janik, L.J., Skjemstad, J.O., Kögel-Knabner, I., 2001. Quantification of carbon derived from lignite in soils using mid-infrared spectroscopy and partial least squares. Organic Geochemistry 32, 831-839.

Schaefer, J., Stejskal, E.O., 1976. Carbon-13 nuclear magnetic resonance of polymers spinning at magic angle. Journal of the American Chemical Society 98, 1031-1032.

Schmidt, M.W.I., 1998. Organic Matter in Natural Soils and in Soils Contaminated by Atmospheric Organic Particles from Coal Processing Industries. Shaker Verlag, Aachen, Reihe Geowissenschaften, 134 pp.

Schmidt, M.W.I., Noack, A.G., 2000. Black carbon in soils and sediments: Analysis, distribution, implications, and current challenges. Global Biogeochemical Cycles 14, 777-793.

Schmidt, M.W.I., Knicker, H., Hatcher, P.G., Kögel-Knabner, I., 1996. Impact of brown coal dust on the organic matter in particle-size fraction of a Mollisol. Organic Geochemistry 25, 29-39.

Schmidt, M.W.I., Knicker, H., Hatcher, P.G., Kögel-Knabner, I., 1997. Improvement of $^{13}$C and $^{15}$N CPMAS NMR spectra of bulk soils, particle size fractions and organic material by treatment with 10% hydrofluoric acid. European Journal of Soil Science 48, 319-328.

Schmidt, M.W.I., Rumpel, C., Kögel-Knabner, I., 1999a. Particle size fractionation of soil containing coal and combusted particles. European Journal of Soil Science 50, 515-522.

Schmidt, M.W.I., Skjemstad, J.O., Gehrt, E., Kögel-Knabner, I., 1999b. Charred organic carbon in German chernozemic soils. European Journal of Soil Science 50, 351-365.

Schmidt, M.W.I., Knicker, H., Hatcher, P.G., Kögel-Knabner, I., 2000. Airborne contamination of forest soils by carbonaceous particles from industrial coal processing. Journal of Environmental Quality 29, 768-777.

Shindo, H., 1991. Elementary composition, humus composition, and decomposition in soil of charred grassland plants. Soil Science and Plant Nutrition 37, 651-657.

Skjemstad, J.O., Clarke, P., Taylor, J.A., Oades, J.M., McClure, S.G., 1996. The chemistry and nature of protected carbon in soil. Australian Journal of Soil Science 34, 251-271.

Skjemstad, J.O., Clarke, P., Golchin, A., Oades, J.M., 1997. Characterization of soil organic matter by solid-state $^{13}$C NMR spectroscopy. In: Cadisch, G., Giller, K.E. (Eds.), Driven by Nature: Plant Litter Quality and Decomposition. CAB International, pp. 253-271.

Skjemstad, J.O., Dalal, R.C., Janik, L.J., Mc Growan, J.A., 2001. Changes in chemical nature of soil organic carbon in Vertisols under wheat in south-eastern Queensland. Australian Journal of Soil Research 39, 343-359.

Smernik, R.J., Oades, J.M., 2001. Solid-state $^{13}$C-NMR dipolar dephasing experiments for quantifying protonated and non-protonated carbon in soil organic matter and model systems. European Journal of Soil Science 52, 103-120.

Stach, E., Mackowsky, M.T., Teichmüller, M., Taylor, G.H., Chandra, D., Teichmüller, R., 1982. Stach`s Textbook of Coal Petrology. Gebrüder Borntraeger, Berlin.

Stemmer, M., Gerzabek, M.H., Kandeler, E., 1998. Organic matter and enzyme activity in particle-size fractions of soils obtained after low-energy sonication. Soil Biology & Biochemistry 30, 9-17.

Taylor, G.H., Teichmüller, M., Davis, A., Diessel, C.F.K., Littke, R., Robert, P., 1998. Organic Petrology. Gebrüder Borntraeger, Berlin.

Tyson, R.V., 1995. Sedimentary Organic Matter: Organic Facies and Palynofacies. Chapman and Hall, London.

USDA (United States Department of Agriculture), 1998. Keys to Soil Taxonomy. Washington, D. C.

Van Krevelen, D.W., 1993. Coal - Typology, Physics, Chemistry, Constitution. Elsevier.

von Lützow, M., Leifeld, J., Kainz, M., Kögel-Knabner, I., Munch, J.C., 2001. Indications for soil organic matter quality in soils under different management. Geoderma (in press).

Wik, M., Renberg, I., 1987. Distribution in forest soils of carbonaceous particles from fossil fuel combustion. Water, Air and Soil Pollution 33, 125-129.

Wilson, M.A., 1987. N.M.R. Techniques and Applications in Geochemistry and Soil Chemistry. Pergamon Press, 353 pp.

# 6 Contribution of lignin to the refractory SOC pool

## Abstract

The objective of this section is to assess the contribution of lignin to the refractory SOC pool. The CuO oxidation technique was used to determine the amount of lignin and its degree of structural alteration. Bulk samples of eight long-term agroecosystem experiments and particle-size fractions of two of the sites were investigated. For the bulk samples, the amount of lignin phenols declined more than the total OC in the course of C-depletion. The contribution of lignin phenols to total OC was thus lower in the C-depleted versus the fertilized plots. For bulk samples of the depleted plots a greater extent of lignin biodegradation was found compared with the fertilized plots. The analysis of size fractions revealed lower OC-normalized contents of lignin phenols and a higher degree of lignin alteration in fractions < 63 µm of the depleted versus the fertilized plots. These findings indicate that lignin does not accumulate within the refractory C pool of arable soils. The refractory SOC pool shows a lower contribution of lignin as compared with more labile fractions of SOC. If lignin-derived carbon is present in the stable pool it has been extensively modified so that it can no longer be identified from CuO oxidation products.

## 6.1 Introduction

The refractory pool of soil organic carbon (SOC) is that conceptual fraction whose turnover time ranges from hundreds to thousands of years (Falloon and Smith, 2000). The refractory pool contrasts with a pool of labile OC, which is characterized by turnover times of years to decades (Parton et al., 1987). The refractory pool of SOC is relevant for the long-term sequestration of C in soil, and consequently for the role of soils as a long-term terrestrial C sink within the global carbon cycle (Schlesinger, 1991; Trumbore, 1997; Falloon and Smith, 2000).

The stability of OC in soil is, among others, thought to be due to the chemical recalcitrance of specific compounds (Oades, 1995; Sollins et al., 1996). Lignin, which is the second most abundant component of plant residues in terrestrial ecosystems, is generally considered to be recalcitrant. This aromatic biomacromolecule is degraded at a much lower rate than the other major components of plant litter, i.e. cellulosic and non-cellulosic polysaccharides and proteins (Martin et al., 1980; Haider, 1992). The major reasons for the high resistance towards degradation are thought to be the presence of non-hydrolyzable C-O-C and C-C bonds between the monomeric phenylpropanoid units, and the structural complexity, i.e. the variety of ether and C-C bonds linking the different monomeric units (Fengel and Wegener, 1984; Kirk, 1984). According to the current concept, the stable ether and C-C bonds are cleaved by oxidative mechanisms. A common feature of the different degradation pathways seems to be that the ligninolytic enzymes are involved in the generation of lignin radicals by removing electrons from phenolic units. These radicals, because of their chemical instability, subsequently undergo a variety of spontaneous cleavage reactions, including cleavage of intermonomeric bonds and of propyl side-chains (Hammel, 1997). This mechanism results in a depolymerization of the lignin matrix and also in an increasing oxidative alteration of the remnant lignin (Haider, 1992). Another peculiarity of lignin biodegradation compared with other plant compounds is the co-metabolic character of the degradation process (Zeikus, 1981; Haider, 1992). Lignin does not provide a source of energy or of carbon for ligninolytic organisms, and thus for an effective lignin degradation a readily metabolizable co-substrate such as carbohydrates is required.

The CuO oxidation method revealed that particle-size fractions of soils differ in the contribution of lignin to total OC, but also in the degree of lignin alteration (Guggenberger et al., 1994; Guggenberger et al., 1995a; Amelung et al., 1999). Highest lignin contents were noted in the coarse fractions, and lignin contents gradually decreased with decreasing particle-size in soils under different land-use. Furthermore, lignin in finer fractions showed a higher degree of oxidative alteration than in coarser fractions, and thus had undergone more intensive microbial degradation. Regarding the composition of OM in particle-size fractions, a decreasing contribution of plant-derived compounds from sand to silt to clay, and in the same order an increasing contribution of microbially synthesized compounds to SOM was found (Guggenberger et al., 1994). Particle-size fractionation isolates different pools of SOM which comprise lignin moieties in different stages of degradation.

In long-term agroecosystem experiments different treatments with respect to soil management/fertilization are established, a particular plot being under the same type of treatment for decades. Over the long-term, the different management practices lead to different SOC levels in the various experimental plots (Körschens et al., 1998). These field experiments provide a means to study the composition of SOC in relation to different OC levels at a particular site. In this study we will compare two contrasting types of treatment from long-term experiments, i.e. fertilized plots and C-depleted plots (Fig. 1). The group of C-depleted plots comprises unmanured plots and bare fallows, which have a lower SOC content compared with the fertilized counterparts. The unmanured plots have not received farmyard manure, which is an important C-source in addition to the crop residues in the fertilized plots.

The long-term nutrient depletion has led to a reduced input of crop residues in the unmanured versus the fertilized plots. Thus, over decades the supply of OM to the soil has been considerably reduced in the unmanured plots. The soils under bare fallow have not received any organic input. When comparing the two types of plots after several decades of contrasting management, the C-depleted plots are primarily depleted in *labile* compounds compared to the fertilized plots, as indicated in Fig. 1. In contrast to the labile pool, loss of C from the *refractory* pool is small at a time-scale of decades (Rühlmann, 1999). Accordingly, the size of the refractory C pool remains nearly constant (Fig. 1). The total SOC left in the C-depleted plots is relatively enriched in refractory compounds compared with the SOC pool of the fertilized plots. In long-term bare fallows, the SOC should mostly consist of refractory compounds (Rühlmann, 1999).

The aim of this study is to assess the extent to which lignin contributes to the refractory carbon pool of arable soils. To this end, the amount of lignin and its degree of alteration were investigated in C-depleted and fertilized plots of long-term agroecosystem experiments (bulk samples and particle-size fractions).

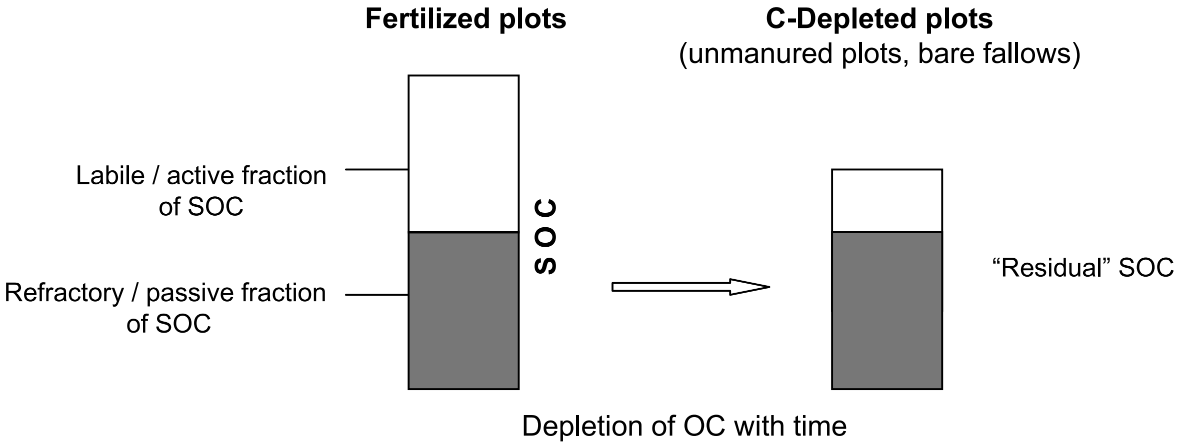


**Fig. 1:** Experimental approach of this study: comparison of C-depleted plots with fertilized plots from long-term agroecosystem experiments.

## 6.2 Materials and methods

### 6.2.1 Long-term experiments and soil sampling

We obtained soil samples from eight long-term agroecosystem experiments established at seven sites in central and eastern Europe, as listed in Table 1. The study sites cover a range of climatic features and soil types (four Luvisols, one Cambisol, two Chernozems). The sites

**Table 1:** Overview of the experimental sites.

| **Location** | **MAT** [a] (°C) | **MAP** [b] (mm) | **Soil type** (FAO) | **Sand** | **Silt** (%) | **Clay** [c] | **Started in** | **Crop rotations** | **Treatments** | |
|---|---|---|---|---|---|---|---|---|---|---|
| Thyrow (Germany) | 8.6 | 520 | Albic Luvisol | 82 | 15 | 3 | 1937 | Potatoes – spring barley – maize – spring barley | I) Fertilized | NPK + farmyard manure (15 t ha$^{-1}$ y$^{-1}$) + lime |
| | | | | | | | | | II) Unmanured | No fertilization/liming |
| Groß Kreutz (Germany) | 8.9 | 537 | Albic Luvisol | 75 | 21 | 4 | 1967 | Potatoes – winter wheat – sugar beet – spring barley | I) Fertilized | N + farmyard manure (3 t ha$^{-1}$ y$^{-1}$) |
| | | | | | | | | | II) Unmanured | No fertilization |
| Skierniewice (Poland) | 7.9 | 527 | Luvisol | 72 | 22 | 6 | 1923 | Fertilized plots (I): Potatoes – spring barley – red clover – winter wheat – rye | I) Fertilized | NPK + farmyard manure (6 t ha$^{-1}$ y$^{-1}$) + lime |
| | | | | | | | | Unmanured plots (II): Arbitrary rotation | II) Unmanured | No fertilization/liming |
| Puch (Germany) | 7.9 | 927 | Orthic Luvisol | 9 | 73 | 18 | 1983 [d] | Maize – winter wheat – winter barley | I) Fertilized | NPK + farmyard manure (10 t ha$^{-1}$ y$^{-1}$) + lime |
| | | | | | | | 1953 [e] | | II) Bare fallow | |
| Lauterbach (Germany) | 6.3 | 900 | Dystric Cambisol | 42 | 40 | 18 | 1966 | Potatoes – winter wheat - grass | I) Fertilized | NPK + farmyard manure + lime |
| | | | | | | | | | II) Unmanured | No fertilization, but liming |
| Bad Lauchstädt (Germany) | 8.6 | 490 | Haplic Chernozem | 7 | 70 | 23 | | | | |
| *Experiment A* | | | | | | | 1902 [f] | Potatoes – winter wheat – sugar beet – spring barley | I) Fertilized | NPK + farmyard manure (15 t ha$^{-1}$ y$^{-1}$) |
| | | | | | | | | | II) Unmanured | No fertilization |
| | | | | | | | 1956 [g] | | III) Bare fallow | |
| *Experiment B* | | | | | | | 1983 | Potatoes – maize - sugar beet | I) Fertilized | Farmyard manure (200 t ha$^{-1}$ y$^{-1}$) |
| | | | | | | | | | II) Bare fallow | |
| Prague (Czech Republic) | 8.1 | 450 | Luvi-haplic Chernozem | 20 | 51 | 29 | 1958 | | I) Bare fallow + fertilization | Farmyard manure (80 t ha$^{-1}$ y$^{-1}$) |
| | | | | | | | | | II) Bare fallow | |
| | | | | | | | | | III) Bare fallow + soil tillage | |

[a] Mean annual temperature
[b] Mean annual precipitation
[c] Sand (2000-63 µm), silt (63-20 µm), clay (< 2 µm)
[d] Fertilized treatment since 1983
[e] Bare fallow since 1953
[f] Fertilized / unmanured plots since 1902
[g] Bare fallow since 1956

**Table 2:** Crop yields and estimated amounts of C-input to the soil in the plots under contrasting management of Thyrow and Bad Lauchstädt.

| | **Thyrow** | | | **Bad Lauchstädt (Experiment A)** | | |
|---|---|---|---|---|---|---|
| | Fertilized | Unmanured | **Unmanured / Fertilized** (%)[a] | Fertilized | Unmanured | **Unmanured / Fertilized** (%) |
| **Yields** (t ha$^{-1}$)[b] | | | | | | |
| 1998 | Spring barley | Spring barley | | Spring barley | Spring barley | |
| | 2.5 | 0.0 | – | 5.1 | 2.3 | 44 |
| | | | | Winter wheat | Winter wheat | |
| | | | | 8.0 | 4.4 | 55 |
| | | | | Potatoes | Potatoes | |
| | | | | 33.5 | 11.8 | 35 |
| | | | | Sugar beet | Sugar beet | |
| | | | | 9.4 | 3.8 | 40 |
| 1999 | Maize for | Maize for | | Spring barley | Spring barley | |
| | silage | silage | | 9.7 | 3.3 | 34 |
| | 6.0 | 1.1 | 19 | Winter wheat | Winter wheat | |
| | | | | 9.7 | 6.0 | 62 |
| | | | | Potatoes | Potatoes | |
| | | | | 35.9 | 15.5 | 43 |
| | | | | Sugar beet | Sugar beet | |
| | | | | 12.6 | 2.6 | 21 |
| **Estimated C-input** (t C ha$^{-1}$) | | | | | | |
| 1998 | | | | | | |
| Crop residues[c] | 0.2 | 0 | | 0.6 | 0.3 | |
| Farmyard manure[d] | 1.5 | 0 | | 1.5 | 0 | |
| Sum | 1.7 | 0 | – | 2.1 | 0.3 | 12 |
| 1999 | | | | | | |
| Crop residues | 0.5 | 0.08 | | 0.8 | 0.4 | |
| Farmyard manure | 1.5 | 0 | | 1.5 | 0 | |
| Sum | 2.0 | 0.08 | 4 | 2.3 | 0.4 | 16 |

[a] $\frac{\text{Unmanured plot} \times 100}{\text{Fertilized plot}}$

[b] Oral communication (M. Körschens, M. Baumecker)
Yields of spring barley and winter wheat given as 86% dry matter (dm), potatoes as fresh matter, sugar beet as dry matter of sugar, maize as dry matter

[c] Estimation of crop residues (including roots) according to the following equations, derived from long-term measurements at Thyrow and Bad Lauchstädt (oral communication, M. Körschens):

Cereals residues (t dm ha$^{-1}$) = yields (t ha$^{-1}$) x 0.2
Potatoes residues (t dm ha$^{-1}$) = yields (t ha$^{-1}$) x 0.05
Maize residues (t dm ha$^{-1}$) = above-ground phytomass (t ha$^{-1}$) x 0.1
Sugar beet - not considered
C content of crop residues: 40% of dry matter (Körschens et al., 1998)

[d] Farmyard manure-C (t ha$^{-1}$) = fresh matter (t ha$^{-1}$) x 0.1 (Körschens et al., 1998)

vary in soil texture and are arranged according to increasing clay contents of the soils. The soils from Thyrow, Groß Kreutz and Skierniewice represent a group of highly sandy soils, whereas the soils from Bad Lauchstädt and Puch are dominated by silt.

The same set of contrasting treatments was chosen at each experimental site (Table 1):

i) Fertilized plots, with the combined addition of mineral and organic fertilization (treatment I);
ii) C-depleted plots, either unmanured plots with the same crop rotation as in the fertilized treatment, but without any fertilization, or bare fallows (treatments II and III).

Table 2 outlines the differences in crop yields and estimated C-inputs between the fertilized and unmanured plots from Thyrow and Bad Lauchstädt (Experiment A). Whereas at Thyrow only one crop of the rotation is grown in a season, at Bad Lauchstädt all the crops are grown simultaneously each year on the plots under different treatment. Although at both sites crop yields are reduced in the absence of fertilization, the relative decrease is much stronger for Thyrow than for Bad Lauchstädt, as shown for the years 1998 and 1999. In the unmanured soils, crop residues represent the only C-source. Thus, the residual amounts of C-input in the depleted plots compared with the fertilized plots are low. The discrepancy in C-input between the two management regimes is more pronounced in the sandy soil from Thyrow than in the loamy soil from Bad Lauchstädt.

Except for the bare fallows in Prague and Bad Lauchstädt, all treatments are replicated at least three-fold on separate plots arranged randomly within the experimental stations. The bare fallow plots at Prague and Bad Lauchstädt (Experiment A) are 2 to 3 $m^2$ in size and have no replications. The bare fallow of Experiment B at Bad Lauchstädt is replicated twice. Soil samples were taken in summer 1998 from a depth 0-20 cm. Soil material collected at ten sampling points on each of the replication plots of a particular treatment was mixed. Mixed samples were air-dried. Subsequently, components > 2 mm were removed by dry sieving. For analysis of carbon and lignin in the bulk samples, an aliquot of the sieved material was ground using a ball mill.

### 6.2.2 Particle-size fractionation (soils from Thyrow and Bad Lauchstädt)

The procedure involved ultrasonic dispersion of the soil samples by the two-step method used by Amelung et al. (1998), and isolation of the particle-size fractions by a combination of wet sieving (fractions 2000-20 µm), SPLITT-fractionation (fractions 20-0.2 µm) (Keil et al., 1994), and centrifugation (< 0.2 µm). Briefly, a suspension of air-dried soil < 2 mm (soil-to-water ratio 1:3 for Thyrow, 1:5 for Bad Lauchstädt) was ultrasonicated at 60 J $ml^{-1}$ using a probe-type ultrasonic disintegrator (Branson 250, Branson). Thereafter, primary particles 2000-250 µm (coarse and medium sand) including particulate OM, released by the first dispersion step, were removed by wet sieving. According to Amelung and Zech (1999), the removal of particulate OM after a first step of weak dispersion is recommended in order to avoid mechanical disruption of the particulate OM during the subsequent high-energy ultrasonification. In a second step, the suspension < 250 µm (soil-to-water ratio 1:5 for Thyrow, 1:10 for Bad Lauchstädt) was ultrasonicated at 440 J ml$^{-1}$ for complete dispersion. The fractions 250-63 (fine sand) and 63-20 µm (coarse silt) were obtained by wet sieving. Two successive SPLITT runs, employing the cutoffs of 6 µm (1st run) and 2 µm (2nd run), yielded the fractions 20-6 µm (medium silt), 6-2 µm (fine silt) and 2-0.2 µm (coarse clay). The fine clay (< 0.2 µm) was isolated by repeated centrifugation. Except for the 250 µm limit, we followed the limits of the German classification system (AG Boden, 1994). All fractions were freeze-dried. Aliquots were ground in a ball mill prior to the analysis for carbon and lignin.

### 6.2.3 Density fractionation

Prior to lignin analysis, the separates 2000-250 and 250-63 µm of the C-depleted soil from Thyrow were subjected to density fractionation using sodium polytungstate solution [$Na_6(H_2W_{12}O_{40}) \times H_2O$, Sometu, Berlin, Germany] of 1.8 g cm$^{-3}$ density. OC contents in these separates were very low (0.5 to 0.6 g kg$^{-1}$ fraction mass). Density fractionation allowed the organic matter to be concentrated in the light fraction ($\leq$ 1.8 g cm$^{-3}$). The light material, which represented approximately 90% of the total OC in the fractions, was then analyzed for lignin.

### 6.2.4 Carbon analysis

Total carbon in bulk samples, particle-size and density fractions was determined by dry combustion in a Vario EL elemental analyzer (Elementar Analysen-Systeme, Hanau, Germany). Inorganic carbon was determined by elemental analysis of subsamples after ignition at 550°C for 3 h (removal of organic matter). The samples did not contain any inorganic carbon.

Amounts of total OC in bulk samples of the depleted plots (g C kg$^{-1}$ soil) were related to those of the corresponding fertilized plots:

$$\frac{OC_{\text{depleted}} \times 100}{OC_{\text{fertilized}}} = OC_{\text{residual}} \qquad (1)$$

| | |
|---|---|
| $OC_{\text{depleted}}$ | OC amount in bulk samples from depleted plots (g $kg^{-1}$ soil) |
| $OC_{\text{fertilized}}$ | OC amount in bulk samples from fertilized plots (g $kg^{-1}$ soil) |
| $OC_{\text{residual}}$ | Residual OC in depleted plots (% of fertilized plot) |

### 6.2.5 Analysis of lignin

The procedure involved digestion of lignin by alkaline CuO oxidation, purification of the extracts and quantification of the phenolic oxidation products by gas chromatography using a flame ionization detector (GC-FID) (Kögel-Knabner, 1995). 50-800 mg of soil sample (bulk samples, particle-size fractions) or 50 mg of plant materials / light fraction material (see section 6.2.3) were placed into a teflon vessel and mixed with 15 ml 2 M NaOH, 250 mg CuO, 50 mg $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ and 50 mg glucose. The digestion of lignin was carried out in a pressure bomb (Groteklaes, Jülich, Germany) at 172 °C for 2 h in a $N_2$ atmosphere. After cooling, the suspension was centrifuged and the supernatant saved. The sediment was washed and centrifuged as before. The combined supernatants were acidified with 6 M HCl to pH 2 and allowed to stand for at least 1 h in the dark for precipitation of humic acids. After centrifugation, an aliquot (50-150 ml) of the supernatant was applied onto a pre-conditioned disposable $C_{18}$ column (Bakerbond spe $^{TM}$, J.T. Baker, USA). After the solution had passed through the column, the column was dried in a $N_2$ stream for 1 h. The lignin phenols were eluted from the column by applying 5 x 0.5 ml ethyl acetate. Ethyl acetate in the vials was evaporated under a stream of $N_2$. The residue was dissolved in pyridine, containing phenyl acetic acid as the internal standard for GC quantification. Then, BSTFA [bis-(trimethylsilyl)-trifluoroacetamide] was added as a silylating agent. Samples were measured on a GC 8000 series gas chromatograph (Fisons Instruments) using the following operation conditions:

i) fused silica capillary column, stationary phase with 5% diphenyl- and 95% dimethylsiloxane, 15 m length;
ii) split injection (split ratio 1:20), injector temperature 250°C; FID temperature 320°C;
iii) temperature program: 100°C for 10 min, ramp of 2°C $min^{-1}$ up to 150°C, ramp of 15°C $min^{-1}$ up to 300°C, 300°C for 8 min;
iv) Helium as carrier gas, with a constant pressure of 100 kPa.

Vanillyl (V) and syringyl (S) units were calculated from the corresponding aldehydes, ketones and carboxylic acids: V = vanillin + acetovanillone + vanillic acid; S = syringaldehyde + acetosyringone + syringic acid. Cinnamyl (C) units were derived from the sum of p-coumaric acid and ferulic acid (Hedges and Ertel, 1982). The sum of the three structural units (VSC = V + S + C) is considered to reflect the lignin amount in a sample. $Ac/Al_{V,S}$ are the mass ratios of acid to aldehyde units for vanillyl and syringyl units, respectively. S/V is the mass ratio of syringyl to vanillyl units.

Analyses were done in triplicate for bulk samples and in duplicate for particle-size fractions / plant materials. The mean relative standard deviation was 7% for the sum of VSC, 9% for vanillyl (V), 10% for syringyl (S) and 9% for cinnamyl (C) units (mean for all analyzed samples). The mean recovery rate of VSC in particle-size fractions relative to VSC amounts in the bulk samples was 97% ± 12%.

The C-depleted plots were compared with the fertilized counterparts with regard to OC-normalized VSC contents (VSC g $kg^{-1}$ OC). The contrasting treatments were also compared regarding amounts of VSC (VSC normalized to soil mass, VSC g $kg^{-1}$ soil). The amount of VSC remaining in the depleted plot was related to that in the corresponding fertilized plot, and denoted as "residual" VSC:

$$\frac{\mathrm{VSC}_{\mathrm{depleted}} \times 100}{\mathrm{VSC}_{\mathrm{fertilized}}} = \mathrm{VSC}_{\mathrm{residual}} \qquad (2)$$

| | |
|---|---|
| $\mathrm{VSC}_{\mathrm{depleted}}$ | Amount of VSC in the depleted plots (g $kg^{-1}$ soil) |
| $\mathrm{VSC}_{\mathrm{fertilized}}$ | Amount of VSC in the fertilized plots (g $kg^{-1}$ soil) |
| $\mathrm{VSC}_{\mathrm{residual}}$ | Residual VSC in the depleted plots (% of fertilized plot) |

Equation 2 was applied to data from bulk samples and individual particle-size fractions.

For particle-size fractions of both treatments, net amounts of VSC were calculated by multiplying the VSC content in a given fraction with the mass proportion of that fraction:

$$\frac{\mathrm{VSC}_{\mathrm{fraction}} \times \mathrm{Mass}_{\mathrm{fraction}}}{100} = \mathrm{VSC} \qquad (3)$$

| | |
|---|---|
| $\mathrm{VSC}_{\mathrm{fraction}}$ | Content of VSC for a given particle-size fraction (g $kg^{-1}$ fraction) |

| | |
|---|---|
| Mass $_{\text{fraction}}$ | Mass proportion of the fraction (% of soil mass) |
| VSC | Amount of VSC in a given particle-size fraction (g $kg^{-1}$ soil) |

### 6.2.6 Statistics

Differences in lignin parameters (VSC contents, Ac/Al-ratios, S/V ratio) between the two groups of treatments were tested by means of the *t*-test for paired samples (bulk soils). The *t*-test and linear regression analysis were performed with the software package Statistica 5.1 (Statsoft).

## 6.3 Results and discussion

### 6.3.1 VSC contents

#### *6.3.1.1 Bulk samples*

For all experiments, OC-normalized contents of lignin phenols substantially decreased in the C-depleted plots compared with the fertilized counterparts (Table 3a). On average, VSC contents declined from 21 g $kg^{-1}$ OC in the fertilized plots to 7 g $kg^{-1}$ OC in the depleted plots, this change being highly significant. The net amounts of lignin phenols were considerably reduced in the C-depleted plots (Table 3b): On average, the amount of VSC left in the depleted plots was 18% of the amount in the fertilized plots. The decrease of lignin phenols was more pronounced than the decline of total OC in all the long-term experiments, with an average residual amount of 18% for VSC and 52% for OC. Consequently, the remaining SOC in the depleted plots showed a lower contribution of lignin phenols than the SOC of the fertilized plots (Table 3a).

Leifeld (1998) observed that VSC contents (relative to OC) in unmanured plots from agroecosystem experiments were lower compared with plots receiving organic amendments, which is in agreement with our results. Note that the data presented here refer only to the unaltered lignin determined by the CuO oxidation technique. We have no information about the fate of modified lignin moieties that have undergone structural alterations such as demeth(ox)ylation, cleavage of the propyl side-chain from the aromatic ring, and which were not identified by the used analytical procedure.

**Table 3a:** OC-normalized contents of VSC in bulk samples from the contrasting treatments of long-term agroecosystem experiments.

| | | **VSC** (g kg$^{-1}$ OC) | | |
|---|---|---|---|---|
| **Experimental site** | | **Fertilized** | **Depleted** | **Δ (Depl. - Fert.)** |
| Thyrow | | 21.7 | 4.9 | - 16.8 |
| Groß Kreutz | | 25.4 | 13.0 | - 12.4 |
| Skierniewice | | 13.2 | 4.6 | - 8.6 |
| Puch | | 15.9 | 6.5 | - 9.4 |
| Lauterbach | | 16.8 | 8.0 | - 8.7 |
| Bad Lauchstädt | | | | |
| *Experiment A* | | | | |
| | Unmanured plot | 19.1 | 5.4 | - 13.7 |
| | Bare fallow | | 1.7 | - 17.4 |
| *Experiment B* | | 28.9 | 9.8 | - 19.0 |
| Prague | Bare fallow | 26.0 | 5.6 | - 20.4 |
| | Bare fallow + tillage | | 7.8 | - 18.3 |
| Mean ± SD (*n*=8 experiments) | | 20.9 ± 5.5 | 7.1 ± 3.1 | - 13.7 ± 4.5 *** |

*** Difference between the treatments significant at the 0.001 probability level (paired *t*-test)

### 6.3.1.2 Particle-size fractions (Thyrow and Bad Lauchstädt)

#### 6.3.1.2.1 OC-normalized VSC contents

For two soils of contrasting texture (sandy soil from Thyrow, loamy soil from Bad Lauchstädt), the behavior of lignin in the course of C-depletion was investigated in particle-size fractions. In all separates of the depleted (= unmanured) plots, VSC contents were lower than in the separates of the fertilized plots, except for the coarse fractions (2000-63 µm) from Bad Lauchstädt (Fig. 2). The decline of VSC contents recorded for bulk samples of the C-depleted plots is therefore the consequence of a considerable decrease in all the separates (Thyrow) and medium-fine separates (Bad Lauchstädt), respectively.

OM in sand separates consists of plant debris in first stages of decay (Christensen, 1996; Amelung et al., 1998). The VSC contents in these separates should thus be related to the composition of the OM that was recently supplied to the soil. For Bad Lauchstädt, the organic

**Table 3b:** Amounts of VSC and total OC in bulk samples derived from the contrasting treatments of long-term agroecosystem experiments.

| | VSC | | | OC | | |
|---|---|---|---|---|---|---|
| | **Fertilized** | **Depleted** | **Residual amounts** [a] | **Fertilized** | **Depleted** | **Residual amounts** [b] |
| **Experimental site** | (g kg$^{-1}$ soil) | | (% of fertilized plot) | (g kg$^{-1}$ soil) | | (% of fertilized plot) |
| Thyrow | 0.15 | 0.02 | 11 | 6.8 | 3.2 | 47 |
| Groß Kreutz | 0.26 | 0.05 | 21 | 10 | 4.1 | 40 |
| Skierniewice | 0.12 | 0.02 | 18 | 8.8 | 4.4 | 51 |
| Puch | 0.19 | 0.05 | 24 | 12 | 7.0 | 58 |
| Lauterbach | 0.81 | 0.24 | 30 | 48 | 30 | 62 |
| Bad Lauchstädt | | | | | | |
| *Experiment A* | | | | | | |
| Unmanured plot | 0.46 | 0.09 | 19 | 24 | 16 | 67 |
| Bare fallow | | 0.03 | 6 | | 15 | 62 |
| *Experiment B* | 1.19 | 0.19 | 16 | 41 | 20 | 48 |
| Prague Bare fallow | 0.76 | 0.08 | 11 | 29 | 14 | 50 |
| Bare fallow + tillage | | 0.10 | 13 | | 13 | 44 |
| Mean ± SD (*n*=8 experiments) | | | 18 ± 6 | | | 52 ± 8 |

[a] Residual amounts of VSC in the depleted plots (equation 2)

[b] Residual amounts of total OC in the depleted plots (equation 1)

input in the two experimental treatments had similar lignin contents, whereas for Thyrow the input to the C-depleted plot had less lignin than the fertilized plot (Fig. 2). VSC concentrations in the coarse fractions were in the range of values determined for cereal straw and potato foliage (Fig. 2).

Although the contrasting treatments at a particular site differed in the magnitude of VSC contents, they had a similar pattern of contents across the fractions (Fig. 2). Within a particular soil, the concentration of phenolic oxidation products was highest in the sand and coarse silt, and decreased in fractions < 20 µm with decreasing particle-size. This agrees with results published for a number of soils (e.g. Guggenberger et al., 1994; Amelung et al., 1999). The diminishing content of intact lignin with decreasing particle-size may be the result of both the progressive degradation of lignin and the increasing input of microbially derived compounds to OC.

#### *6.3.1.2.2 Amounts of VSC*

In the C-depleted plots from both sites, net amounts of VSC were considerably reduced over the whole range of separates (Fig. 3, calculations according to equations 2 and 3). The VSC residues in the depleted plots - expressed relative to the amount in the fertilized counterparts - ranged from 8 to 34% (Thyrow) and from 10 to 39% (Bad Lauchstädt). As a result of this decline, the residual VSC amounts in bulk samples were 11% for Thyrow and 19% for Bad Lauchstädt. For a given site, the various size fractions behaved similarly, except for the fine clay, which tended to retain a slightly higher proportion of lignin phenols (Fig. 3).

OM in the sand fraction is turned over within several years to decades (Christensen, 1996). The decrease noted in coarse fractions therefore probably reflects the reduced lignin input in the depleted plots over the last years. The decrease of lignin in silt and clay fractions, being similar to that in the sand fraction, also seems to reflect the continuously reduced lignin input.

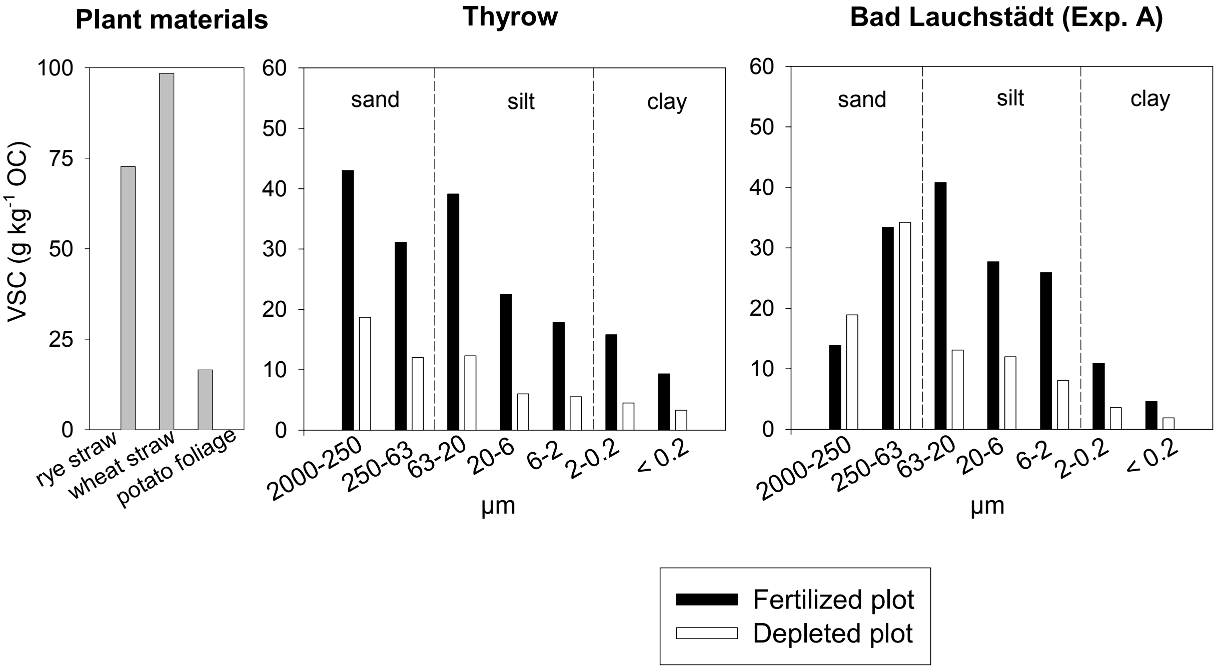


**Fig. 2:** VSC contents in the fertilized and C-depleted (=unmanured) plots of the experiments from Thyrow and Bad Lauchstädt.

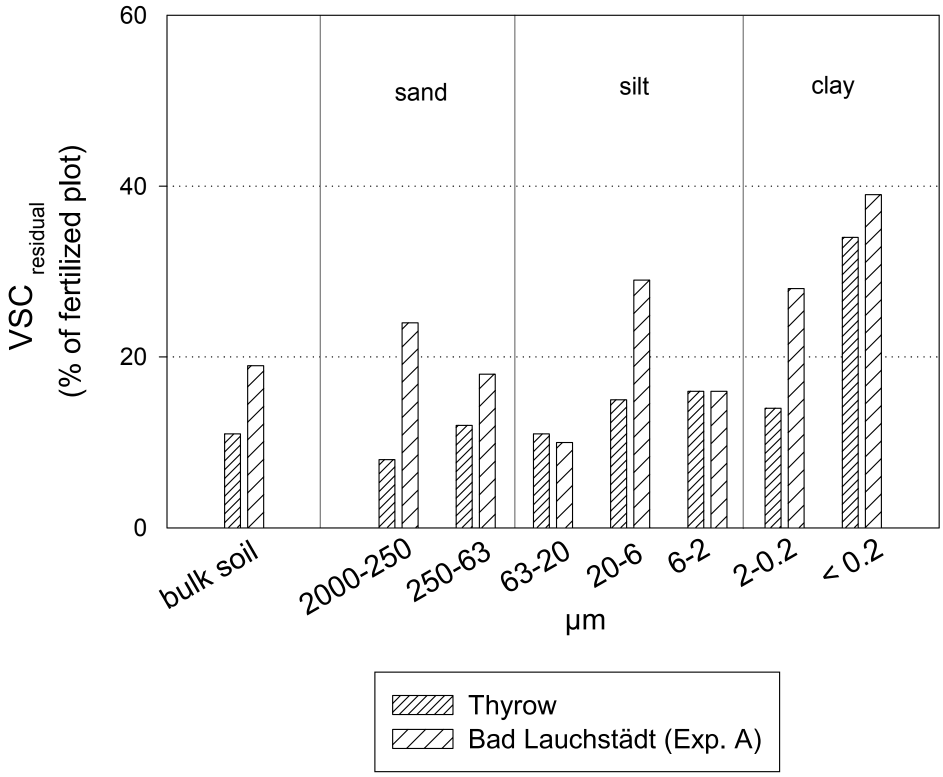


**Fig. 3:** Residual amounts of VSC in the various separates of the C-depleted (=unmanured) plots from Thyrow and Bad Lauchstädt.

### 6.3.2 Acid-to-aldehyde ratios and syringyl-to-vanillyl-ratio

#### *6.3.2.1 Bulk samples*

Table 4 compares the contrasting treatments with respect to the acid-to-aldehyde ratios for vanillyl/syringyl units ($Ac/Al_{V,S}$) and the ratio of syringyl-to-vanillyl units (S/V). These ratios were shown to serve as indicators for the degree of microbial lignin alteration/degradation. The $Ac/Al_V$ and $Ac/Al_S$ ratios reflect the degree of side-chain oxidation of the two phenylpropanoid units (Kögel, 1986; Hedges et al., 1988). In incubation experiments, ongoing lignin degradation resulted in a steady increase of the Ac/Al ratios within the remnant lignin (Hedges et al., 1988; Miltner and Zech, 1998). The S/V ratio can be used as an indicator for the decomposition degree of angiosperm lignin: syringyl units are preferentially degraded over vanillyl units, resulting in a depletion of syringyl relative to vanillyl units in the course of lignin degradation (Hedges et al., 1988; Miltner and Zech, 1998). We assumed that the Ac/Al and S/V ratios of lignin in the organic input material are similar for the different treatments at a site. In this case, deviations in the ratios between the contrasting treatments can be interpreted in relation to lignin biodegradation.

For the $Ac/Al_V$ and $Ac/Al_S$ ratios, in most of our long-term experiments an increase was recorded in the depleted compared with the fertilized plots. However, the extent of increase varied between the experimental sites as indicated by the delta ($\Delta$) values in Table 4. The highest increase for both ratios occurred in the sandy soils from Thyrow and Skierniewice. For the eight experiments, the increase of the ratios along with C-depletion was statistically significant. The S/V ratio was lower in the depleted plots of all experiments with one exception (Experiment A, Bad Lauchstädt) (Table 4). The extent of change was again most pronounced for Thyrow and Skierniewice. The average change of the S/V ratio, i.e. the decrease of syringyl relative to vanillyl units in the depleted plots, was significant at the 0.01 probability level. The average changes, i.e. the increase in the Ac/Al ratios and the decrease in the S/V ratio in the C-depleted soils, are consistent with a higher degree of lignin biodegradation in the depleted versus the fertilized soils.

The range of values for the $Ac/Al_V$ and $Ac/Al_S$ ratios and the S/V ratio noted in the present study is in line with the results reported for other arable soils (Sanger et al., 1997; Leifeld, 1998; Schmidt, 1998). The S/V ratios are in the range of values noted for non-woody angiosperm tissues (Hedges and Mann, 1979), this type of tissue presumably dominating the OM-input in arable soils.

**Table 4:** Acid-to-aldehyde ratio for vanillyl ($\text{Ac/Al}_{V}$) and syringyl units ($\text{Ac/Al}_{S}$), and syringyl-to-vanillyl ratio (S/V) for the contrasting treatments from long-term agroecosystem experiments (bulk samples).

| | **$\text{Ac/Al}_{V}$** | | | **$\text{Ac/Al}_{S}$** | | | **S/V** | | |
|---|---|---|---|---|---|---|---|---|---|
| **Experimental site** | **Fertilized** | **Depleted** | **$\Delta$(Depl. - Fert.)** | **Fertilized** | **Depleted** | **$\Delta$(Depl. - Fert.)** | **Fertilized** | **Depleted** | **$\Delta$(Depl. - Fert.)** |
| Thyrow | 0.28 | 0.55 | + 0.27 | 0.42 | 0.66 | + 0.24 | 1.8 | 1.2 | - 0.65 |
| Groß Kreutz | 0.14 | 0.22 | + 0.08 | 0.38 | 0.43 | + 0.05 | 2.0 | 1.7 | - 0.29 |
| Skierniewice | 0.18 | 0.56 | + 0.38 | 0.44 | 0.71 | + 0.27 | 1.8 | 1.1 | - 0.63 |
| Puch | 0.28 | 0.34 | + 0.06 | 0.44 | 0.60 | + 0.16 | 1.7 | 1.3 | - 0.40 |
| Lauterbach | 0.29 | 0.44 | + 0.15 | 0.48 | 0.47 | - 0.01 | 1.5 | 1.3 | - 0.20 |
| Bad Lauchstädt | | | | | | | | | |
| *Experiment A* | | | | | | | | | |
| Unmanured plot | 0.31 | 0.29 | - 0.02 | 0.53 | 0.62 | + 0.09 | 1.6 | 1.7 | + 0.10 |
| Bare fallow | | 0.36 | + 0.06 | | 0.69 | + 0.16 | | 1.3 | - 0.27 |
| *Experiment B* | 0.21 | 0.31 | + 0.10 | 0.40 | 0.47 | + 0.07 | 1.7 | 1.6 | - 0.06 |
| Prague Bare fallow | 0.20 | 0.34 | + 0.14 | 0.41 | 0.40 | - 0.01 | 1.8 | 1.7 | - 0.17 |
| Bare fallow + tillage | | 0.23 | + 0.03 | | 0.40 | - 0.01 | | 1.8 | - 0.09 |
| Mean ± SD (*n*=8 experiments) | 0.23 ± 0.06 | 0.38 ± 0.12 | + 0.14 ± 0.12 * | 0.44 ± 0.04 | 0.55 ± 0.11 | + 0.11 ± 0.10 * | 1.7 ± 0.15 | 1.4 ± 0.23 | - 0.30 ± 0.23 ** |

*, ** Difference between the two treatments significant at the 0.05 and 0.01 probability level, respectively (paired *t*-test)

### *6.3.2.2 Particle-size fractions (Thyrow and Bad Lauchstädt)*

Figs. 4a and 4b show the $Ac/Al_V$ and $Ac/Al_S$ ratios in size fractions for the fertilized and C-depleted (=unmanured) plots of the two sites. Lowest values were generally observed in coarse fractions (sand, coarse silt), highest values in the clay fraction. For the sandy soil (Thyrow), however, a stronger increase of the two ratios from coarse to fine separates occurred in the C-depleted compared with the fertilized plot. For this soil, in fractions < 63 µm, $Ac/Al_V$ and $Ac/Al_S$ ratios were considerably higher in the depleted plot. The effect of C-depletion on the Ac/Al ratios was less pronounced for the loamy soil (Bad Lauchstädt). Here, $Ac/Al_V$ ratios were similar in the two treatments, whereas $Ac/Al_S$ ratios were slightly higher in fractions < 63 µm of the C-depleted plot. These results reveal that the increase of the Ac/Al ratios noted in the bulk samples from the two sites (see Table 4) was always due to an increase in fractions < 63 µm. The largest divergence between the treatments occurred in fine fractions. For both sites, the contrasting treatments did not differ in the $Ac/Al_V$ and $Ac/Al_S$ ratios in the fractions 2000-63 µm. The values here were close to those for fresh plant materials (cereal straw, potato foliage) (Figs. 4a and 4b).

The relative increase of the $Ac/Al_V$ and $Ac/Al_S$ ratios reflects progressing oxidative cleavage of side-chains in the respective structural units from coarse to fine separates, and this points to an increasing degree of lignin biodegradation with decreasing particle-size (Guggenberger et al., 1994). The values of the two ratios indicate that the sand fractions in both treatments comprises undecomposed / weakly decomposed lignin from relatively recent organic input. Differences in the ratios between the treatments were mainly manifest in the fine separates. Within a soil sample, the fine separates comprised the lignin moieties with the highest degree of oxidative alteration reached under a certain treatment. The higher ratios in fine-medium separates of the C-depleted plots indicate that lignin is more oxidatively altered and thus lignin degradation proceeded further under these conditions than in the fertilized soils.

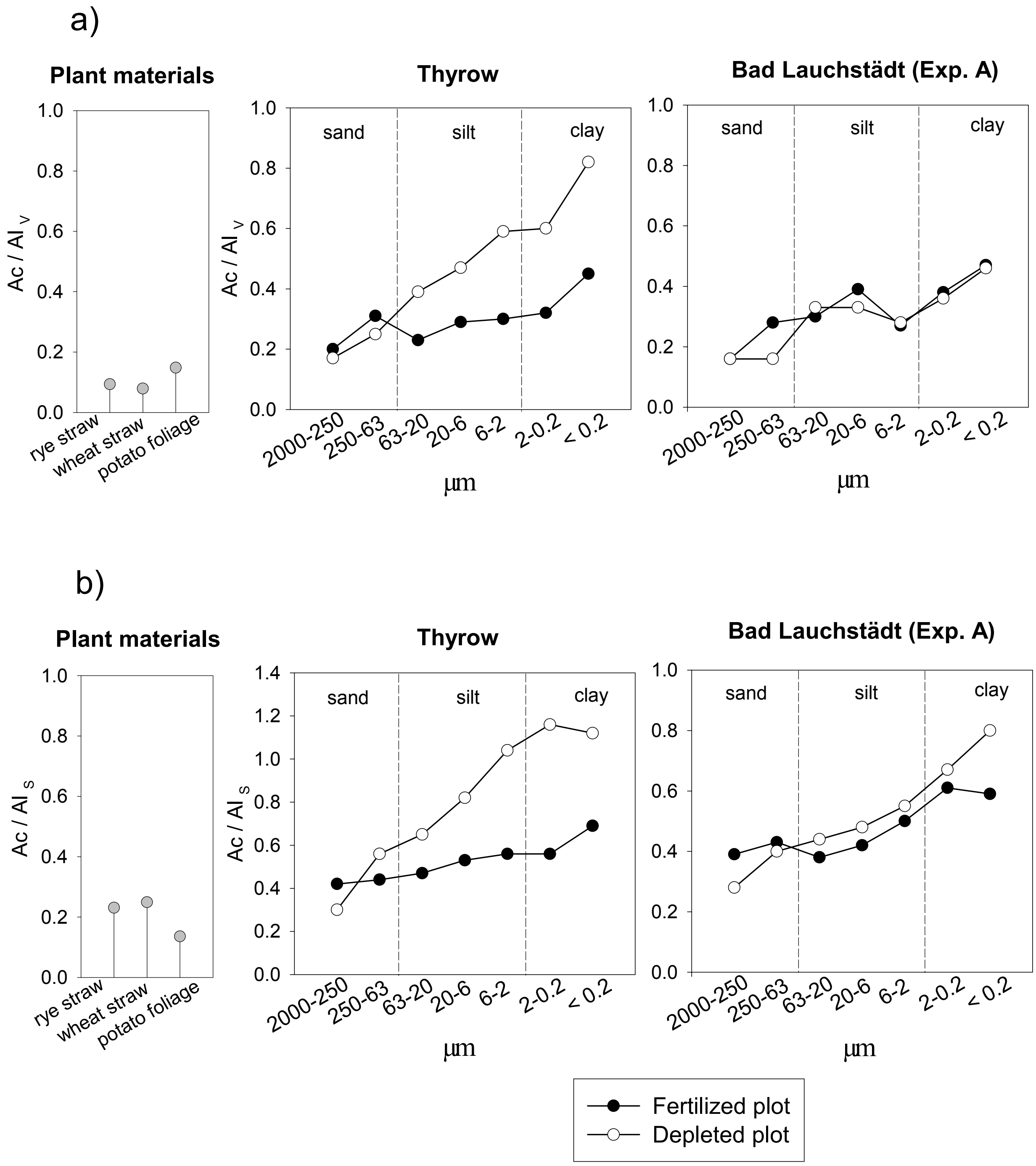


**Fig. 4:** Ac/Al$_V$ ratios (a) and Ac/Al$_S$ ratios (b) in the fertilized and C-depleted (=unmanured) plots from Thyrow and Bad Lauchstädt.

### 6.3.3 Contribution of lignin to the refractory C pool

The stronger decline of lignin phenols compared with total OC in bulk samples (eight long-term experiments) indicates a higher contribution of lignin to the labile SOC pool than to the refractory C pool remaining in the C-depleted plots. Lignin clearly does not accumulate within the refractory C pool of arable soils. These conclusions are supported by the findings on particle-size fractions, especially the fine fractions (< 6 µm), from two of the experimental sites. It was shown previously (chapter 2.3.2) that the fractions < 6 µm contain the major part of refractory SOC, whereas C in coarse-medium fractions may be attributed to a compartment of more labile SOC. Although the lignin phenol contents (relative to OC) were lower in nearly all fractions of the depleted plots, the decrease in separates < 6 µm emphasizes that lignin does not accumulate within the refractory C pool. The residual amounts of VSC were low over the whole range of separates. This pattern contrasts with that for total OC, where residual amounts were markedly higher in fine separates (< 6 µm) compared with coarse fractions (chapter 2.3.2). Due to this greater retention, total OC in fine separates can be considered to be more stable than OC in coarse separates. In contrast, the pattern of relative residuals of lignin indicates that there is no (pronounced) long-term stabilization of lignin in any of the fractions. Lignin in fine fractions appears to have a labile nature similar to the lignin in coarser fractions.

The higher degree of oxidative lignin alteration in the depleted versus the fertilized plots underlines that lignin is not preserved in the former plots. The lower contribution of lignin phenols to SOC together with a greater structural alteration in the depleted plots point towards a progressive degradation of lignin in soil. This is especially true for lignin in fine-medium particle-size fractions. The Ac/Al ratios show that lignin exists in various stages of degradation within a particular soil sample, from fresh lignin in coarse separates to more altered lignin in fine separates. The present data give evidence that lignin, regardless of its degree of structural alteration, is not subject to stabilization processes. Although lignin is a comparatively recalcitrant compound during biodegradation, it does not provide a significant contribution to the refractory C pool of the examined arable soils.

It should be stressed that these conclusions are based solely on amounts and Ac/Al ratios of lignin phenols that have an intact substitution pattern and were identified by GC analysis. Intermediates of lignin degradation, which could not be identified, may contribute to the refractory C pool. Testing this hypothesis would require other analytical approaches, e.g. involving carbon labelling in lignin units to trace the behavior of lignin-derived carbon at different time-scales. In the course of oxidative lignin degradation, functional groups such as hydroxyl, keto and carboxyl groups are formed within the lignin matrix (Dijkstra et al., 1998). Haider (1992, 1999) proposed that the modified lignin, enriched in polar functional groups relative to the original lignin, may more readily interact with mineral surfaces and possibly become stabilized by this association. The present study does not indicate mechanisms for the long-term stabilization of basically intact lignin.

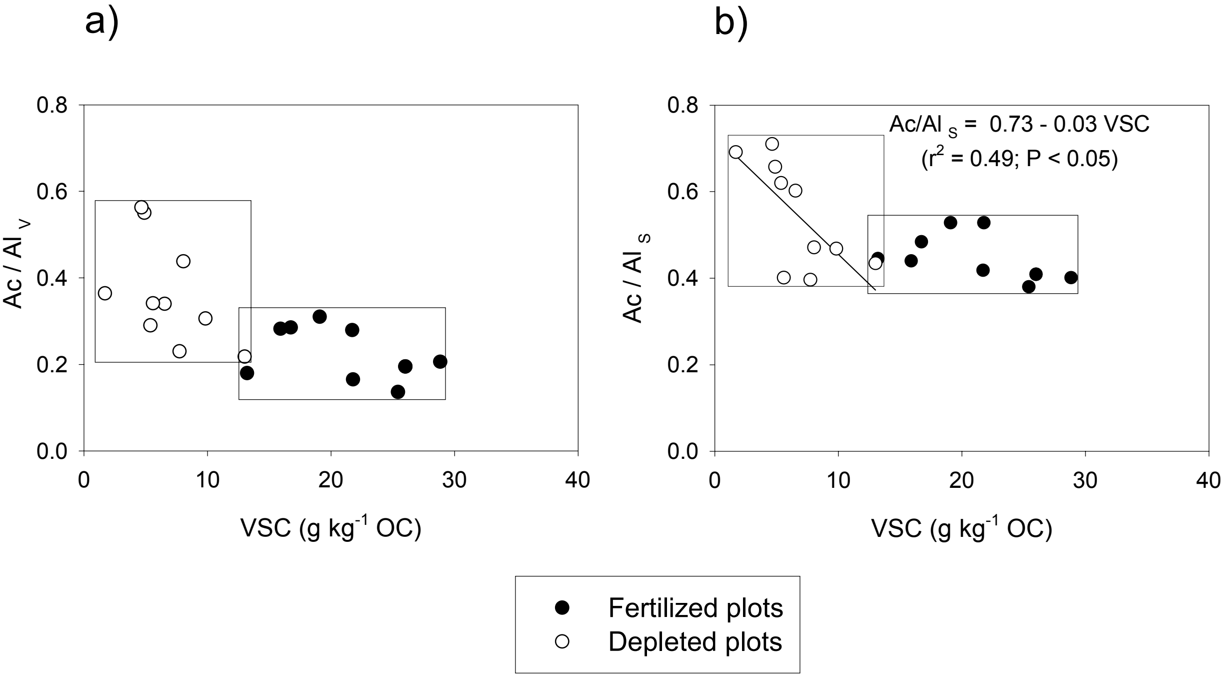


**Fig. 5:** Ac/Al$_V$ ratios versus VSC contents (a) and Ac/Al$_S$ ratios versus VSC contents (b) in bulk samples derived from contrasting treatments of the long-term experiments.

### 6.3.4 Acid-to-aldehyde ratios versus VSC contents

#### *6.3.4.1 Bulk samples*

Figs. 5a and 5b summarize the Ac/Al$_V$ and Ac/Al$_S$ ratios as well as the lignin phenol contents from the various study sites. The C-depleted plots (unmanured plots and bare fallows) from all sites are treated as one group, in the same way as the fertilized plots. VSC contents ranged from approximately 2 to 30 g kg$^{-1}$ OC, with the fertilized plots varying between 13 to 30 g kg$^{-1}$ OC, and the depleted plots covering the lower range of values from 2 to 13 g kg$^{-1}$ OC. Apart from two datapoints, the contrasting treatments did not overlap with respect to VSC contents. In contrast, the two groups overlapped regarding the Ac/Al$_V$ and Ac/Al$_S$ ratios. Nevertheless, the highest ratios among the samples were found in the C-depleted plot group. These results show that for a number of different experimental sites VSC contents are a more sensitive parameter for differentiating the contrasting management regimes than the Ac/Al ratios, i.e. the degree of alteration of the identified lignin. The study sites vary in the details of management practice, e.g. the amount of farmyard manure in the fertilized plots (Table 1) and possibly the lignin content of this manure. They also vary in climatic features and physico-chemical properties of the soils, these factors presumably directly or indirectly affecting lignin biodegradation (e.g. Amelung et al., 1999; Rodionov et al., 1999). Soil texture may be important because it influences structural properties/aggregation, which in turn affect aeration and accessibility of OM to microbes (Baldock and Skjemstad, 2000). These aspects may all have an effect on the lignin content in a sample and may explain part of the VSC variability within the dataset of the fertilized and depleted plots (Figs. 5a and 5b).

In the group of depleted plots, $Ac/Al_S$ ratios increased linearly with decreasing VSC contents (Fig. 5b). The more a sample is depleted in lignin, the more the syringyl units within the lignin are oxidatively altered. This indicates that the (remnant) lignin in samples of low VSC content has undergone intensive microbial degradation. A high degree of oxidative alteration of lignin/syringyl units in bulk soil samples is reached only at rather low VSC contents (C-depleted plots). For the other three subgroups of data shown in Figs. 5a and 5b, VSC contents and Ac/Al ratios were not correlated.

### *6.3.4.2 Particle-size fractions (Thyrow and Bad Lauchstädt)*

Fig. 6 plots the Ac/Al ratios against the concentrations of CuO oxidation products (VSC g $kg^{-1}$ OC) in particle-size fractions, for both the fertilized (Fig. 6a) and the depleted plots (Figs. 6b and 6c) at the two study sites. For both treatments, VSC contents and Ac/Al ratios were linearly related across the separates, except for the relation between $Ac/Al_S$ ratios and VSC contents in the depleted plot from Bad Lauchstädt. The declining VSC contents with decreasing particle-size were accompanied by linearly increasing Ac/Al ratios of both vanillyl and syringyl units. For the fertilized plots the data of the two study sites showed a similar trend and were combined (Fig. 6a). For the depleted plots, the relation between Ac/Al ratios and VSC contents was analyzed separately for each site (Figs. 6b and 6c). Although both treatments show a linear relationship, the treatments differed in the slope and the y-intercept, especially in the sandy soil (Thyrow). The fertilized soils covered a wide range of VSC contents (from 5 to 43 g VSC $kg^{-1}$ OC) and showed a rather slow increase of Ac/Al ratios with decreasing contents (Fig. 6a). In the depleted plots, VSC contents varied in a lower range (mostly 2 to 18 g VSC $kg^{-1}$ OC) (Figs. 6b and 6c). For the sandy soil (Thyrow), the absolute value of the slope in the depleted plot was higher than that for the combined fertilized plots by a factor of 10. This indicates a stronger increase of the degree of oxidative lignin alteration with decreasing VSC contents, or decreasing particle-size, than in the fertilized soils.

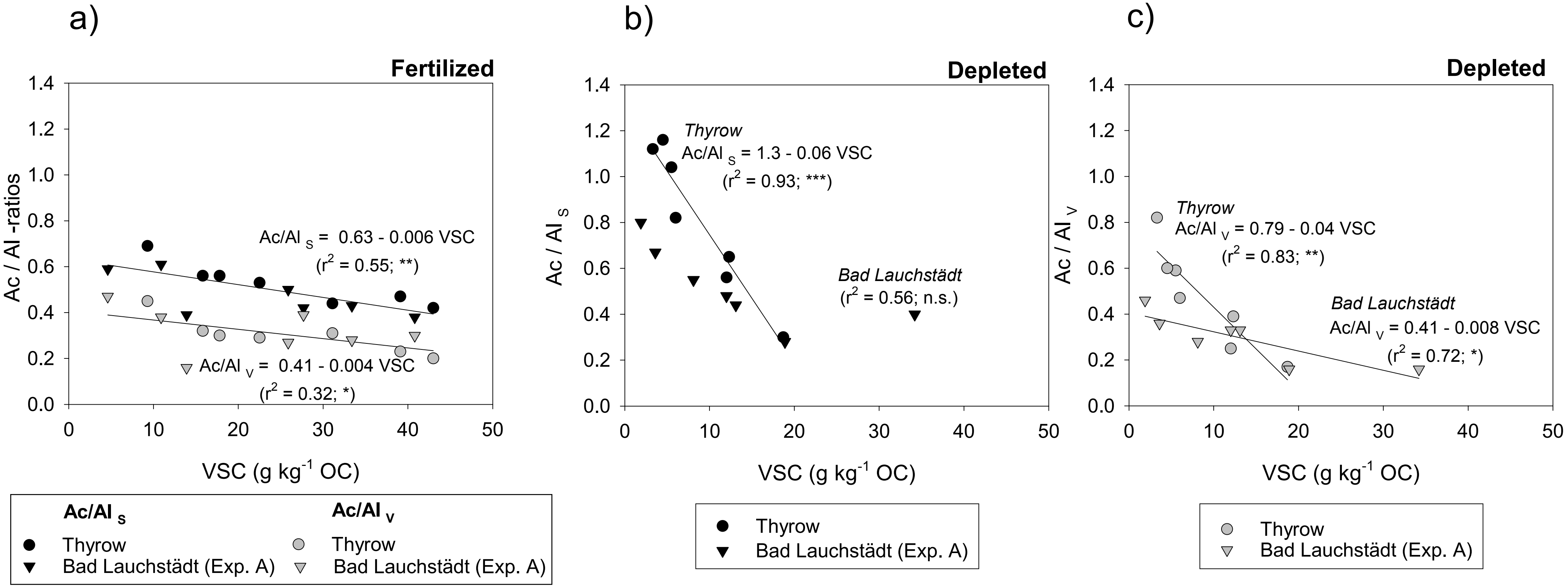


**Fig. 6:** Ac/Al$_V$ ratios and Ac/Al$_S$ ratios versus VSC contents in particle-size fractions of the fertilized plots (a) and the C-depleted (=unmanured) plots (b and c) from Thyrow and Bad Lauchstädt (*, **, ***: Significant at the 0.05, 0.01, and 0.001 probability level, respectively. n.s.: Not significant).

Especially for Thyrow, the contrasting management regimes resulted in different magnitudes of VSC contents in the soil and, moreover, in different slopes in the relationship between Ac/Al ratios and VSC contents. A negative correlation between the $Ac/Al_V$ ratio and lignin contents across the size separates was also noted by Guggenberger et al. (1995b) for a soil under different land-use regimes, including an arable rotation.

## 6.4 References

AG Boden, 1994. Bodenkundliche Kartieranleitung, 4. Auflage. E. Schweizerbart`sche Verlagsbuchhandlung, Stuttgart.

Amelung, W., Zech, W., 1999. Minimisation of organic matter disruption during particle-size fractionation of grassland epipedons. Geoderma 92, 73-85.

Amelung, W., Zech, W., Zhang, X., Follett, R.F., Tiessen, H., Knox, E., Flach, K.-W., 1998. Carbon, nitrogen, and sulfur pools in particle-size fractions as influenced by climate. Soil Science Society of America Journal 62, 172-181.

Amelung, W., Flach, K.W., Zech, W., 1999. Lignin in particle-size fractions of native grassland soils as influenced by climate. Soil Science Society of America Journal 63, 1222-1228.

Baldock, J.A., Skjemstad, J.O., 2000. Role of the mineral matrix and minerals in protecting natural organic materials against decomposition. Organic Geochemistry 31, 697-710.

Christensen, B.T., 1996. Carbon in primary and secondary organomineral complexes. In: Carter, M.R., Stewart, B.A. (Eds), Structure and Organic Matter Storage in Agricultural Soils. Advances in Soil Science. CRC Press, Boca Raton, pp. 97-165.

Dijkstra, E.F., Boon, J.J., Van Mourik, J.M., 1998. Analytical pyrolysis of a soil profile under Scots pine. European Journal of Soil Science 49, 295-304.

Falloon, P.D., Smith, P., 2000. Modelling refractory soil organic matter. Biology and Fertility of Soils 30, 388-398.

Fengel, D., Wegener, G., 1984. Wood: Chemistry, Ultrastructure, Reactions. de Gruyter, Berlin New York.

Guggenberger, G., Christensen, B.T., Zech, W., 1994. Land-use effects on the composition of organic matter in particle-size separates of soils: I. Lignin and carbohydrate signature. European Journal of Soil Science 45, 449-458.

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

Guggenberger, G., Zech, W., Haumaier, L., Christensen, B.T., 1995b. Land-use effects on the composition of organic matter in particle-size separates of soils: II. CPMAS and solution $^{13}C$ NMR analysis. European Journal of Soil Science 46, 147-158.

Haider, K., 1992. Problems related to the humification processes in soils of temperate climates. In: Stotzky, G., Bollag, J.-M. (Eds), Soil Biochemistry, Volume 7. Marcel Dekker, pp. 55-94.

Haider, K., 1999. Von der toten organischen Substanz zum Humus. Zeitschrift für Pflanzenernährung und Bodenkunde 162, 363-371.

Hammel, K.E., 1997. Fungal degradation of lignin. In: Cadisch, G., Giller, K.E. (Eds), Driven by Nature: Plant Litter Quality and Decomposition. CAB International, pp. 33-45.

Hedges, J.I., Mann, D.C., 1979. The characterization of plant tissues by their lignin oxidation products. Geochimica et Cosmochimica Acta 43, 1803-1807.

Hedges, J.I., Ertel, J.R., 1982. Characterization of lignin by gas capillary chromatography of cupric oxide oxidation products. Analytical Chemistry 54, 174-178.

Hedges, J.I., Blanchette, R.A., Weliky, K., Devol, A.H., 1988. Effects of fungal degradation on the CuO oxidation products of lignin: A controlled laboratory study. Geochimica et Cosmochimica Acta 52, 2717-2776.

Keil, R.G., Tsamakis, E., Fuh, C.B., Giddings, J.C., Hedges, J.I., 1994. Mineralogical and textural controls on the organic composition of coastal marine sediments: Hydrodynamic separation using SPLITT-fractionation. Geochimica et Cosmochimica Acta 58, 879-893.

Kirk, T.K., 1984. Degradation of lignin. In: Gibson, D.T. (Ed), Microbial Degradation of Organic compounds. Marcel Dekker, New York, pp. 399-435.

Kögel, I., 1986. Estimation and decomposition pattern of the lignin component in forest humus layers. Soil Biology & Biochemistry 18, 589-594.

Kögel-Knabner, I., 1995. Composition of soil organic matter. In: Nannipieri, P., Alef, K. (Eds), Methods in Applied Soil Microbiology and Biochemistry. Academic Press, pp. 66-78.

Körschens, M., Weigel, A., Schulz, E., 1998. Turnover of soil organic matter (SOM) and long-term balances - tools for evaluating sustainable productivity of soils. Zeitschrift für Pflanzenernährung und Bodenkunde 161, 409 - 424.

Leifeld, J., 1998. Einfluß von Kompostanwendung auf den Umsatz der organischen Substanz in Böden. Reihe Geowissenschaften. Shaker Verlag, Aachen.

Martin, J.P., Haider, K., Kassim, G., 1980. Biodegradation and stabilization after 2 years of specific crop, lignin, and polysaccharide carbons in soils. Soil Science Society of America Journal 44, 1250-1255.

Miltner, A., Zech, W., 1998. Beech leaf litter lignin degradation and transformation as influenced by mineral phases. Organic Geochemistry 28, 547-463.

Oades, J.M., 1995. An overview of processes affecting the cycling of organic carbon in soils. In: Zepp, R.G., Sonntag, C. (Eds), Role of Nonliving Organic Matter in the Earth's Carbon Cycle. John Wiley & Sons Ltd., pp. 293-303.

Parton, W.J., Schimel, D.S., Cole, C.V., Ojima, D.S., 1987. Analysis of factors controlling soil organic matter levels in Great Plains grasslands. Soil Science Society of America Journal 51, 1173-1179.

Rodionov, A., Amelung, W., Urusevskaja, I., Zech, W., 1999. Klimaeinfluß auf Lignin und Polysaccharide in Partikelgrößen-Fraktionen zonaler Steppenböden Rußlands. Zeitschrift für Pflanzenernährung und Bodenkunde 162, 231-238.

Rühlmann, J., 1999. A new approach to estimating the pool of stable organic matter in soil using data from long-term field experiments. Plant and Soil 213, 149-160.

Sanger, L.J., Cox, P., Splatt, P., Whelan, M.J., Anderson, J.M., 1997. The characterization of a lignin-derived organic matter fraction in soils developed under different vegetation types. Journal of Applied Ecology 34, 14-20.

Schlesinger, W.H., 1991. Biogeochemistry - An Analysis of Global Change. Academic Press, San Diego.

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

Sollins, P., Homann, P., Caldwell, B.A., 1996. Stabilization and destabilization of soil organic matter: mechanisms and controls. Geoderma 74, 65-105.

Trumbore, S.E., 1997. Potential response of soil organic carbon to global environmental change. Proceedings of the National Academy of Science 94, 8284-8291.

Zeikus, J.G., 1981. Lignin metabolism and the carbon cylce. Polymer biosynthesis, biodegradation, and environmental recalcitrance. Advances in Microbial Ecology 5, 211-243.

# 7 Contribution of non-cellulosic polysaccharides to the refractory SOC pool

## Abstract

The objectives of this section are to assess the contribution of non-cellulosic polysaccharides to the refractory SOC pool and to investigate the composition of the refractory polysaccharide fraction. For bulk samples from eight long-term experiments and particle-size fractions from two of the experiments, individual sugars were determined following hydrolysis with trifluoroacetic acid (TFA). OC-normalized contents of polysaccharides (neutral sugars and galacturonic acid) were similar in bulk samples of the C-depleted and fertilized plots. The contrasting treatments showed similar polysaccharide contents especially in separates < 6 µm. As for net amounts, the separates < 6 µm in the C-depleted plots retained between 50 and 100% of the amount in the fertilized plots. Within a particular soil, the fine separates were those with the highest mass ratio of (galactose+mannose)-to-(arabinose+xylose) (GM/AX). Comparing the treatments, the GM/AX ratio was higher in bulk samples of the C-depleted versus the fertilized plots. These results indicate that the refractory C pool has a similar proportion of polysaccharides as the labile C pool. Refractory polysaccharides may be mainly associated with fine separates and show a dominant contribution of microbial sugars. The stable sugar pool is enriched in microbial sugars compared with polysaccharides of the labile C pool. These results provide evidence that polysaccharides, mainly those of microbial origin, are stabilized over the long-term within fine separates of arable soils.

## 7.1 Introduction

Carbohydrates represent an important fraction of soil organic carbon (SOC), whereby in cultivated soils the carbohydrate-C accounts for approximately 10% of total OC on average (Cheshire, 1979). The soil carbohydrate pool is composed of polysaccharides derived from various sources, i.e. plant and animal tissues, plant mucilage, cellular tissues and extracellular products of soil microbes. The presence of carbohydrates in soil has manifold effects on biological, physical and chemical soil properties, reflecting the diverse properties and functions of polysaccharides in soil (Greenland and Oades, 1975; Lowe, 1978). They represent the major carbon and energy source for soil organisms, thus governing biological activity. Polysaccharides, mainly those of microbial origin, act as binding agents between soil particles, thus influencing soil structure / aggregation. Since polysaccharides interact with metal cations, they may also affect soil chemical properties.

Carbohydrates are usually considered to be readily degradable compounds. The non-cellulosic and cellulosic fractions in plant residues are degraded at a much higher rate than lignin for example (e.g. Martin et al., 1980; Martin and Haider, 1986). Microbially synthesized polysaccharides were also found to be attacked rather rapidly, although saccharides of some microbial species may be relatively resistant against decomposition (Martin et al., 1966; Greenland and Oades, 1975). On the other hand, carbohydrates account for a considerable proportion of SOC. Based on model experiments, different mechanisms for sugar stabilization have been proposed (Cheshire, 1977): i) a close association of polysaccharides with mineral colloids, ii) an interaction of polysaccharides with metal ions (Fe, Cu, Zn) through uronic acid and mannose units, iii) an interaction of sugars with tannins. It was proposed that enzymes may show a reduced affinity towards polysaccharide-metal complexes as compared with pure polysaccharides (Martin et al. , 1966). A reduced degradation of polysaccharides in the presence of tannins was explained by a reduced availability of the sugars to enzymes/microbes, and by an enzyme inhibition through tanning, respectively. A further protection against rapid degradation may arise from the special role of polysaccharides as “bridges” between mineral particles, being directly involved in the build-up of stable (micro)-aggregates. The promotion of aggregation occludes the polysaccharides themselves within aggregates and consequently reduces accessibility to decomposer organisms (Oades, 1984; Puget et al., 1999).

By the natural $^{13}$C labelling technique it was shown that particle-size fractions represent different pools of SOC in terms of turnover time (Balesdent, 1996; Christensen, 1996; Feller and Beare, 1997). Organic compounds in sand-sized fractions (particulate organic matter) are turned over rather rapidly, within several years, whereas OC associated with fractions < 50/20 µm shows markedly higher turnover times, thus being involved in the mid- and long-term dynamics of SOC. Balesdent (1996), seeking fractions of SOC that are related to C dynamics, employed both particle-size fractionation and various chemical fractionation procedures (e.g. alkaline extraction, acid treatment). The author concluded that only particle-size fractionation allowed the isolation of SOC fractions with different turnover times.

In the present study, SOC is classified into two major pools according to turnover, namely a *refractory* pool, encompassing compounds with estimated turnover times from hundreds to thousands of years, and a *labile* pool comprising compounds which are turned over within years to several decades. To characterize these OC pools in arable soils, we will compare two contrasting treatments from long-term agroecosystem experiments, i.e. fertilized plots and C-depleted plots. The group of C-depleted plots comprises unmanured plots and bare fallows, both being depleted in total SOC in comparison with the fertilized plots, which receive high doses of inorganic and organic manure (Körschens et al., 1998). The C-depleted plots are mainly depleted in labile compounds compared with the fertilized plots, while the refractory C pool should not be affected by C-depletion at a time-scale of decades (Rühlmann, 1999). Therefore, the SOC pool of the C-depleted plots is relatively enriched in refractory compounds compared with the SOC of the fertilized counterparts.

The objectives of this paper are (i) to assess the contribution of non-cellulosic polysaccharides to the refractory C pool in arable soils, and (ii) to examine the composition of the refractory polysaccharide fraction. This was achieved by comparing the polysaccharide signature of C-depleted plots with that of fertilized plots of long-term agroecosystem experiments.

## 7.2 Materials and methods

### 7.2.1 Long-term experimental sites and soil sampling

We obtained soil samples from eight long-term agroecosystem experiments located in central and eastern Europe (see Table 1, chapter 6.2.1). The study sites cover a range of climatic features and soil types. Samples were obtained from contrasting experimental plots of the long-term field trials: a) Fertilized plots, with the combined addition of mineral and organic fertilization, and b) C-depleted plots, either unmanured plots with the same crop rotation as in the fertilized treatment, but without any fertilization, or bare fallows. Except for the bare fallows, all treatments were replicated at least three-fold on separate plots arranged randomly within the experimental station. Soil samples were taken in summer 1998 from a depth 0-20 cm. Soil material collected at ten sampling points on each of the replication plots of a particular treatment was mixed. Mixed samples were air-dried and components > 2 mm were subsequently removed by dry sieving. For elemental analysis and determination of polysaccharides in the bulk soil samples, an aliquot of the sieved material was ground by a ball mill.

### 7.2.2 Particle-size fractionation (soils from Thyrow and Bad Lauchstädt)

Soil samples were dispersed ultrasonically in two steps, involving a first step of low-energy input (60 J $ml^{-1}$) followed by high-energy sonification (440 J $ml^{-1}$) (Amelung et al., 1998). Soil suspensions were sonificated using a probe-type ultrasonic disintegrator (Branson 250, Branson). After the first dispersion step, primary particles 2000-250 µm (coarse and medium sand) including particulate organic matter (OM) were removed by wet sieving. In this manner, a physical disruption of particulate OM, which is likely to occur at energy levels > 60 J $ml^{-1}$, was avoided (Amelung and Zech, 1999). After treating the suspensions < 250 µm at 440 J $ml^{-1}$, separation of the size fractions was accomplished by a combination of several methods: i) wet sieving, yielding the fractions 250-63 (fine sand) and 63-20 µm (coarse silt); ii) SPLITT-fractionation (Keil et al., 1994) for isolating the fractions 20-6 (medium silt), 6-2 (fine silt) and 2-0.2 µm (coarse clay); iii) centrifugation for isolating particles < 0.2 µm (fine clay). All fractions were freeze-dried. Aliquots were ground in a ball mill prior to elemental analysis and determination of polysaccharides.

### 7.2.3 Density fractionation

Prior to polysaccharide determination, the separates 2000-250 and 250-63 µm of the C-depleted soil from Thyrow were subjected to density fractionation using sodium polytungstate solution [$Na_6(H_2W_{12}O_{40}) \times H_2O$, Sometu, Berlin, Germany] of a density 1.8 g $cm^{-3}$. OC contents in these separates were very low (0.5 to 0.6 g $kg^{-1}$ fraction mass), and thus high weights would be necessary to reach concentrations which are above the limit of determination. Density fractionation allowed the organic matter to be concentrated in the light fraction (≤ 1.8 g $cm^{-3}$). The light material, which represented approximately 90% of the separates` total OC, was then analyzed for polysaccharides.

### 7.2.4 Carbon analysis

Total carbon in bulk samples, particle-size and density fractions was determined by dry combustion in a Vario EL elemental analyzer (Elementar Analysen-Systeme, Hanau, Germany). Inorganic carbon was determined by elemental analysis of subsamples after ignition at 550°C for 3 h (removal of organic matter). The samples did not contain any inorganic carbon.

Values of total OC in bulk samples of the depleted plots were related to those of the corresponding fertilized plots:

$$\frac{OC_{\text{depleted}} \times 100}{OC_{\text{fertilized}}} = OC_{\text{residual}} \qquad (1)$$

| | |
|---|---|
| $OC_{\text{depleted}}$ | OC amount in bulk samples from depleted plots (g $kg^{-1}$ soil) |
| $OC_{\text{fertilized}}$ | OC amount in bulk samples from fertilized plots (g $kg^{-1}$ soil) |

| | |
|---|---|
| OC $_{\text{residual}}$ | Residual OC in depleted plots (% of fertilized plot) |

### 7.2.5 Determination of neutral sugars and uronic acids

Neutral sugars and uronic acids from non-cellulosic carbohydrates were analyzed according to the method proposed by Amelung et al. (1996). The procedure involved hydrolysis of samples with 4 M trifluoroacetic acid (TFA), clean-up of the hydrolyzates by using a combination of cation exchange and XAD-7 resins, and gas chromatographic (GC) analysis of the monomers after a two-step derivatization. 50 mg of plant material (cereal straw, potato foliage) / light fraction material (see section 7.2.3) or 50-500 mg of soil sample (bulk samples, particle-size fractions) were hydrolyzed with 10 ml 4 M TFA at 105°C for 4 h. After filtration, the hydrolyzate was dried in a rotary evaporator and then re-dissolved in 3 ml deionized water. The solution was successively passed through two glass columns filled with XAD-7 adsorption resin (Sigma-Aldrich) and with a strongly acidic cation exchange resin (Merck). The effluent from the 2nd column was collected and freeze-dried. The dried sample was dissolved in N-methyl-pyrrolidone (NMP), which contained O-methylglucose as an internal standard for GC quantification. The sugar monomers were transformed into O-methyl-oximes by reaction of the carbonyl group with O-methylhydroxylaminehydrochloride (75°C for 30 min). Finally, BSTFA [bis-(trimethylsilyl)-trifluoroacetamide] was added for silylation of the hydroxyl groups (75°C for 5 min). Gas chromatographic analysis was performed on a GC 8000 series gas chromatograph (Fisons Instruments) equipped with a flame ionization detector (FID), using the following conditions:

i) fused silica capillary column, stationary phase with 5% diphenyl- and 95% dimethylsiloxane, 30 m length;
ii) split injection (split ratio 1:20), injector temperature 250°C; FID temperature 300°C;
iii) temperature program: 160°C for 4 min, ramp of 8°C min$^{-1}$ up to 185°C, 185 °C for 5 min, ramp of 2°C min$^{-1}$ up to 220°C, 220°C for 3 min;
iv) Helium as carrier gas, with a constant pressure of 80 kPa.

The following sugar monomers were analyzed and the sum reported as *neutral sugars*: D (-) arabinose, D (+) xylose, D (-) ribose, D (+) glucose, D (+) galactose, D (+) mannose, the deoxyhexoses L (+) rhamnose and L (-) fucose. Concentrations of D (-) fructose were found to be below the limit of determination in almost all samples and are therefore not reported. To assess the proportion of microbially-derived sugars in relation to plant sugars, mass ratios of hexoses/deoxyhexoses-to-pentoses were calculated for bulk soils and particle-size fractions:

[(galactose + mannose) / (arabinose + xylose)] (GM/AX) and [(rhamnose + fucose) / (arabinose + xylose)] (RF/AX).

We measured the two *uronic acids* commonly found in soils, D (-) galacturonic and D (+) glucuronic acid. Results for D (+) glucuronic acid in most samples were not reproducible and the concentrations were often below the limit of determination. Data for glucuronic acid are therefore not reported.

Data for *non-cellulosic polysaccharide-C* were derived from the sum of neutral sugar-C and galacturonic acid-C, assuming a C content of 40% for neutral sugars and 37% for galacturonic acid:

$$\text{Polysaccharide-C} = \text{neutral sugar-C} + \text{galacturonic acid-C} \qquad (2)$$

Analyses were done in triplicate for bulk samples and in duplicate for particle-size fractions / plant materials. The mean relative standard deviation was 7% for the sum of neutral sugars and 12% for galacturonic acid (mean for all analyzed samples). The mean recovery in particle-size fractions relative to the amounts in bulk samples was 94% ± 14% for the sum of neutral sugars and 68% ± 7% for galacturonic acid.

The C-depleted plots were compared with the fertilized counterparts with regard to OC-normalized contents of polysaccharides (neutral sugars / galacturonic acid g $kg^{-1}$ OC). Moreover, amounts of polysaccharide-C (normalized to soil mass, polysaccharide-C g $kg^{-1}$ soil) left in the C-depleted plots were related to the respective amounts in the fertilized plots:

$$\frac{\text{Polysaccharide-C}_{\text{depleted}} \times 100}{\text{Polysaccharide-C}_{\text{fertilized}}} = \text{Polysaccharide-C}_{\text{residual}} \qquad (3)$$

| | |
|---|---|
| Polysaccharide-C $_{\text{depleted}}$ | Amount of polysaccharide-C in depleted plots (g $kg^{-1}$ soil) |
| Polysaccharide-C $_{\text{fertilized}}$ | Amount of polysaccharide-C in fertilized plots (g $kg^{-1}$ soil) |
| Polysaccharide-C $_{\text{residual}}$ | Residual amount of polysaccharide-C in depleted plots<br>(% of fertilized plot) |

Equation 2 was applied to data from bulk soil samples and individual particle-size fractions.

For particle-size fractions of both treatments, net amounts of polysaccharide-C were calculated by multiplying the polysaccharide-C content in a given fraction with the mass proportion of that fraction:

$$\frac{\text{Polysaccharide-C}_{\text{fraction}} \times \text{Mass}_{\text{fraction}}}{100} = \text{Polysaccharide-C} \qquad (4)$$

| | |
|---|---|
| Polysaccharide-C $_{\text{fraction}}$ | Content of polysaccharide-C for a given particle-size fraction (g $\text{kg}^{-1}$ fraction) |
| Mass $_{\text{fraction}}$ | Mass proportion of that fraction (% of soil mass) |
| Polysaccharide-C | Amount of polysaccharide-C in a given particle-size fraction (g $\text{kg}^{-1}$ soil) |

### 7.2.6 Determination of specific surface area (SSA)

Surface area was measured in separates < 6 µm after oxidation of the organic matter with peroxodisulfate at 80°C for 16 h (Meier and Menegatti, 1997). The freeze-dried, OM-free fractions were outgassed at 70°C for 23 h and then analyzed by $N_2$ adsorption (77 K) using an Autosorb 1 surface area analyzer (Quantachrome Corporation). Surface area values were obtained by using the multi-point BET approach.

### 7.2.7 Statistics

Differences between the C-depleted and fertilized plots with respect to polysaccharide contents and hexoses-to-pentoses ratios in bulk samples were tested by means of the *t*-test for paired samples (Statistica 5.1, Statsoft).

## 7.3 Results and discussion

### 7.3.1 Contents of polysaccharides

#### *7.3.1.1 Bulk samples*

In most of the eight experiments, OC-normalized contents of neutral sugars and galacturonic acid in the depleted plots were similar to those in the fertilized plots (Table 1a). On average, the contrasting treatments were not statistically different in these contents. The net amounts of polysaccharide-C declined over the course of C-depletion, with relative residues from 41 to

**Table 1a:** Contents of neutral sugars and galacturonic acid in bulk samples derived from the contrasting treatments of long-term agroecosystem experiments.

| | **Σ Neutral sugars** ($g\ kg^{-1}$ OC) | | | **Galacturonic acid** ($g\ kg^{-1}$ OC) | | |
|---|---|---|---|---|---|---|
| **Experimental site** | **Fertilized** | **Depleted** | **Δ (Depl. - Fert.)** | **Fertilized** | **Depleted** | **Δ (Depl. - Fert.)** |
| Thyrow | 107 | 166 | + 59 | 4.0 | 5.1 | + 1.1 |
| Groß Kreutz | 190 | 197 | + 6 | 5.5 | 4.6 | - 0.9 |
| Skierniewice | 163 | 141 | - 22 | 5.0 | 4.7 | - 0.2 |
| Puch | 205 | 159 | - 45 | 6.0 | 6.3 | + 0.3 |
| Lauterbach | 195 | 195 | 0 | 4.1 | 5.6 | + 1.4 |
| Bad Lauchstädt | | | | | | |
| *Experiment A* | | | | | | |
| Unmanured plot | 116 | 104 | - 12 | 4.5 | 4.0 | - 0.5 |
| Bare fallow | | 99 | - 17 | | 5.1 | + 0.6 |
| *Experiment B* | 124 | 151 | + 27 | 5.2 | 3.9 | - 1.3 |
| Prague Bare fallow | 149 | 169 | + 20 | 4.0 | 3.2 | - 0.8 |
| Bare fallow + tillage | | 153 | + 4 | | 3.3 | - 0.7 |
| Mean ± SD (*n*=8 experiments) | 156 ± 36 | 159 ± 28 | + 3 ± 30 NS | 4.8 ± 0.7 | 4.8 ± 0.9 | 0.0 ± 0.9 NS |

NS Difference between the two treatments not significant (paired *t*-test)

**Table 1b:** Amounts of polysaccharide-C and total OC in bulk samples derived from the contrasting treatments of long-term agroecosystem experiments.

| | **Polysaccharide-C** [a] | | | **Total OC** | | |
|---|---|---|---|---|---|---|
| | **Fertilized** | **Depleted** | **Residual amounts** [b] | **Fertilized** | **Depleted** | **Residual amounts** [c] |
| Experimental site | (g $kg^{-1}$ soil) | | (% of fertilized plot) | (g $kg^{-1}$ soil) | | (% of fertilized plot) |
| Thyrow | 0.30 | 0.22 | 73 | 6.8 | 3.2 | 47 |
| Groß Kreutz | 0.81 | 0.33 | 41 | 10 | 4.1 | 40 |
| Skierniewice | 0.59 | 0.26 | 44 | 8.8 | 4.4 | 51 |
| Puch | 1.01 | 0.46 | 46 | 12 | 7.0 | 58 |
| Lauterbach | 3.83 | 2.40 | 63 | 48 | 30 | 62 |
| Bad Lauchstädt | | | | | | |
| *Experiment A* | | | | | | |
| Unmanured plot | 1.15 | 0.69 | 60 | 24 | 16 | 67 |
| Bare fallow | | 0.62 | 54 | | 15 | 62 |
| *Experiment B* | 2.13 | 1.21 | 57 | 41 | 20 | 48 |
| Prague Bare fallow | 1.77 | 1.00 | 56 | 29 | 14 | 50 |
| Bare fallow + tillage | | 0.80 | 45 | | 13 | 44 |
| Mean ± SD (*n*=8 experiments) | | | 54 ± 10 | | | 52 ± 8 |

[a] see equation 2

[b] Residual amounts of polysaccharide-C in the depleted plots (equation 3)

[c] Residual amounts of total OC in the depleted plots (equation 1)

73% in the depleted plots (Table 1b). The net decline of polysaccharide-C approximately paralled the decrease of total OC, with mean residues of 54 and 52% for polysaccharide-C and total OC, respectively (Table 1b). Due to this parallel change, similar polysaccharide contents (relative to total OC) were found in the C-depleted and fertilized plots (Table 1a).

The finding that the concentration of polysaccharides relative to total OC remained quite constant for a given site, even if the total SOC was subject to a large decrease, agrees with previous observations (Oades, 1967; Dalal and Henry, 1988). In the latter study, carbohydrates declined to a similar extent as the bulk OC upon cultivation of virgin soils. In a four- and ten-year field experiment, non-cellulosic polysaccharide contents in plots with organic amendments were not essentially different from those in unmanured plots (Leifeld, 1998).

### *7.3.1.2 Particle-size fractions (Thyrow and Bad Lauchstädt)*

#### *7.3.1.2.1 OC-normalized contents of polysaccharides*

Figs. 1a and 1b show the neutral sugar and galacturonic acid contents in size separates for the sandy soil from Thyrow and the loamy soil from Bad Lauchstädt. Galacturonic acid accounted for 2 to 5% of neutral sugar contents in the separates of the various samples. Comparing polysaccharide contents (neutral sugars and galacturonic acid) in the contrasting treatments the following trends were found in size separates: i) OC in the coarse sand fraction (2000-250 µm) was enriched in polysaccharides in the depleted plots relative to the fertilized plots. As OM in this fraction mainly originates from recent organic input (Christensen, 1996), these differences may be related to different compositions of the input material in the two treatments. The contents of neutral sugars and galacturonic acid in this fraction were in the range of values for the analyzed plant materials (Figs. 1a and 1b). ii) Polysaccharide contents in fractions 250-6 µm decreased under the conditions of the C-depleted plots. For Bad Lauchstädt, contents successively declined from the fertilized plot to the unmanured plot to the bare fallow. iii) As opposed to the separates 250-6 µm, the fine fractions (< 6 µm) of the depleted plots maintained polysaccharide contents similar to those in the fertilized plots.

In bulk samples of the depleted plots, OC-normalized polysaccharide contents were similar to those in the fertilized plots (Table 1a). The results on particle-size fractions suggest that the fine separates were largely responsible for this similarity. The pattern of neutral sugar concentrations in the range of fractions in this study is in line with the results from other soils. This is especially valid regarding the relatively high contents in fractions < 2 µm (Oades et al., 1987; Guggenberger et al., 1994) and the relative depletion of sugars for OC in fractions 250-20 µm (Amelung et al., 1999).

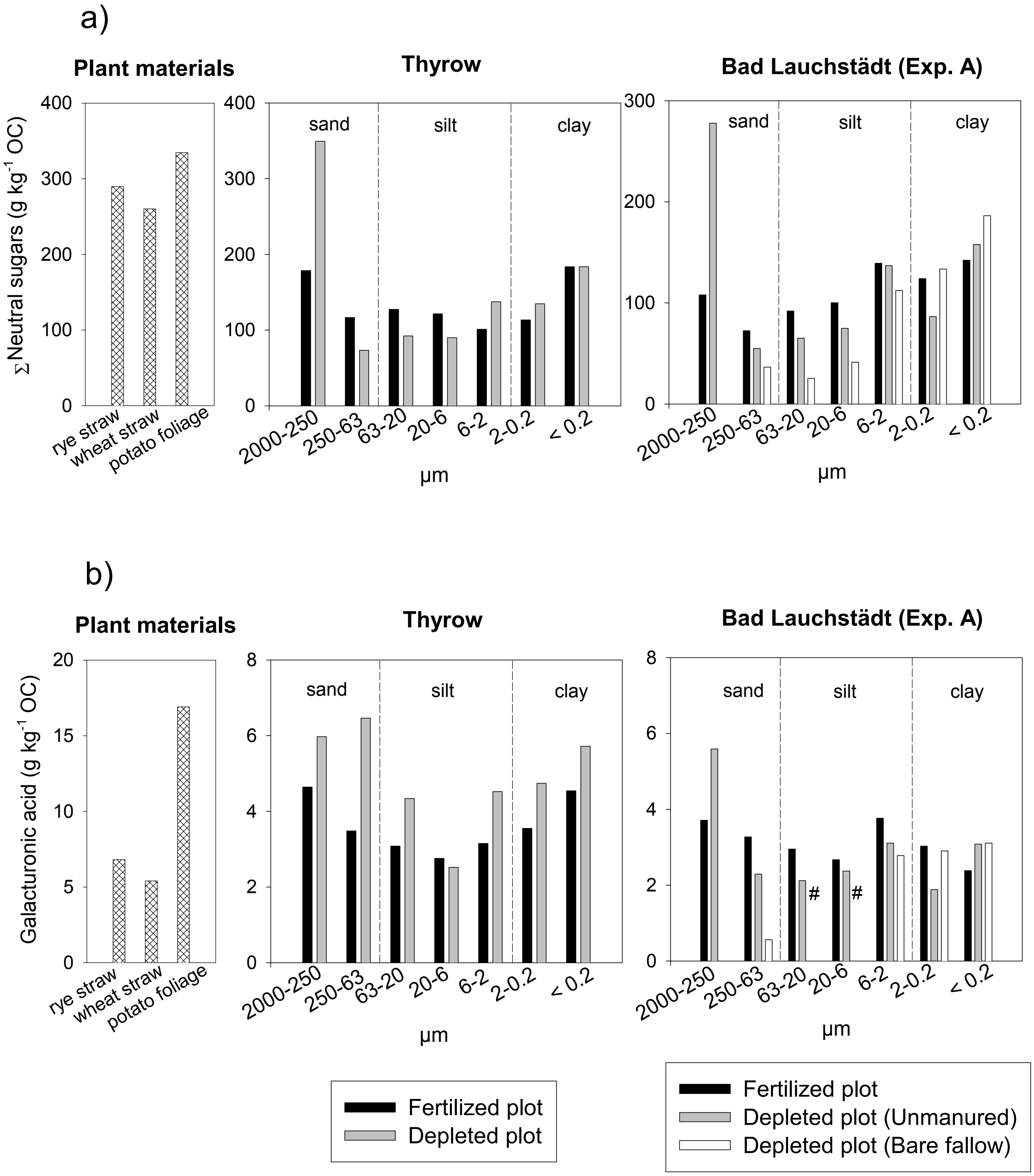


**Fig. 1:** OC-normalized contents of neutral sugars (a) and galacturonic acid (b) in particle-size fractions of the contrasting treatments from Thyrow and Bad Lauchstädt.
(#: Galacturonic acid concentrations in the bare fallow (Bad Lauchstädt) below the limit of determination)

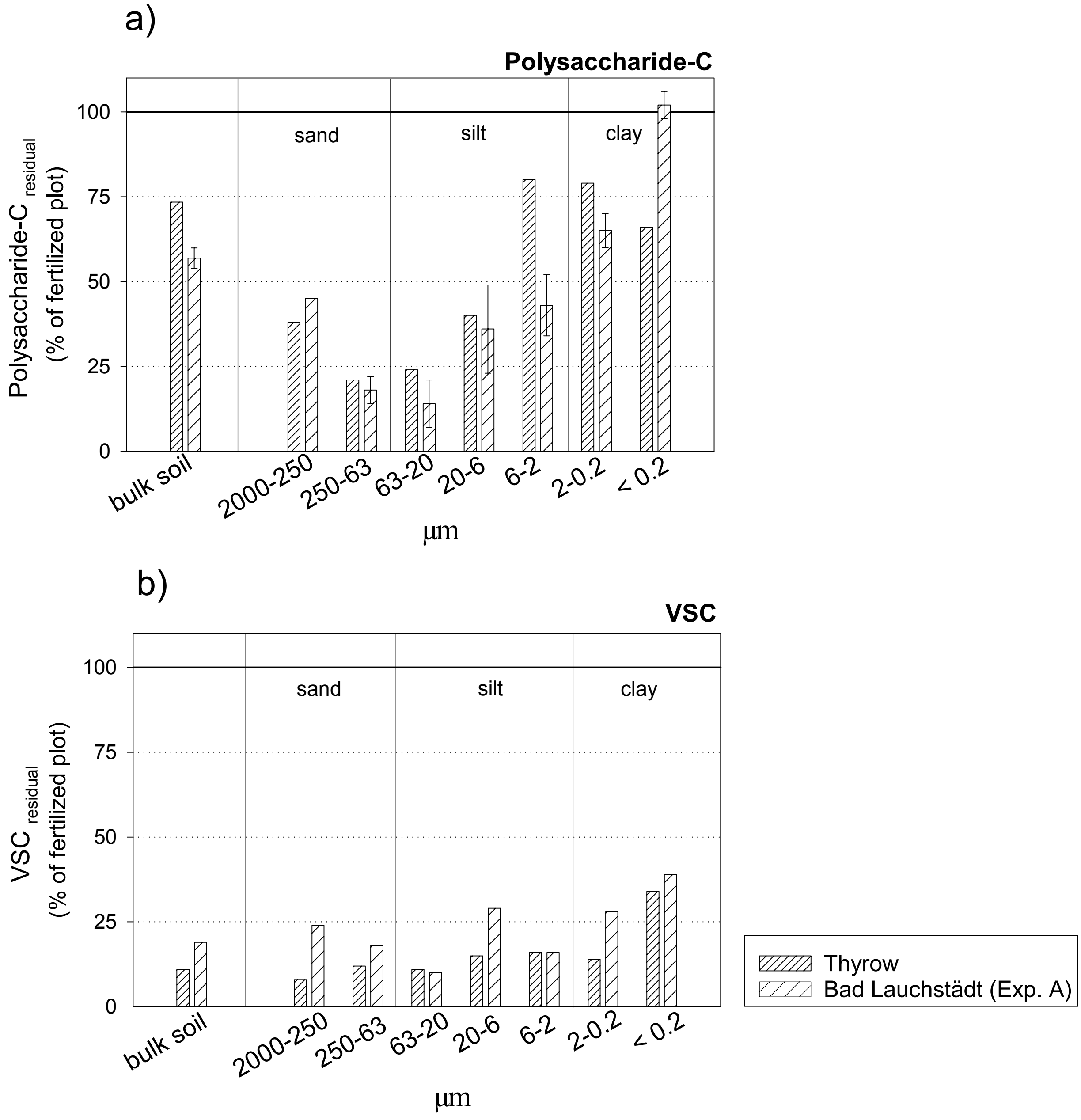


**Fig. 2:** Residual amounts of polysaccharide-C (a) and lignin phenols (b) in bulk samples and particle-size fractions of the C-depleted plots from Thyrow and Bad Lauchstädt.
For polysaccharide-C, the mean percentage for the unmanured soil and the bare fallow from Bad Lauchstädt is represented, together with the range covered by the individual values (error bars).

### *7.3.1.2.2 Amounts of polysaccharides and polysaccharide distribution*

The amount of polysaccharide-C remaining in a given fraction of the depleted plots was expressed as a percentage of the amount in the same fraction of the fertilized counterparts (see equations 2, 3 and 4) (Fig. 2a). For both sites, residual polysaccharide-C was lowest in fractions 250-20 µm and reached highest values in fine fractions (< 6 µm). For the loamy soil

(Bad Lauchstädt), residual polysaccharide-C gradually increased in fractions < 20 µm and reached a maximum in the fine clay.

The sandy soil from Thyrow and the loamy soil from Bad Lauchstädt differed in their polysaccharide distribution pattern among the separates (Table 2). The sandy soil generally showed a higher contribution from coarse separates (2000-63 µm) compared with the loamy soil. For both soils, however, the distribution was shifted towards finer separates as a consequence of C-depletion: In the depleted plots a higher percentage of total polysaccharides was associated with fractions < 6 µm (Thyrow) and < 2 µm (Bad Lauchstädt).

Upon cultivation of native sites, the net decrease of carbohydrates was least for the clay (< 2 µm), as an average trend for three study sites (Dalal and Henry, 1988). This lower decrease in the clay (compared with coarser separates) in a process of SOC-depletion is in line with the behavior of polysaccharides in separates of our arable soils.

**Table 2:** Distribution of polysaccharides among the size fractions in the contrasting treatments from Thyrow and Bad Lauchstädt (Experiment A).

| | **Polysaccharide-C** (% of total polysaccharide-C) | | | |
|---|---|---|---|---|
| | **Thyrow** | | **Bad Lauchstädt** | |
| **Particle-size fractions** (µm) | **Fertilized** | **Depleted** | **Fertilized** | **Depleted** [a] |
| 2000 - 63 | 32 | 21 | 7 | 1 |
| 63 - 6 | 21 | 13 | 14 | 4 |
| 6 - 2 | 17 | 24 | 29 | 19 |
| 2 – 0.2 | 22 | 26 | 30 | 39 |
| < 0.2 | 9 | 17 | 21 | 38 |

[a] Bare fallow

### *7.3.1.2.3 Polysaccharides versus mineral surface area*

One mechanism responsible for stabilizing readily degradable compounds such as carbohydrates in soil is the interaction of these compounds with mineral surfaces (Lynch and Cotnoir, 1956; Baldock and Skjemstad, 2000). To assess whether this mechanism is important for non-cellulosic sugars in the soils from Thyrow and Bad Lauchstädt, polysaccharide-C contents (normalized to fraction mass) were analyzed in relation to specific mineral surface area in fine separates (Figs. 3a and 3b). For the sandy soil (Thyrow), polysaccharide-C increased in proportion to mineral surface area (SA) in both treatments (Fig. 3a). This relationship in separates < 6 µm indicates that mineral SA is a key variable for polysaccharide storage in the sandy soil. For the loamy soil (Bad Lauchstädt), polysaccharide-C was positively related to mineral SA only in the bare fallow (Fig. 3b). The SOC pool of long-term bare fallows should mainly comprise refractory compounds (Rühlmann, 1999). The data on the bare fallow suggest that mineral SA controls the accumulation of refractory carbohydrates in this soil. However, SA may not be the most relevant factor determining polysaccharide concentrations in the fertilized and unmanured plots. Although Thyrow and Bad Lauchstädt represent different soil types, they agree with respect to the polysaccharide-SA relationship in fine separates of the C-depleted plots. This relation suggests that for both soils a (long-term) stabilization of sugars is largely due to an interaction of sugars with mineral surfaces.

A positive correlation between mineral SA and carbohydrate concentration was also reported for particle-size fractions of coastal sediments (Bergamaschi et al., 1997). Model experiments showed that polysaccharides of various origin interact with both external and internal surfaces of clay minerals (Cheshire, 1979; Cheshire and Hayes, 1990; Chenu, 1993). Electron microscopic investigations of soils demonstrated that mineral particles/clay plates do interact with plant mucilage as well as bacterial cells, fungal hyphae and their extracellular polysaccharides (Oades, 1984). There may be different reasons for polysaccharide stabilization provided by minerals. A general hypothesis which explains a surface area-controlled stabilization of OC is the enzyme size exclusion model proposed by Mayer (1994). According to this, polysaccharides would be localized within the small intraparticle pores of minerals and therein be protected against the attack of enzymes, which are too large to enter the pores. On the other hand, soil micrographs showed that microbial cells and associated mucilages may be coated by fine mineral particles which are attached to the mucilage (Oades, 1984). In this case, microbes and their extracellular compounds may be protected against degradation due to an encapsulation by mineral particles.

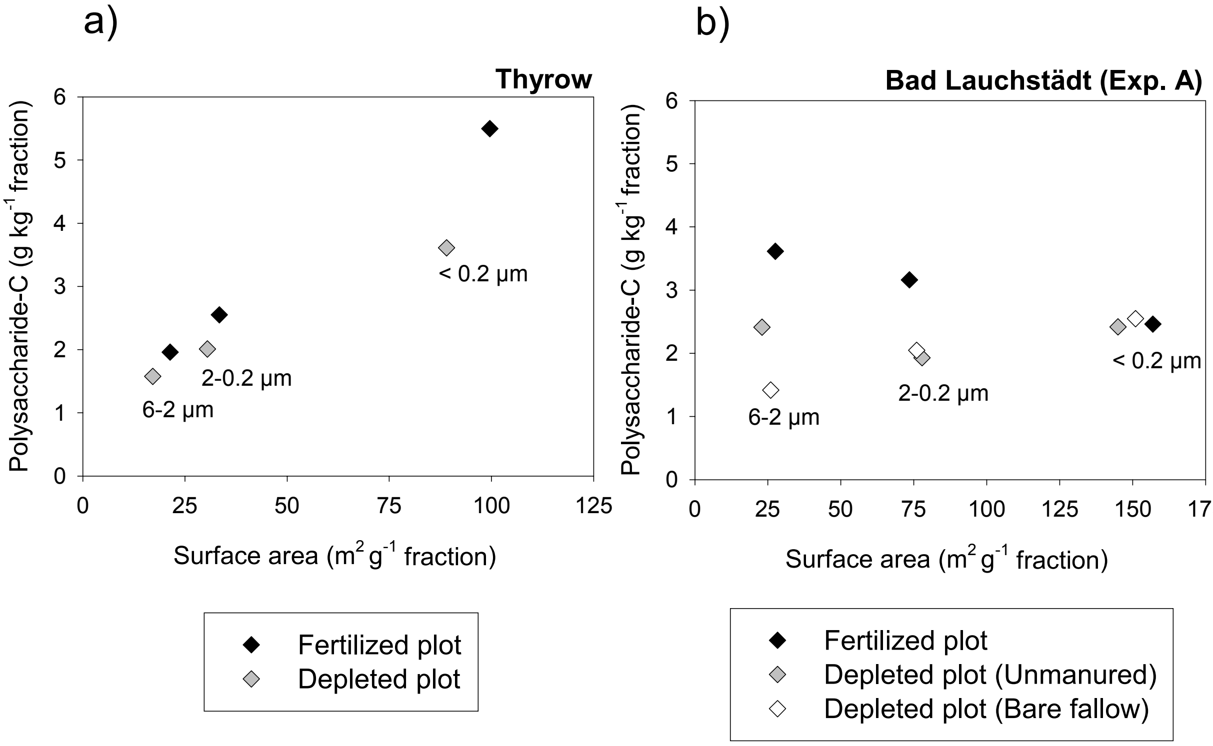


**Fig. 3:** Polysaccharide-C versus mineral surface area in fractions < 6 µm of the contrasting treatments from Thyrow (a) and Bad Lauchstädt (b).

### 7.3.2 Mass ratios of hexoses-to-pentoses

#### *7.3.2.1 Bulk samples*

For nearly all experiments, the mass ratio of (galactose+mannose)-to-(arabinose+xylose) (GM/AX) in bulk samples of the depleted plots was elevated compared with the corresponding fertilized plots (Table 3). On average, GM/AX ratios were significantly higher in the group of depleted plots. For the mass ratio of (rhamnose+fucose)-to-(arabinose+xylose) (RF/AX), there was no consistent trend among the study sites when comparing the C-depleted with the fertilized plots (Table 3). Since the RF/AX ratios in both groups did not fit a normal distribution, variations in this ratio between the two treatments were not further evaluated statistically.

Microbial sugars were reported to show (distinctly) higher GM/AX and RF/AX ratios in comparison with sugars in plant tissues, e.g. in different types of straw (Cheshire, 1979; Murayama, 1984; Oades, 1984). Provided that sugars from microbes and plants show clearly different ratios, the relative contribution of microbial and plant sugars to the total sugar pool may be estimated from the hexoses-to-pentoses ratio in a soil sample. Furthermore, a relative change in the bulk ratio may indicate a shift in the proportion of microbial relative to plant

**Table 3:** Mass ratios of hexoses-to-pentoses in bulk samples derived from the contrasting treatments of long-term agroecosystem experiments.

| | **GM / AX** [a] | | | **RF / AX** [b] | | |
|---|---|---|---|---|---|---|
| **Experimental site** | **Fertilized** | **Depleted** | **Δ (Depl. - Fert.)** | **Fertilized** | **Depleted** | **Δ (Depl. - Fert.)** |
| Thyrow | 0.86 | 1.33 | + 0.47 | 0.54 | 0.57 | + 0.03 |
| Groß Kreutz | 0.74 | 1.00 | + 0.27 | 0.41 | 0.44 | + 0.03 |
| Skierniewice | 0.90 | 1.05 | + 0.15 | 0.38 | 0.54 | + 0.16 |
| Puch | 0.93 | 1.41 | + 0.48 | 0.45 | 0.68 | + 0.23 |
| Lauterbach | 0.87 | 0.81 | - 0.06 | 0.55 | 1.28 | + 0.74 |
| Bad Lauchstädt | | | | | | |
| *Experiment A* | | | | | | |
| Unmanured plot | 1.07 | 1.17 | + 0.10 | 0.59 | 0.61 | + 0.02 |
| Bare fallow | | 1.21 | + 0.14 | | 0.54 | - 0.05 |
| *Experiment B* | 0.86 | 1.16 | + 0.30 | 0.52 | 0.45 | - 0.06 |
| Prague Bare fallow | 0.90 | 1.15 | + 0.24 | 1.03 | 0.69 | - 0.34 |
| Bare fallow + tillage | | 0.98 | + 0.07 | | 1.06 | + 0.03 |
| Mean ± SD (*n*=8 experiments) | 0.89 ± 0.09 | 1.13 ± 0.18 | + 0.24 ± 0.17 ** | 0.56 ± 0.19 | 0.68 ± 0.26 | + 0.12 ± 0.26 |

[a] [(galactose + mannose)/(arabinose + xylose)]

[b] [(rhamnose + fucose)/(arabinose + xylose)]

** Difference between the two treatments significant at the 0.01 probability level (paired *t*-test) sugars within the soil carbohydrate pool. In the present study, the comparison of the ratios between the contrasting treatments is based on the following assumptions: i) The microbial sugar pool shows markedly higher ratios of hexoses-to-pentoses than polysaccharides in plant tissues. ii) The ratios of plant polysaccharides in the organic input materials are similar for the two treatments at a site; the composition of microbial sugars is also similar for the two treatments. Under these assumptions, the significant increase of the GM/AX ratio in the depleted plots indicates a higher contribution of microbial sugars to the polysaccharide pool in these plots compared with the fertilized plots. The increased contribution of microbial sugars, as indicated by the GM/AX ratio, however, for most experiments did not result in higher RF/AX ratios in the depleted plots. By contrast, in many studies increases in the proportion of galactose and mannose have been related to an increase in rhamnose and fucose (e.g. Cheshire, 1979; Guggenberger et al., 1994). Our findings from bulk soil samples suggest that the GM/AX ratio is a more sensitive indicator for changes in the contribution of microbial relative to plant sugars than the RF/AX ratio.

#### *7.3.2.2 Particle-size fractions (Thyrow and Bad Lauchstädt)*

The general trend for the soil samples regardless of treatment was the increase of the GM/AX ratio with decreasing particle-size (Fig. 4). While for the sandy soil (Thyrow) this ratio increased steadily from the sand to the fine clay, the loamy soil (Bad Lauchstädt) showed an increase up to the coarse clay (2-0.2 μm), followed by a decrease from the coarse to the fine clay (< 0.2 μm). According to Oades (1984), plant tissues show GM/AX ratios of < 0.5, while microbial sugars are dominated by hexoses and have a GM/AX ratio of > 2. The sand fractions of all samples showed ratios around 0.5, which was close to the values obtained for cereal straw (Fig. 4). This suggests a predominance of carbohydrates from plant tissues in the sand fractions. The increase of the GM/AX ratio with decreasing particle-size indicates an increasing contribution of microbial sugars to the carbohydrate pool for both study sites. Assuming that microbial sugars show a ratio of > 2, the values for the fine fractions generally suggest a high / dominant contribution from microbes to the sugar pool of these separates (Fig. 4). For Bad Lauchstädt, the lower ratio in the fine clay (relative to the coarse clay) indicates a relative accumulation of pentose-rich material, possibly of plant origin.

Comparing the contrasting treatment types, for Bad Lauchstädt a close resemblance in the whole range of fractions is evident, except for higher ratios in fractions 6-0.2 μm of the unmanured soil compared with the fertilized one (Fig. 4). In the Thyrow soil, C-depletion led to higher ratios in fractions < 20 μm, especially 6-0.2 μm. This suggests that the sugar pool of fine-intermediate fractions in the depleted plot is enriched in microbial sugars relative to the fertilized plot.

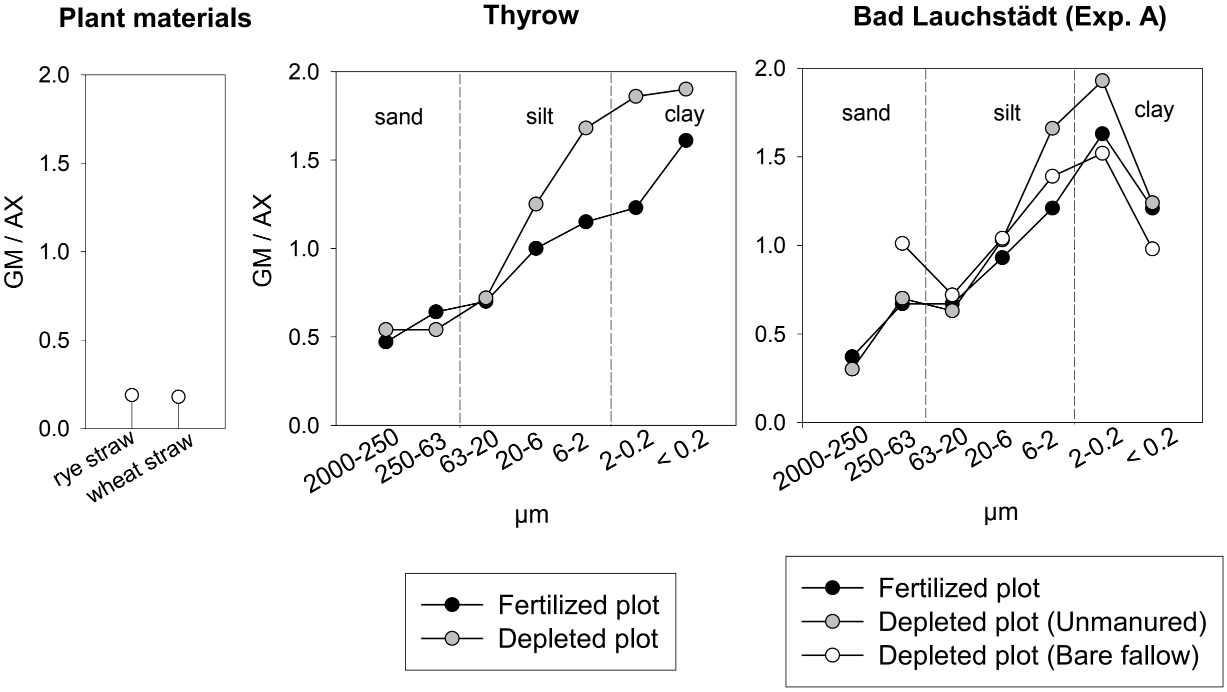


**Fig. 4:** Mass ratios of (galactose+mannose)-to-(arabinose+xylose) (GM/AX) in particle-size fractions of the contrasting treatments from Thyrow and Bad Lauchstädt.

Gradually increasing GM/AX ratios from coarse towards fine fractions has been observed for soils under various land-use, as reviewed by Feller and Beare (1997). These ratios for coarse, intermediate and fine fractions in our arable soils were of a similar magnitude as those reported for comparable size classes in other soils, e.g. under arable rotation and pasture (Guggenberger et al., 1994), under native grassland (Amelung et al., 1999), and under arable land (Cheshire and Mundie, 1981). Since most studies did not further subdivide the clay fraction (< 2 µm), there is no information on sugar composition in coarse subfractions of clay versus the fine clay. Turchenek and Oades (1979), isolating coarse, medium and fine clay from Australian soils, observed a drop of the GM/AX ratio when comparing the coarse clay with finer fractions, similarly to the Bad Lauchstädt soil. In some soils there may be a preferential stabilization of pentose-rich saccharides in fine clay fractions compared with the coarse clay, although this seems to contradict the trend of a steady increase in hexoses with decreasing particle-size.

### 7.3.3 Contribution of polysaccharides to the refractory C pool

The findings on polysaccharide contents in bulk samples from the contrasting treatments indicate that the refractory C pool is characterized by a similar contribution of polysaccharides as the labile C pool. A fraction of soil polysaccharides is thus subject to long-term stabilization. Particle-size fractionation for two of the study sites showed residual amounts of polysaccharides (left in the depleted plots) to be much higher in fine separates (< 6 µm) than in coarser ones. OM in separates 2000-20 µm has been shown to respond rapidly to changes in soil management and to be turned over within years to decades (Carter, 1996; Christensen, 1996). The relative decline of polysaccharides noted in the coarse fractions may thus be directly related to the reduced organic inputs in the depleted versus the fertilized plots. The higher polysaccharide retention in fine fractions indicates that these compounds are stabilized within these fractions. The higher residues together with the shift in polysaccharide distribution towards fine separates (in the course of C-depletion) indicate that refractory polysaccharides are mostly associated with the fine separates. For fractions < 6 µm, polysaccharide contents were found to be related to mineral surface area. This suggests that the interaction of sugars with mineral surfaces in the fine separates is a relevant mechanism for their long-term stabilization.

From the GM/AX ratios it becomes evident that the stable polysaccharide pool, associated with fine separates, consists mostly of microbially-derived sugars. The stable nature of microbial sugars is emphasized by the higher ratios in fractions < 20 µm as a consequence of C-depletion (soil from Thyrow). This increase indicates that microbial sugars were preserved to a higher extent than plant polysaccharides in these fractions, and microbial sugars showing a higher stability. Stabilized polysaccharides are relatively enriched in microbial sugars compared with polysaccharides of the labile C pool, as follows from the higher GM/AX ratios in bulk samples of the C-depleted plots. Microbial resynthesis of sugars in soil appears to be an essential pathway for the formation of stable carbohydrates. According to the present results, the refractory C pool in arable soils does not differ from the labile pool in the relative contribution of non-cellulosic polysaccharides, but it differs in the origin of polysaccharides.

### 7.3.4 Long-term stabilization in soil: polysaccharides *versus* lignin

In the following, non-cellulosic polysaccharides and lignin will be compared with regard to their long-term stability in soil. The effect of C-depletion on polysaccharides and lignin is exemplified by the results from particle-size fractions of two soils (Thyrow and Bad Lauchstädt) (Figs. 2a and 2b). Fig. 2a shows the relative residues for polysaccharide-C (see equations 2, 3 and 4); in Fig. 2b, relative residues for the sum of lignin phenols (VSC = V + S + C; vanillyl- (V), syringyl- (S), cinnamyl units (C)) are given. Residues for lignin phenols were only analyzed for the unmanured plots from the two sites. The major difference between the two compounds is noticeable in the fine fractions: While lignin phenol residues in fine fractions were similar to those in coarse fractions, the residual level of polysaccharides was markedly higher in fine fractions. For polysaccharides, the high residues in fine fractions may largely explain the high residuals in the bulk samples (Fig. 2a). Compared with the residual level of polysaccharides in bulk samples (74% and 56% for Thyrow and Bad Lauchstädt), that of lignin phenols was low (11% and 19% for Thyrow and Bad Lauchstädt) (Figs. 2a and 2b).

As mentioned above, OM in the coarse separates is considered to be a labile pool of SOC, and both lignin and polysaccharides in these separates may show a rapid turnover. Given the higher polysaccharide residues in fine separates compared with the coarse ones, it follows that polysaccharides are stabilized within the former. By contrast, since the residual level of lignin phenols in fine fractions is not essentially higher than in coarse separates, lignin in fine fractions may not be more stable. Lignin does not appear to be stabilized at the long-term in any fraction. Polysaccharides and lignin are thus different from the viewpoint of long-term stability in soil. Our data indicate that sugars, mainly those of microbial origin, are stabilized over the long-term within fine separates. Lignin is considered to be a recalcitrant compound, resisting microbial attack when following the decay of plant residues at the short/mid-term (Paul and Clark, 1989). However, lignin does not seem to be subject to stabilization processes effective over the long-term.

In bulk samples of the C-depleted plots, OC-normalized contents of polysaccharides were similar (see chapter 7.3.1.1), but contents of lignin phenols were significantly lower (chapter 6.3.1.1) compared with the fertilized plots. These OC-normalized results indicate that the two compounds actually differ in their contribution to the refractory C pool: The polysaccharide contribution to the refractory C pool is comparable to the labile C pool, whereas lignin is quantitatively less important within the refractory OC than within labile OC pools.

## 7.4 References

Amelung, W., Zech, W., 1999. Minimisation of organic matter disruption during particle-size fractionation of grassland epipedons. Geoderma 92, 73-85.

Amelung, W., Cheshire, M.V., Guggenberger, G., 1996. Determination of neutral and acidic sugars in soil by capillary gas-liquid chromatography after trifluoroacetic acid hyrdolysis. Soil Biology & Biochemistry 28, 1631-1639.

Amelung, W., Zech, W., Zhang, X., Follett, R.F., Tiessen, H., Knox, E., Flach, K.-W., 1998. Carbon, nitrogen, and sulfur pools in particle-size fractions as influenced by climate. Soil Science Society of America Journal 62, 172-181.

Amelung, W., Flach, K.W., Zech, W., 1999. Neutral and acidic sugars in particle-size fractions as influenced by climate. Soil Science Society of America Journal 63, 865-873.

Baldock, J.A., Skjemstad, J.O., 2000. Role of the mineral matrix and minerals in protecting natural organic materials against decomposition. Organic Geochemistry 31, 697-710.

Balesdent, J., 1996. The significance of organic separates to carbon dynamics and its modelling in some cultivated soils. European Journal of Soil Science 47, 485-493.

Bergamaschi, B.A., Tsamakis, E., Keil, R.G., Eglinton, T.I., Montlucon, D.B., Hedges, J.I., 1997. The effect of grain size and surface area on organic matter, lignin and carbohydrate concentration, and molecular composition in Peru Margin sediments. Geochimica et Cosmochimica Acta 61, 1247-1260.

Carter, M.R., 1996. Analysis of soil organic matter storage in agroecosystems. In: Carter, M.R., Stewart, B.A. (Eds.), Structure and Organic Matter Storage in Agricultural Soils. Advances in Soil Science. CRC Press. pp. 3-8.

Chenu, C., 1993. Clay- or sand-polysaccharide associations as models for the interface between micro-organisms and soil: water related properties and microstructure. Geoderma 56, 143-156.

Cheshire, M.V., 1977. Origin and stability of soil polysaccharide. Journal of Soil Science 28, 1-10.

Cheshire, M.V., 1979. Nature and Origin of Carbohydrates in Soils. Academic Press, London New York.

Cheshire, M.V., Mundie, C.M., 1981. The distribution of labelled sugars in soil particle size fractions as a means of distinguishing plant and microbial carbohydrate residues. Journal of Soil Science 32, 605-618.

Cheshire, M.V., Hayes, M.H.B., 1990. Composition, origins, structures, and reactivities of soil polysaccharides. In: De Boodt, M.F., Hayes, M.H.B., Herbillon, A.J. (Eds.), Soil Colloids and their Associations in Aggregates. NATO ASI Series, Series B: Physics. Plenum Press. pp. 307-336.

Christensen, B.T., 1996. Carbon in primary and secondary organomineral complexes. In: Carter, M.R., Stewart, B.A. (Eds.), Structure and Organic Matter Storage in Agricultural Soils. Advances in Soil Science. CRC Press, Boca Raton. pp. 97-165.

Dalal, R.C., Henry, R.J., 1988. Cultivation effects on carbohydrate contents of soils and soil fractions. Soil Science Society of America Journal 52, 1361-1365.

Feller, C., Beare, M.H., 1997. Physical control of soil organic matter dynamics in the tropics. Geoderma 79, 69-116.

Greenland, D.J., Oades, J.M., 1975. Saccharides. In: Gieseking, J.E. (Ed.), Soil Components - Organic Components. Springer-Verlag, pp. 213-261.

Guggenberger, G., Christensen, B.T., Zech, W., 1994. Land-use effects on the composition of organic matter in particle-size separates of soils: I. Lignin and carbohydrate signature. European Journal of Soil Science 45, 449-458.

Keil, R.G., Tsamakis, E., Fuh, C.B., Giddings, J.C., Hedges, J.I., 1994. Mineralogical and textural controls on the organic composition of coastal marine sediments: Hydrodynamic separation using SPLITT-fractionation. Geochimica et Cosmochimica Acta 58, 879-893.

Körschens, M., Weigel, A., Schulz, E., 1998. Turnover of soil organic matter (SOM) and long-term balances - tools for evaluating sustainable productivity of soils. Zeitschrift für Pflanzenernährung und Bodenkunde 161, 409 - 424.

Leifeld, J., 1998. Einfluß von Kompostanwendung auf den Umsatz der organischen Substanz in Böden. Shaker Verlag, Aachen.

Lowe, L.E., 1978. Carbohydrates in soil. In: Schnitzer, M., Khan, S.U. (Eds.), Soil Organic Matter. Elsevier, Amsterdam. pp. 65-93.

Lynch, D.L., Cotnoir, L.J., 1956. The influence of clay minerals on the breakdown of certain organic substances. Proceedings of the Soil Science Society of America 20, 367-370.

Martin, J.P., Haider, K., 1986. Influence of mineral colloids on turnover rates of soil organic carbon. In: Interactions of Soil Minerals with Natural Organics and Microbes, SSSA Special Publication. pp. 283-303.

Martin, J.P., Ervin, J.O., Shepherd, R.A., 1966. Decomposition of the iron, aluminum, zinc and copper salts or complexes of some microbial and plant polysaccharides in soil. Proceedings of the Soil Science Society of America 30, 196-200.

Martin, J.P., Haider, K., Kassim, G., 1980. Biodegradation and stabilization after 2 years of specific crop, lignin, and polysaccharide carbons in soils. Soil Science Society of America Journal 44, 1250-1255.

Mayer, L.M., 1994. Relationship between mineral surfaces and organic carbon concentrations in soils and sediments. Chemical Geology 114, 347-363.

Meier, L.P., Menegatti, A.P., 1997. A new, efficient, one-step method for the removal of organic matter form clay-containing sediments. Clay Minerals 32, 557-563.

Murayama, S., 1984. Changes in the monosaccharide composition during decomposition of straw under field conditions. Soil Science and Plant Nutrition, 367-381.

Oades, J.M., 1967. Carbohydrates in some Australian soils. Australian Journal of Soil Research 5, 103-115.

Oades, J.M., 1984. Soil organic matter and structural stability: mechanisms and implications for management. Plant and Soil 76, 319-337.

Oades, J.M., Vassallo, A.M., Waters, A.G., Wilson, M.A., 1987. Characterization of organic matter in particle size and density fractions from a red-brown earth by solid-state $^{13}$C N.M.R. Australien Journal of Soil Research 25, 71-82.

Paul, E.A., Clark, F.E., 1989. Soil Microbiology and Biochemistry. Academic Press, San Diego.

Puget, P., Angers, D.A., Chenu, C., 1999. Nature of carbohydrates associated with water-stable aggregates of two cultivated soils. Soil Biology & Biochemistry 31, 55-63.

Rühlmann, J., 1999. A new approach to estimating the pool of stable organic matter in soil using data from long-term field experiments. Plant and Soil 213, 149-160.

Turchenek, L.W., Oades, J.M., 1979. Fractionation of organo-mineral complexes by sedimentation and density techniques. Geoderma 21, 311-343.

# 8 Synthesis: Refractory SOC studied in long-term agroecosystem experiments

This work aimed at characterizing the refractory SOC in arable soils. The main questions addressed here are: the distribution of refractory C in the range of particle-size fractions, its chemical composition, and stabilization mechanisms responsible for its accumulation.

## 8.1 Stability of OC and N in particle-size fractions (chapter 2)

Residual contents of OC / N left in the various fractions of the C-depleted plots - relative to the contents in the fertilized plots - were taken as a measure for OM stability, with highest residuals corresponding to the highest degree of stability among the separates. Coarse and fine separates differed considerably in their residual OM level and consequently in OM stability: Residuals for OC and N in fine separates (< 6 µm) were in most cases higher than in coarser ones, highest values being found in the clay fraction (< 2 µm). From this pattern of residuals and OC distributions among the separates in the C-depleted plots it was concluded that most of the refractory OC is associated with fine separates, primarily with the clay fraction.

## 8.2 Chemical structure of refractory OC

### 8.2.1 SOC composition as revealed by $^{13}$C NMR and contribution of airborne contaminants (chapters 4 and 5)

For four out of seven study sites, an increase of the aromatic C proportion at the expense of the O/N-alkyl C intensity was noted in the C-depleted compared with the fertilized plots (bulk samples from Thyrow, Skierniewice, Bad Lauchstädt, Prague). For bulk samples from the other sites (Groß Kreutz, Lauterbach, Puch), relative signal distributions in the two treatments were rather similar.

Relating the relative proportions of the C-species in the depleted plots to the total SOC content in the fertilized counterparts, a net decline for all the C-species in the course of C-depletion was evident at all sites. Therefore, within bulk samples none of the C-species appeared to be completely inert. However, the extent of decline was different for the species. According to mean values for the seven sites, the losses of C were highest for O/N-alkyl C and smallest for aromatic C. Consequently, the C-species differ in stability, with strongest differences existing between O/N-alkyl and aromatic C (average trend for the seven sites).

For three of the sites (Thyrow, Skierniewice, Bad Lauchstädt), the trends noted for the signal distribution of bulk samples, i.e. the increase in aromatic C, decrease in O/N-alkyl C, similarity in alkyl / carboxyl C, were recorded in an analogous manner for nearly all size separates; however, the shifts in signal distribution were most pronounced in coarse-intermediate separates. Focusing on the soils from Thyrow, Skierniewice, and Bad Lauchstädt, the data from bulk samples ($^{13}$C NMR) and particle-size fractions ($^{13}$C NMR and coal petrographic analysis) indicated as follows:

- The data from bulk samples indicated that the refractory SOC as a whole is relatively enriched in aromatic C, but depleted in O/N-alkyl C compared with more labile OC pools.
- In coarse-intermediate separates (2000-20 μm), the considerable increase of aromatic C intensities with C-depletion was attributed to an accumulation of airborne carbonaceous particles (coal, coke, char) relative to plant litter compounds. It was concluded that these highly aromatic contaminants represent a refractory C compartment in separates 2000-20 μm.
- Fine separates (< 6 μm) generally showed the highest stability of OC, as mentioned before. Within these separates, aromatic C appeared to be the most stable C-species (especially Thyrow and Bad Lauchstädt). The combination of conventional and dipolar dephasing NMR data suggested that only in the loamy soil (Bad Lauchstädt) part of aromatic C is present in contaminants and/or pyrogenic C, whereas in the sandy soils (Thyrow, Skierniewice) there may be no essential contribution of such compounds, and thus native SOM dominates within the fine separates.
- For the 20-6 μm fraction, conventional $^{13}$C NMR spectra showed a relatively high aromaticity at all sites. Since fractions in the range 2000-20 μm were proven to contain airborne contaminants, it may be hypothesized that such compounds are also present in the 20-6 μm fraction. Other studies showed that contamination of a soil with airborne particles can affect a broad range of particle-size classes (e.g. Rumpel et al., 1998).

This work points out that atmospheric input of carbonaceous particles can provide a substantial contribution to the refractory OC of soils in the industrialized central/eastern European region. At the study sites, this contribution of contaminants apparently is one of the reasons for the finding that the refractory SOC pool is enriched in aromatic C.

### 8.2.2 Contribution of lignin and polysaccharides (chapters 6 and 7)

Contents of lignin phenols (normalized to total OC) were lower in bulk samples of the C-depleted plots compared with the fertilized ones for all study sites. Contents were lower in the whole range of separates, including the fine ones. The lignin in the C-depleted plots exhibited a higher degree of oxidative alteration, indicating a higher extent of lignin biodegradation than in the fertilized plots. This work shows that the lignin detected by CuO oxidation, with an intact substitution pattern of phenol units, does not provide a significant contribution to the refractory SOC pool. Although lignin represents a recalcitrant fraction in plant litter, it is obviously not subject to a pronounced long-term stabilization. It can, however, not be excluded that intermediates of lignin degradation are stabilized in soil.

The MBTH-method showed that the polysaccharide fraction in both treatments is dominated by non-cellulosic compounds. OC-normalized contents of total polysaccharides (cellulosic and non-cellulosic) were similar in bulk samples of the contrasting treatments, mainly due to the similarity in non-cellulosic polysaccharides. Cellulose contents were generally low; in the depleted plots, contents further declined (below the limit of detection in several experiments). The similarity in non-cellulosic sugars was confirmed by the analysis of individual sugars. The results on non-cellulosic polysaccharides obtained by the two methods (MBTH versus individual sugars) were found to be correlated ($n = 18$, $r = 0.56$; $P < 0.05$). Analysis of non-cellulosic sugars (individual sugars) in particle-size fractions showed that residual amounts were highest in fine separates of the C-depleted plots; from hexoses-to-pentoses ratios it was inferred that the sugar pool in fine separates has a high / dominant contribution of microbial sugars. These findings indicated as follows: i) The concentration of non-cellulosic polysaccharides within the refractory C pool is similar to labile C pools; long-term stabilization of soil polysaccharides is thus quantitatively more important than the stabilization of lignin. ii) Refractory polysaccharides are mostly associated with fine separates, analogous to the total refractory C, and are predominantly of microbial origin.

In summary, lignin and polysaccharide data indicate that the refractory C pool in arable soils is relatively depleted in plant compounds (primary resources, i.e. lignin, cellulose, plant hemicelluloses) compared with labile C pools. On the other hand, the data on polysaccharides suggest that microbially-derived compounds (secondary resources) are quantitatively important constituents of refractory C. The decomposition of plant litter is accompanied by re-synthesis of polysaccharides by microbes, resulting in an enrichment of microbially-derived relative to plant sugars in the course of decomposition (Kögel-Knabner, 1993; Dijkstra et al., 1998). According to the present results, microbial synthesis of polysaccharides seems to be an essential step for stabilizing this compound class over the long-term in soils.

### 8.2.3 Comparison of results: $^{13}$C NMR *versus* compound classes determined by chemical degradative methods

Results from $^{13}$C NMR spectroscopy, as a non-destructive technique, will be compared with the chemolytic methods for the specific analysis of lignin and polysaccharides, with regard to the following aspects: i) Are the contents of the compound classes correlated with signal intensities of the corresponding shift ranges in the $^{13}$C NMR spectrum? ii) Are changes / trends observed for lignin / polysaccharide contents in the course of C-depletion in agreement with changes of NMR signal intensities?

#### *8.2.3.1 Lignin (see chapters 4, 5, and 6)*

CuO oxidation revealed a decrease of lignin phenol contents with C-depletion. In NMR spectra of complex organic mixtures, the presence of lignin is considered to be indicated by clear peaks at 150 ppm (phenolic C) and 56 ppm (methoxyl C). Following C-depletion, in bulk samples and coarse separates, the 56 ppm peak was reduced (in line with a decrease of lignin phenols), and there was a lower resolution in the range 140 to 160 ppm, so that in many cases at 150 ppm a distinct peak could not be identified any more, probably due to overlapping signals from C-substituted/protonated aromatic compounds (resonances between 110 to 140 ppm). In the C-depleted plots of most sites, a broad signal in the aromatic range (110 to 140 / 160 ppm) was found, with a peak around 130 ppm. Especially in these plots, (quantitative) information on lignin from the NMR spectra is not precise. For four study sites, signal intensities of total aromatic C (110 to 160 ppm) clearly increased with C-depletion, which can mainly be ascribed to higher intensities of C-substituted/protonated aromatic C (110 to 140 ppm). Combining the data from CuO oxidation and $^{13}$C NMR, lower contents of lignin moieties, but a relative enrichment of C-substituted/protonated aryl compounds (resonance between 110 to 140 ppm) are evident for the SOC of the C-depleted plots.

Values for lignin phenols were not correlated with the intensities of aromatic species from NMR spectroscopy, neither with O-aryl nor with total aromatic signals (data from all the bulk samples, $n = 18$). The lack of correlation between CuO oxidation products and total aromatic

C suggests that the aromatic pool is dominated by compounds other than lignin or lignin-derived products.

#### 8.2.3.2 *Polysaccharides (see chapters 4, 5, and 7)*

For bulk samples, contents of total polysaccharides (MBTH-method) were correlated with O-alkyl C (60 to 110 ppm) intensities ($n = 18$, $r = 0.64$, $P < 0.001$). Non-cellulosic polysaccharides determined in particle-size fractions (by analysis of individual sugars) were related to O-alkyl C signals ($n = 31$, $r = 0.50$, $P < 0.01$), although cellulose may also contribute to the total O-alkyl signal in some of the fractions. Chemolytic methods and NMR spectroscopy yield analogous results as for the relative contribution of polysaccharides to OC. However, when comparing the magnitude of this contribution, yields of total carbohydrates (following hydrolysis) are much smaller than relative intensities of O-alkyl C. Among the reasons which are discussed for this phenomenon in the literature is incomplete hydrolysis of soil carbohydrates. In this work, the effectiveness of $H_2SO_4$ hydrolysis was checked by NMR analysis of the hydrolysis residue. By combining the data from NMR and the MBTH-method, it appeared that i) hydrolysis was efficient for removing the majority of polysaccharides, however, ii) O-alkyl compounds that were hydrolyzable, but could not be identified as sugars by the used colorimetric procedure, make up a considerable proportion of the total O-alkyl C signal. The fact that there are hydrolyzable O-alkyl C compounds that could not be identified may have several reasons, e.g. destruction of sugars during hydrolysis, sugars being involved in secondary reactions, incomplete breakdown of polysaccharides into monomeric units, or the existence of „pseudo polysaccharides".

Considering the effect of C-depletion, the two approaches for several sites gave divergent results: Intensities of O/N-alkyl C (45-110 ppm) decreased, whereas the level of (non-cellulosic) polysaccharides did not essentially change (results from bulk samples and fine separates). Combining the two methods suggests that the decrease of the O/N-alkyl C signal in the C-depleted plots is due to a lower contribution of the non-polysaccharide compounds in the 45 to 110 ppm range, such as lignin, proteins, and possibly hydrolyzable-non identified O-alkyl compounds, whereas the contribution of polysaccharides is similar. This would imply that these other compounds in the range 45 to 110 ppm are, on average, less stable than polysaccharides. An important mechanism for stabilizing polysaccharides appears to be the interaction with mineral surfaces, as stated in the next chapter.

## 8.3 Stabilization mechanisms (chapters 3, 5, and 7)

The fine separates (< 6 µm) showed the highest OC contents, and the OC pool of these separates contained the highest proportion of refractory compounds. This work tried to assess whether the stabilization by mineral surfaces is a relevant protection mechanism for OC in the fine separates.

From the correlation approach two different pictures were obtained: i) In the sandy soils (Thyrow, Skierniewice), total OC contents were correlated with the surface area (SA) of total minerals and of the two subfractions oxides and silicates, as well as with iron oxide contents. However, in the linear regression equations (see Fig. 2, chapter 3.3.1.1), y-intercept values are high, meaning that part of OC can not be explained on the basis of surface area / iron oxides. For both soils, total mineral SA in fine separates was dominated by the contribution from oxides. ii) By contrast, in the loamy soil (Bad Lauchstädt), OC contents were not related at all to mineral SA or iron oxide contents. A linear correlation between OC and surface area implies that SA is a key variable for the accumulation of OC, and this points to a SA-dependent stabilization mechanism, as represented by the physical association of organic compounds with the surface of mineral particles. For the sandy soils, the findings indicated that (long-term) C stabilization is - to a large part - due to an interaction of organic compounds with mineral surfaces. Given the dominant SA contribution from oxides in the sandy soils, it was concluded that OC mainly interacts with these mineral compounds, so that the oxides are to a major part responsible for C stabilization.

Density fractionation within the fine separates showed that the refractory pool of C is represented by C which is associated with minerals (recovered in the fraction > 2 g $cm^{-3}$), for the sandy soil (Thyrow) and the loamy soil. For the sandy soils, these results confirmed the previous conclusion based on the OC-SA correlation. For the loamy soil, the combination of the two approaches revealed that, on the one hand, the interaction with minerals is a relevant stabilization mechanism, but on the other hand, mineral SA may not be the only factor that controls stabilization processes.

Regardless of the pattern noted for total OC, contents of polysaccharides were related to mineral SA in the soils of contrasting texture (sandy soil from Thyrow, loamy soil). This indicates that for polysaccharides in particular the association with minerals is an important protection mechanism.

Airborne contaminants identified in coarse-intermediate separates of two sites (Thyrow, Bad Lauchstädt) were found to contribute to the refractory C pool. This is probably a consequence of their chemical recalcitrance. After dispersion, the contaminants could be recovered as „light" OM ($\rho \leq 1.9$ g $cm^{-3}$), indicating that they were not closely / strongly associated with minerals, and thus, their high stability may not be the result of an interaction with minerals. From the OC balances it seemed that the contribution of these recalcitrant contaminants to the refractory SOC is (much) smaller than that of OC stabilized within fine separates, for both sites.

Summarizing, for the three sites of different texture examined in this work, the major stabilization mechanism seems to be the interaction of OC with mineral surfaces within fine particle-size separates. In addition to this, inherently recalcitrant compounds such as airborne contaminants and pyrogenic C play a role for the refractory SOC at the sites, mainly in coarse-intermediate separates, but presumably also in fine separates at one site (loamy soil).

Given the great importance of organo-mineral interactions for C stability, revealed by the present work, the following questions / aspects should be considered in future investigations:

How are the various C-species and compound classes distributed among density subfractions of a particle-size separate? Are there differences in the degree of association with the mineral phase?

Are there C-species and compound classes which show a preferential association with particular mineral constituents (oxides, silicates)? What are the consequences for OC stability?

## 8.4 References

Dijkstra, E.F., Boon, J.J., Van Mourik, J.M., 1998. Analytical pyrolysis of a soil profile under Scots pine. European Journal of Soil Science 49, 295-304.

Kögel-Knabner, I., 1993. Biodegradation and humification processes in forest soils. In: Bollag, J.-M., Stotzky, G. (Eds.), Soil Biochemistry, vol. 8. Marcel Dekker, Inc. pp. 101-135.

Rumpel, C., Knicker, H., Kögel-Knabner, I., Hüttl, R.F., 1998. Airborne contamination of immature soil (Lusatian mining district) by lignite-derived material: its detection and contribution to the soil organic matter budget. Water, Air and Soil Pollution 105, 481-492.

# 9 Summary

The objective of this work is to characterize the refractory soil organic carbon (SOC) in arable soils, i.e. the C pool which shows very high turnover times. The experimental approach is the comparison of SOC, in quantitative (amounts) and qualitative terms (structural composition of SOC), in two contrasting treatments of long-term agroecosystem experiments: C-depleted plots are compared with the fertilized counterparts at a particular site; the SOC of the first plots is considered to be relatively enriched in refractory C compounds. Samples were obtained from eight long-term experiments established in central and eastern Europe.

The methods used are:

i) Particle-size fractionation for three of the study sites (two highly sandy soils, one loamy soil) to separate the different OC pools „mixed" within bulk samples and to obtain a detailed picture of the distribution and the properties of refractory C. At these three sites, the C-depleted plots differed from the fertilized ones in the overall C composition ($^{13}$C NMR), which indicated that the refractory C pool is structurally different from labile pools.

ii) Various techniques for characterizing SOC composition including those which allow the identification of airborne carbonaceous particles ($^{13}$C NMR spectroscopy, chemolytic methods for the analysis of lignin and polysaccharides, coal petrographic analysis).

iii) Techniques for examining the mineral matrix with respect to mineral surface area ($N_2$-BET), contents and fractionation of iron oxides (dithionite/oxalate extraction), composition of silicates (XRD).

The following results refer mainly to those three sites which were subjected to particle-size fractionation. From the high residual contents of OC in fine separates (< 6 µm) and the OC distributions of the C-depleted plots, with a peak in fine separates, it follows that most of the refractory SOC is associated with these separates, primarily with the clay (< 2 µm). The results from different methods (linear correlation between OC contents and mineral surface area, preservation of OC within the heavy subfractions $\rho > 2$ g cm$^{-3}$) indicate that the interaction of organic compounds with mineral surfaces is an important / or major mechanism for stabilizing OC at the long-term in the fine separates of the three soils examined.

The combination of the different methods to examine the chemical composition of SOC leads to the following conclusions:

i) Refractory C is enriched in aromatic compounds compared with more labile C pools, namely in C-substituted / protonated aryl C compounds ($^{13}$C NMR). For separates 2000-20 µm of the investigated sites, the high aromaticity of refractory C can be explained by the presence of airborne contaminants (coal, coke, char) (coal petrographic analysis). For fine separates, NMR data suggested that only in one of the three sites (loamy soil) contaminants may be present and contribute to the refractory C.

ii) Refractory C is relatively depleted in total O/N-substituted alkyl C compounds ($^{13}$C NMR), but apparently not with respect to the compound class of polysaccharides. The contribution of polysaccharides to the refractory pool seems to be similar to labile C pools. Stable polysaccharides are mainly found in fine separates, analogous to the total refractory C, and are predominantly of microbial origin.

iii) Refractory C is depleted in lignin compared with labile pools. The data from CuO oxidation suggest that lignin is not stable at the long-term in none of the separates, although lignin is a recalcitrant aromatic compound in plant litter. In contrast to polysaccharides, lignin is apparently not subject to stabilization processes.

iv) Refractory C does not differ from labile pools in the contribution of alkyl and carboxyl C compounds ($^{13}$C NMR).

Based on the present results, the following two aspects should be underlined:

Airborne contaminants have to be taken into consideration as constituents of the refractory C in soils of industrialized regions.

Regarding native SOC compounds, the present findings suggest that the refractory C is depleted in plant-derived compounds (primary resources), whereas secondary resources (microbes and their products) play an important role for the stabilized SOM.

# 10 Zusammenfassung

Die vorliegende Arbeit befaßt sich mit der Charakterisierung des refraktären C-Pools in Ackerböden, d.h. jener Fraktion der organischen Bodensubstanz, welche sehr langsam umgesetzt wird. Es wurden acht Dauerfeldversuche (in Mittel- und Ost-Europa) beprobt, wobei jeweils zwei extrem unterschiedliche Varianten verglichen wurden: C-verarmte Varianten, welche einen hohen Anteil an refraktärem C enthalten, und Volldüngungsvarianten als Vergleichsbasis.

Das verwendete Methodenspektrum umfaßt:

i) Korngrößenfraktionierung für drei Standorte (zwei sandige Böden, ein lehmiger Boden), um ein differenziertes Bild von der Verteilung und den Eigenschaften des refraktären C innerhalb der Böden zu erhalten. Diese Standorte wurden für die physikalische Fraktionierung ausgewählt, da deutliche Unterschiede in der Zusammensetzung des Corg-Pools ($^{13}$C NMR) zwischen C-verarmten und Volldüngungsvarianten bestehen.

ii) Methoden zur Kennzeichnung der Zusammensetzung und Struktur des organischen Kohlenstoffs, einschließlich solcher zur Erfassung von luftbürtigen Einträgen ($^{13}$C NMR Spektroskopie, naßchemische Verfahren zur Bestimmung von Lignin und Polysacchariden, kohlepetrographische Untersuchungsmethoden).

iii) Methoden zur Kennzeichnung der mineralischen Fraktion, und zwar der mineralischen Oberfläche ($N_2$-BET), Gehalte und Fraktionierung der Fe-Oxide (Dithionit/Oxalat-Extraktion), Zusammensetzung der Silikatfraktion (Röntgenbeugung).

Die meisten der folgenden Ergebnisse beziehen sich auf jene drei Standorte, von welchen Korngrößen-Fraktionen untersucht wurden. Aufgrund der hohen Corg-Restgehalte in feinen Fraktionen sowie der Tatsache, daß in den C-verarmten Varianten der meiste Corg in diesen feinen Fraktionen zu finden ist, scheint der Großteil des refraktären Corg in Fraktionen < 6 µm lokalisiert zu sein, wobei die Tonfraktion (< 2 µm) quantitativ am wichtigsten ist. Die Ergebnisse zweier unterschiedlicher Ansätze (vorhandene Korrelation zwischen Corg-Gehalten und mineralischer Oberfläche, Stabilisierung von C innerhalb der schweren Subfraktion $\rho > 2$ g cm$^{-3}$) weisen darauf hin, daß die Interaktion von organischen Verbindungen mit der Oberfläche von Mineralpartikeln ein wichtiger Stabilisierungsmechanismus innerhalb der feinen Fraktionen ist.

Die verschiedenen Methoden zur strukturchemischen Charakterisierung des refraktären Corg ergeben folgendes Bild:

i) Der refraktäre C weist einen höheren Aromatengehalt als labile C Pools auf, und zwar einen höheren Anteil an C-substituieren / nicht protonierten Aryl-Verbindungen ($^{13}$C NMR). Für Fraktionen 2000-20 µm von zwei Standorten kann dies auf das Vorhandensein von luftbürtigen Kontaminanten (Kohle, Koks, Verbrennungsrückstände) zurückgeführt werden (Kohle-Petrographie). Für die feinen Fraktionen haben die NMR Ergebnisse allerdings nur für einen der drei Standorte einen Verdacht auf Kontamination ergeben.

ii) Der refraktäre C ist an O/N-substituierten Alkyl-Verbindungen verarmt im Vergleich zu labilen Pools ($^{13}$C NMR), was aber nicht für die Stoffgruppe der Polysaccharide im speziellen gilt. Der Polysaccharid-Gehalt des refraktären C-Pools ist ähnlich jenem von labilen Pools. Stabile Polysaccharide finden sich vor allem in den feinen Fraktionen, analog zum gesamten refraktärem C, und sind vorwiegend mikrobieller Herkunft.

iii) Der refraktäre C ist an Lignin verarmt im Vergleich zu labileren C-Fraktionen. In keiner Korngrößenfraktion ist das als rekalzitrant (schwer abbaubar) geltende Lignin langfristig stabil, was bedeutet, daß es - im Gegensatz zu den Polysacchariden - keinen Stabilisierungsprozessen unterliegt.

iv) Der refraktäre C unterscheidet sich nicht von labilen Pools im Hinblick auf den Beitrag von Alkyl- und Carboxyl-Verbindungen ($^{13}$C NMR).

Aufgrund dieser Ergebnisse sollten die folgenden zwei Aspekte hervorgehoben werden:

Luftbürtige Einträge können einen Beitrag zum refraktären C-Pool in Ackerböden industrialisierter Gebiete leisten und sollten in dieser Hinsicht berücksichtigt werden.

In Bezug auf die native organische Bodensubstanz scheint es, daß der refraktäre C-Pool an pflanzlichen Komponenten (Primär-Ressourcen) verarmt ist, während Sekundär-Ressourcen (Mikroorganismen und die von ihnen synthetisierten Verbindungen) vermutlich eine wichtige Rolle spielen.

# Dank

Herzlichen Dank an all jene, die in vielfältiger Weise geholfen und mich unterstützt haben:

– Prof. Dr. Ingrid Kögel-Knabner für die Betreuung in den verschiedenen Phasen der Arbeit mit vielen guten Vorschlägen und Diskussionsbeiträgen sowie die gute Zusammenarbeit;
– Prof. Dr. Martin Gerzabek von der Universität für Bodenkultur Wien bzw. dem Forschungszentrum Seibersdorf (Österreich) für die Übernahme des Zweitgutachtens;
– Dr. Heike Knicker für die Einführung und Betreuung in Sachen NMR-Spektroskopie sowie für alle weitere Unterstützung;
– Prof. Dr. Udo Schwertmann für die wertvollen Gespräche über Eisenoxide, mineralische Oberflächen, und über manches mehr;
– alle Mitarbeiter des Lehrstuhls für Bodenkunde, Freising-Weihenstephan, für die freundliche Atmosphäre und die Hilfestellung bei Problemen verschiedenster Art; besonders danken möchte ich Horst Fechter, Ulrike Maul, Michaela Henn, Elfriede Schuhbauer, Irmgard Neumair, Dr. Bettina Haas, Dr. Martin Pichler, Dr. Kai Totsche und Dr. Helge Stanjek; Dr. Werner Häusler war bei der Röntgenbeugungsanalyse behilflich;
– Dr. Bertrand Ligouis von der Universität Tübingen für die Durchführung der kohlepetrographischen Untersuchungen und die kompetente Hilfe bei der Interpretation der Ergebnisse;
– Dr. Wulf Amelung von der Universität Bayreuth für das Beantworten von praktischen Fragen zur Einzelzuckeranalyse;
– Prof. Dr. Martin Körschens (UFZ Leipzig-Halle GmbH, Sektion Bad Lauchstädt) für die gute Zusammenarbeit innerhalb des Projekts und die rege Unterstützung bei der Probenahme; für ihr Entgegenkommen bei der Probenahme sei Michael Baumecker (Versuchsstation Thyrow), Prof. Dr. Stanislaw Mercik (Universität Warschau) sowie Dr. G. Pommer (Bayerische Landesanstalt für Bodenkultur und Pflanzenbau, Freising) gedankt;
– die Deutsche Forschungsgemeinschaft für die Finanzierung des Projektes „Identifizierung und stoffliche Charakterisierung der refraktären und intermediären organischen Substanz in Böden in Abhängigkeit von Standort und Bewirtschaftung“, im Rahmen dessen diese Arbeit zustande kam.