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Review Article

Organo-mineral associations in temperate soils: Integrating biology, mineralogy, and organic matter chemistry§

Ingrid Kögel-Knabner[1]*, Georg Guggenberger[2], Markus Kleber[3], Ellen Kandeler[4], Karsten Kalbitz[5], Stefan Scheu[6], Karin Eusterhues[1,7], and **Peter Leinweber[8]**

[1] Lehrstuhl für Bodenkunde, Department für Ökologie und Ökosystemmanagement, Wissenschaftszentrum Weihenstephan, Technische Universität München, 85350 Freising-Weihenstephan, Germany
[2] Institut für Agrar- und Ernährungswissenschaften, Martin-Luther-Universität Halle-Wittenberg, Weidenplan 14, 06108 Halle/Saale, Germany
[3] Department of Crop and Soil Science, Oregon State University, 3017 Ag & Life Sciences Bldg., Corvallis, OR 97331–7306, USA
[4] Institut für Bodenkunde und Standortslehre, Fachgebiet Bodenbiologie, Universität Hohenheim, Emil-Wolff-Straße 27, 70599 Stuttgart, Germany
[5] Lehrstuhl für Bodenökologie, Universität Bayreuth, 95440 Bayreuth, Germany
[6] Institut für Zoologie, Technische Universität Darmstadt, Schnittspahnstraße 3, 64287 Darmstadt, Germany
[7] present address: Chemisch-Geowissenschaftliche Fakultät, Friedrich-Schiller-Universität, Burgweg 11, 07749 Jena, Germany
[8] Institut für Landnutzung, Universität Rostock, Justus-von-Liebig-Weg 6, 18051 Rostock, Germany

Abstract

We summarize progress with respect to (1) different approaches to isolate, extract, and quantify organo-mineral compounds from soils, (2) types of mineral surfaces and associated interactions, (3) the distribution and function of soil biota at organo-mineral surfaces, (4) the distribution and content of organo-mineral associations, and (5) the factors controlling the turnover of organic matter (OM) in organo-mineral associations from temperate soils. Physical fractionation achieves a rough separation between plant residues and mineral-associated OM, which makes density or particle-size fractionation a useful pretreatment for further differentiation of functional fractions. A part of the OM in organo-mineral associations resists different chemical treatments, but the data obtained cannot readily be compared among each other, and more research is necessary on the processes underlying resistance to treatments for certain OM components. Studies using physical-fractionation procedures followed by soil-microbiological analyses revealed that organo-mineral associations spatially isolate C sources from soil biota, making quantity and quality of OM in microhabitats an important factor controlling community composition. The distribution and activity of soil microorganisms at organo-mineral surfaces can additionally be modified by faunal activities. Composition of OM in organo-mineral associations is highly variable, with loamy soils having generally a higher contribution of polysaccharides, whereas mineral-associated OM in sandy soils is often more aliphatic. Though highly reactive towards Fe oxide surfaces, lignin and phenolic components are usually depleted in organo-mineral associations. Charred OM associated with the mineral surface contributes to a higher aromaticity in heavy fractions. The relative proportion of OC bound in organo-mineral fractions increases with soil depth. Likewise does the strength of the bonding. Organic molecules sorbed to the mineral surfaces or precipitated by Al are effectively stabilized, indicated by reduced susceptibility towards oxidative attack, higher thermal stability, and lower bioavailability. At higher surface loading, organic C is much better bioavailable, also indicated by little ^{14}C age. In the subsurface horizons of the soils investigated in this study, Fe oxides seem to be the most important sorbents, whereas phyllosilicate surfaces may be comparatively more important in topsoils. Specific surface area of soil minerals is not always a good predictor for C-stabilization potentials because surface coverage is discontinuous. Recalcitrance and accessibility/aggregation seem to determine the turnover dynamics in fast and intermediate cycling OM pools, but for long-term OC preservation the interactions with mineral surfaces, and especially with Fe oxide surfaces, are a major control in all soils investigated here.

Key words: OM / organic matter stabilization / microhabitat / fractionation / specific surface area / NMR / SEM / ^{14}C age

Accepted July 13, 2007

* Correspondence: Prof. Dr. I. Kögel-Knabner;
e-mail: koegel@wzw.tum.de

§ **Topical Issue** *Soils as a source and sink for CO_2 – Mechanisms and regulation of organic matter stabilisation in soils* (editors: I. Kögel-Knabner and E. Matzner). Synthesis of the DFG Priority Program SPP 1090 (German Research Foundation—"Deutsche Forschungsgemeinschaft").

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1 Introduction

Inverse correlations between grain size and organic carbon (OC) content have frequently been ascribed to stabilization of organic matter (OM) in soils by close associations between OM and mineral surfaces (*Tiessen* et al., 1984; *Anderson*, 1988; *Oades*, 1988), giving rise to the paradigm of sorptive protection. Mineral surfaces in soils are mainly provided by the very small particles (<2 μm) that constitute the soil clay fraction. This fraction is a mixture of phyllosilicates, oxides, and hydroxides, and, in some soil types, short–range order minerals like allophane and imogolite. Traditional concepts were highly focused on the role of phyllosilicates as dominating mineral surfaces for the formation of organo-mineral associations (*e.g.*, *Stevenson*, 1982; *Theng*, 1979). Later, total mineral surface area has been considered as a predictor for the amount of OM that is stabilized in soils (*Mayer* et al., 1994a; *Saggar* et al., 1996; *Hassink,* 1997; *Six* et al., 2002), but an understanding on the relationship between mineralogy and the chemistry of OM bound in organo-mineral associations is lacking. Most of the available data are from loamy arable topsoils, and information on other soil types and the distribution with depth still is lacking (*von Lützow* et al., 2006).

A major step forward was achieved from the combined efforts of soil-mineralogical, organic-chemical, and microbial research approaches. Increasing evidence emerged on the important influence of soil biota in the formation of organo-mineral assemblages (*Chenu* and *Stotzky*, 2002). Here, the ability of microorganisms to standardize mineral surfaces to their needs by "active support preconditioning" (*Bos* et al., 1999; *Dufrene* et al., 1996) seems to promote mineral-organic associations through the deposition of extracellular polysaccharides and proteins as adhesives. It is well-known that numerous Gram-negative and Gram-positive bacteria exude extracellular polymeric substances (EPS) into their environment (*Omoike* and *Chorover*, 2006). In natural aqueous environments, pristine mineral surfaces become coated rapidly by biogenic organic films (*Bos* et al., 1999). The formation of these "conditioning films" moderates eventual differences in the surface chemistry of the underlying substrate and can thus be seen as an adaptation mechanism that allows microorganisms to colonize highly variable types of mineral surfaces.

Spatial variability has historically been regarded as random noise in the system, but it becomes now evident that soil microorganisms as well as soil enzymes are heterogeneously distributed within the soil matrix (*Ettema* and *Wardle*, 2002; *Kandeler* and *Dick*, 2006; *Fry*, 2007). Still little detailed information is available on the spatial distribution of soil microbiota. This spatial variability and heterogeneity in the distribution of microorganisms and OM with regard to the mineral surfaces requires new approaches to the investigation of possible interactions between OM and mineral surfaces.

In this paper, we summarize the recent results obtained within the PP1090 "Soils as a source and sink for CO_2 – mechanisms and regulation of organic matter stabilisation in soils" and discuss our current understanding of sorptive organo-mineral associations. *A priori* the topics discussed in this manuscript focus on mechanisms at the organic matter–mineral interface. Firstly, we approach this by studies on the separation/isolation or extraction of organo-mineral associations and the quantity of organo-mineral compounds in soils. Then we highlight recent research achievements with respect to the types of mineral surfaces and the associated interactions. Based on that, distribution and content of OM in different soils, its composition and stability in organo-mineral associations are discussed in light of the distribution of microorganisms and microbial enzyme activities at organo-mineral surfaces in soils. For a discussion of protection of OC due to occlusion of OM within clay microstructures and aggregates see *Bachmann* et al. (2008, this issue, pp. 14–26) and *Flessa* et al. (2008, this issue, pp. 36–51). A detailed description of the soils and sites investigated in the PP1090 is given by *Kögel-Knabner* et al. (2008, this issue, pp. 5–13).

2 Approaches to isolate, extract, and quantify organo-mineral compounds in soils

2.1 Physical separation methods

Mineral-bound OM has been defined as all OM that is adsorbed to minerals or entrapped in small micro-aggregates (*Wattel-Koekkoek* et al., 2004; *Chenu* and *Plante*, 2006). To make quantitative estimates of the amount and stability of OM in organo-mineral associations *sensu strictu* and to investigate both their mineral and their organic composition, it is necessary to specifically isolate this fraction from soils. This has been approached by physical aggregate disruption in combination with particle-size and/or density separation or by chemical treatments that are assumed to specifically either attack or isolate organo-mineral components.

Density fractionations have been used for many years in attempts to separate free OM (mostly plant residues) from OM bound to minerals. Organo-mineral fractions obtained from the physical-fractionation approach are generally isolated after disruption of aggregates into so-called primary particles (*Christensen*, 1992, 1996) and subsequent particle and density fractionation (*von Lützow* et al., 2006, 2007). Two techniques are used for aggregate disruption and dispersion, sonication at different energies (*Amelung* and *Zech*, 1999) or end-over-end agitation in water with glass beads (*Balesdent* et al., 1991). *North* (1976) suggested to check complete dispersion by calibration of a clay-sized fraction obtained in proportion to that achieved during standard particle-size analyses. The different aggregate stabilities and densities of organo-mineral associations in soils of different mineralogy have to be considered, when adjusting a fractionation procedure to a specific soil (*Turchenek* and *Oades*, 1979; *Schmidt* et al., 1999a, b). This explains why there is until now no accepted standard protocol with respect to, *e.g.*, input energy to disrupt the aggregates or the critical densities of the heavy liquid to separate the light fraction from mineral-associated OM. *Chenu* and *Plante* (2006) showed that many of the so-called <2 μm "particles" were in fact nanometer- to micrometer-sized micro-aggregates in which OM was

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encrusted by minerals or coated minerals. None-the-less, young plant residues are often well separated from older and thus stable organo-mineral associations by a combination of ultrasonic dispersion followed by particle-size and density fractionation (*Turchenek* and *Oades*, 1979; *Golchin* et al., 1994; *Wander*, 2004; *Schöning* and *Kögel-Knabner*, 2006; *Kaiser* and *Guggenberger*, 2007b). Some authors do not use any dispersion procedure prior to density fractionation (*Sollins* et al., 2006; *Janzen* et al., 1992). Although this avoids the artificial inclusion of more mineral material in the light fraction (*Janzen* et al., 1992), it does not allow a differentiation in primary and secondary particles in the sense of *Christensen* (1992, 1996).

2.2 Chemical extraction and degradation methods

Various extraction methods using desorbing, hydrolyzing, and oxidizing reagents have been used to extract or enrich OM fractions associated with the soil mineral phase in the framework of the PP1090 (*Ellerbrock* et al., 2005; *Eusterhues* et al., 2003, 2005a; *Kleber* et al., 2005; *Mikutta* et al., 2006; *Helfrich* et al., 2007). For a detailed survey of existing procedures see *von Lützow* et al. (2007).

Organic matter extracted from soil with alkaline Na-pyrophosphate (pH 9–10) is supposed to being liberated from metal-organic associations and from mineral surfaces *via* desorption. But Na-pyrophosphate not only liberates metals from metal-organic complexes but also extracts polymeric metal species and small-sized minerals associated with OM like goethite, ferrihydrite, amorphous $Al(OH)_3$, gibbsite, and layered silicates (*Schuppli* et al., 1983; *Kassim* et al., 1984; *Adams* and *Kassim*, 1984; *Evans* and *Wilson*, 1985; *Kaiser* and *Zech*, 1996). Hence, the suitability of pyrophosphate extraction to reliably recover stable OM from soils still remains to be demonstrated, especially in comparison with other more established methods like hydrolysis or oxidation treatments.

Acid hydrolysis using hot 6 N HCl is a common procedure for the quantification of old, biochemically resistant OM fractions in soils (*Paul* et al., 1997, 2001; *Huang* et al., 1999; *Collins* et al., 2000). This is mainly due to the preferential removal of labile O-alkyl C, primarily originating from polysaccharides, and the selective enrichment of more resistant aromatic and aliphatic C components (*Dieckow* et al., 2005). However, fresh plant detritus needs to be removed prior to hydrolysis because plant residues partly resist hydrolysis, which could bias the assessment of stable C in soils. But even then acid hydrolysis does not unambiguously isolate a fully stable OM pool (*Balesdent*, 1996; *Plante* et al., 2006). One should further bear in mind that at low pH, Fe oxides are dissolved and, thus, OM stabilized *via* interaction with this part of the mineral phase may get released.

Chemical degradation of soil OM by oxidizing reagents is an alternative method to preferentially remove OM structures unprotected by the mineral matrix. But it oxidizes preferentially chemically labile components such as polysaccharides and aromatics (*Leifeld* and *Kögel-Knabner*, 2001; *von Lützow* et al., 2007). The organic residuum has been shown to be composed of compounds inherently stable against chemical attack and protected by association with soil minerals (reviewed by *Mikutta* et al., 2005). For example, fossil or black C (*Schmidt* et al., 1999c) and aliphatic compounds such as n-alkanes and n-fatty acids likely originating from aliphatic biopolymers (*Griffith* and *Schnitzer*, 1977; *Martin* et al., 1981; *Schulten* et al., 1996; *Cuypers* et al., 2002) comprise oxidation-resistant OM (*Eusterhues* et al., 2005a). The concentration of oxidation-resistant OM in various soils can vary strongly from 1% to 80% with usually larger relative amounts in subsoils than in topsoils (*Eusterhues* et al., 2003, 2005a; *Kaiser* and *Guggenberger*, 2003). The authors assigned this increasing resistance of OM to oxidation with soil depth to a stronger binding to the mineral phase at smaller surface loading of the minerals by OM. At present, it is still a drawback of the oxidation procedures that they are hardly comparable due to differences in methodology, *i.e.*, oxidant reagent concentration, temperature, solid-to-solution ratios, and number of repetitions, and due to the fact that certain OM components may be removed in preference to others (*e.g.*, lignin-derived aromatics; *Mikutta* et al., 2006). Future research needs to focus on the comparability of methods and on the understanding of underlying processes that cause certain OM components to resist extraction, hydrolysis, or oxidative degradation.

3 Types of mineral surfaces and associated interactions

Three types of functional surface sites on minerals can be distinguished, which offer different bonding possibilities to the OM. Their availability is assumed to control area density as well as the strength of the bonds formed: (1) Surfaces inhabited by single coordinated hydroxyl groups are common to Fe and Al oxides, allophone, and imogolite and the edges of layer silicates; (2) siloxane surfaces with permanent layer charge due to isomorphic substitution. In soils, these surfaces appear at some 1:2–layer-type phyllosilicates, such as vermiculite, illite, and smectite; (3) siloxane surfaces without layer charge. This surface type occurs in both 1:2–layer-type (talc, pyrophyllite) and in 1:1-type minerals (kaolinite group). The results from the PP1090 largely follow this energetic scheme: Strong protection was generally correlated with the presence of hydroxylated mineral phases in acidic soil environments.

In bulk soils and clay fractions of illitic, near-neutral soils, correlations between SOC and CEC (*Kahle* et al., 2002, 2003a) were described. In accordance with the above theoretical considerations, these findings were interpreted to be caused by cation exchange as the dominating bonding mechanism between mineral matrix and OM. The fact that no or negative correlations exist between the Fe of total Fe oxides (Fe_d) and TOC was explained by the presence of well-crystalline and therefore less reactive Fe oxides in the investigated soils or by the decreased number of positively charged surface hydroxyls in near neutral environments. But there is evidence for a specific interaction between Fe oxides and OM in soils of similar mineralogy and pH (Luvisol data, Fig. 7 and 8; *Schöning* et al., 2005b). Most often information on the binding mechanisms is obtained from theoretical considerations, pro-

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cesses found in short-term laboratory experiments, or from statistical correlations. Further studies are needed to directly investigate the specific binding mechanisms found in organo-mineral associations isolated from soils using high-resolution spectroscopic techniques.

Oxidation-resistant OM is a chemically stable, old OM fraction (*Theng* et al., 1986). In a wide range of different soils, its concentration often correlates with the content of Fe oxides and/or clay (*Eusterhues* et al., 2003) or Fe oxides and short–-range order Al silicates (*Kleber* et al., 2005; *Mikutta* et al., 2006). In soils with low amounts of poorly crystalline components (Fe_o : Fe_d < 0.2), the mineral-associated stable OC relates positively to the content of crystalline Fe oxides (*Mikutta* et al., 2006; Fig. 1). These results strongly suggest that in acidic soil environments, stable OM is predominantly associated with Fe oxides and short–range order Al silicates. Such minerals provide large reactive surface areas (*Eusterhues* et al., 2005b), which renders them most suitable for interaction with OM either *via* sportive interactions or coprecipitation. In addition to strong bonds due to ligand exchange, an inherent recalcitrance of the oxidation-resistant OM fraction may lead to its oxidation resistance.

As preferential sorption sites the mouth of micropores (<2 nm) or small mesopores (2–10 nm) of Fe oxides have been suggested (*Kaiser* and *Guggenberger*, 2003). Besides of the boundaries of individual domains, these small pores represent the surface roughness resulting from defects in the surface structure of goethite crystallites (*Weidler* et al., 1998). Consequently, micropores are sites of increased reactivity and thus best suited for sorptive interactions with organic acids and polyelectrolytes (*Kaiser* and *Guggenberger*, 2003; *Mikutta* et al., 2004). There, OM can form strong multiple bindings that disfavor both the desorbability and the oxidative removal of OM, respectively (*Kaiser* and *Guggenberger*, 2007a). Similarly, *Zimmerman* et al. (2004) observed larger desorption hystereses of nitrogenous compounds following sorption to mesoporous sorbents compared with their non-porous analogues. However, in soil and sediment environments, the importance of molecular-sized pores within the mineral matrix for the stabilization of OM is not yet understood. *Mayer* et al. (2004) concluded that other factors than association with mesopores must be responsible for the accumulation of OM in marine sediments because only <10%–20% of OM was found to be actually contained in 2–10 nm pores that are small enough to prevent access of hydrolytic enzymes and microbes. Similar results are reported by *Kaiser* and *Guggenberger* (2007a) for the soil mineral goethite. Close to sorption maximum, the volume ratio between goethite and OM is almost 1. At this surface loading, the configuration of OM is less dense than at lower loading, and OM bridges goethite crystals and crystallites to form small aggregates (*Kaiser* and *Guggenberger*, 2007a). This type of OM with less organic ligands being involved in the bonding is prone to desorption and oxidative degradation, and possibly also to a better biological decomposition.

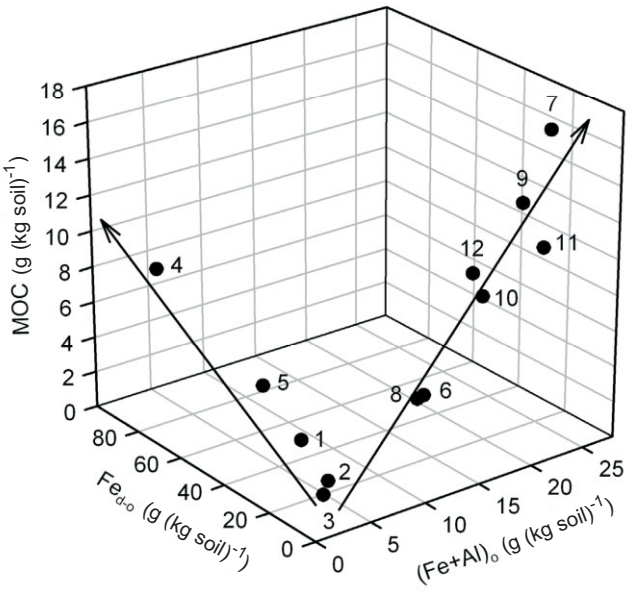

Figure 1: Three-dimensional plot showing the relation of the content of mineral-protected organic C (MOC) with the contents of poorly crystalline minerals and metal-humus complexes [$(Fe + Al)_o$] and crystalline Fe oxides (Fe_{d-o}). In samples 1–5, where little poorly crystalline minerals are present (Fe_o : Fe_d <0.2), a significant relationship of MOC with Fe_{d-o} (r^2 = 0.93) suggests that MOC is mainly associated with crystalline Fe oxides. Arrows are displayed for better visualization only (taken from *Mikutta* et al., 2006).

4 Distribution of microorganisms and microbial enzyme activities at organo-mineral surfaces in soils

4.1 Methods used to study small-scale distribution of soil microorganisms

Different methods have been applied to study the distribution of OM and microorganisms in the soil matrix, such as electron microscopy, repeated washing of soil aggregates, and techniques based on physical soil fractionation (*Stemmer* et al., 1998). Using biological soil thin sections and a combination of image analysis and geostatistical tools, the presence of spatial patterns in the distribution of bacteria was demonstrated at the microscale, with ranges of spatial autocorrelation of 1 mm and below (*Nunan* et al., 2003). For detailed microvisualization, optical-microscope techniques are applied, supported by specific labeling techniques and image analysis (*cf.*, review by *Schmid* et al., 2006; *Watt* et al., 2006). The confocal laser-scanning microscope is a very useful tool to document the spatial arrangement of microbes and their *in situ* activities, as indicated by fluorescence-labeling and reporter technique. Physical fractionation of soils offers the opportunity to study compartimentation of substrates and soil microorganisms at the small scale (*Kandeler* et al., 2005; *Marx* et al., 2005).

4.2 Fungi are more important/abundant in coarse fractions

In general, organic particles >2000 μm, which consist predominantly of particulate residues from plant material, decompose very rapidly while the OM in smaller-size fractions is

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characterized by an increase of the degree of degradation and a decrease in the C : N ratio. The dominance of fungal-derived xylanase activity in coarse fractions has recently been documented for both bulk soil and earthworm casts (Fig. 2, from *Marhan* et al., 2007). Xylanase activity and fungal-derived phospholipid fatty acids show a very close relationship to the particulate OM of the coarse-sand fraction (*Kandeler* et al., 1999a, b, c, 2000, 2001; *Stemmer* et al., 1999; *Poll* et al., 2003). The close vicinity of fungi, extracellular xylanase, and particulate OM guarantees an efficient use of the substrates by the fungal consumer (*Kandeler* and *Dick*, 2006). These results were also supported by the highly significant correlation between xylanase activity and the quality of OM (the signal of hemicelluloses measured by pyrolysis mass spectrometry (Fig. 3)).

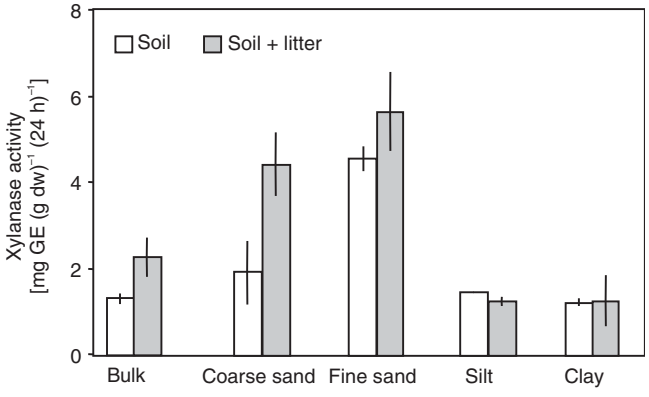

Figure 2: Distribution of xylanase activity in particle-size fractions of a Terra-fusca Rendzina from a 130 y–old beech forest near Göttingen (southern Lower Saxony, Germany). Addition of beech leaf litter (44.5% C, 1.6% N) induced mainly an increase of xylanase activity in the sand fractions (from *Marhan* et al., 2006).

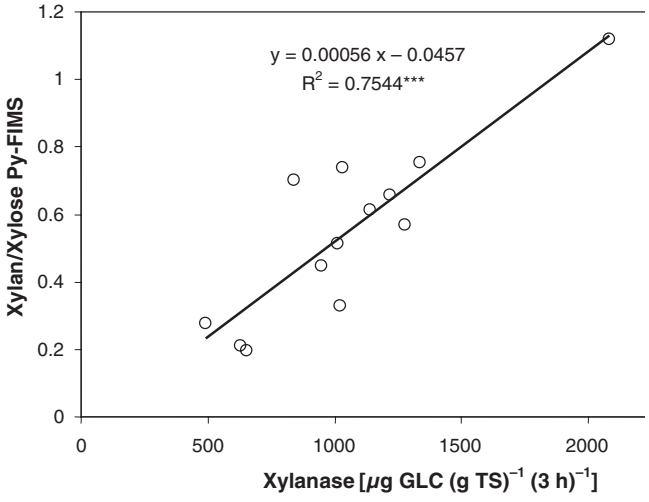

Figure 3: The response of xylanase activity of a Haplic Phaeocem to the abundance of its corresponding substrate. Substrate quality of hemicellulose was measured by its xylan-to-xylose ratio calculated from indicator signals of Py-FIMS. Soil samples (0–20 cm) from the control (unfertilized) and NPK plots of the "Eternal Rye Cultivation" were incubated for 2, 7, 21, and 35 d at 25°C. Xylanase activity was expressed as μg glucose equivalents (GE) per gram of soil and 24 h (Leinweber et al., unpublished).

4.3 Bacteria dominate the small-size fractions

Small-sized fractions contain the most microbial biomass in different soils (*Kanazawa* and *Filip*, 1986; *Jocteur Monrozier* et al., 1991). For example, cellulose-feeding bacteria (*Cytophaga, Cellulomonas*) attach closely with their substrate in these microhabitat, which minimizes the loss of enzymes into the soil solution by diffusion (*Alexander*, 1977). Therefore, enzymes involved in the hydrolysis of high molecular weight C substrates reflect the location and perhaps the quality of OM in agricultural soils (*Speir* and *Ross*, 2002). The bacterial dominance in the finer fraction was also shown by high abundance of bacterial-derived phospholipid fatty acids, high diversity of 16s rRNA gene fragments, and high activities of enzymes involved in the cycling of N and P (*Kandeler* et al., 2000; *Poll* et al., 2003). Clay-sized particles have a higher surface area than coarser particles, which facilitates bacterial growth as well as attachment and protection of microorganisms and extracellular enzymes. Using molecular techniques (RFLP—restriction fragment length polymorphism), we found, that the silt- and clay-sized fraction favored a greater richness of bacterial species (*Sessitsch* et al., 2001; *Kirchmann* et al., 2004; *Selesi* et al., 2007). PLFA pattern of particle-size fractions support these results (*Poll* et al., 2003) showing a rather simple fungal-dominated community in sand-sized fractions and complex bacterial-dominated communities in silt- and clay-sized fractions. Since <0.01% of the soil surface is colonized, spatial isolation of organisms might promote richness of microbial diversity in these fractions. Further evidence for greater habitat diversity in small-sized soil fractions comes from studies of *Sessitsch* et al. (2001) who found aerobic and strictly anaerobic bacteria species in the clay-sized fraction and no strictly anaerobic species in sand-sized fractions.

The high affinity of bacteria to small particle size fractions, as described above, suggests that mineral-organic associations in these fractions show a "microbial fingerprint". Indications for this are low C : N ratios as commonly observed for bacterial biomass (*Knicker*, 2004), enrichment in ^{13}C (*Gleixner* et al., 1993), and a microbial signature of the polysaccharides in the fine fractions (*Guggenberger* et al., 1994; *Kiem* and *Kögel-Knabner*, 2003).

Although studies using particle-size fractions helped to understand the location and functioning of soil microorganisms at the small scale, the fractionation procedure destroys the natural micro-environment of particles and soil microorganisms (especially branching of hyphal organisms) and does not provide any insight into soil microbial processes in the three-dimensional soil system (*Kandeler* and *Dick*, 2006). This disadvantage can partly be overcome by applying slicing techniques of soil cores followed by microanalytical methods and modeling (*Gaillard* et al., 2003; *Poll* et al., 2006). First attempts with these approaches are experimental designs focusing on the description and modeling of two-dimensional gradients of microbial activities that occur in the detritusphere as well as in preferential-flow paths of soils (*Gaston* and *Locke*, 2002; *Poll* et al., 2006, 2007). In a model experiment, bacteria and fungi showed differing utilization strategies of organic substrates at the soil-litter interface. Coupling PLFA

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analysis with stable isotope techniques, *Poll* et al. (2006) revealed that fungi assimilated C directly in the litter, whereas bacteria took up the substrates in the soil and therefore depended more on transport processes than fungi (*cf.*, also *Beare* et al., 1997; *Ekschmitt* et al., 2008, this issue, pp. 27–35).

4.4 Soil animals as drivers of the small-scale distribution of soil microorganisms and microbial-enzyme activities at organo-mineral surfaces

By mixing organic and mineral soil components during the gut passage, decomposer soil invertebrates affect the distribution of microorganisms among soil particle-size fractions, their activity, and, therefore, mobilization and stabilization processes of OM (*Scullion* and *Malik*, 2000; *Marhan* and *Scheu*, 2006). A large number of soil invertebrates are involved in these processes but due to their large size and dominance in numbers and biomass in many terrestrial ecosystems, earthworms are the most important drivers (*Beare* et al., 1995; *Bardgett,* 2005). Both sorption and desorption of OM from mineral surfaces occurs during the gut passage through earthworms (*Shipitalo* and *Protz,* 1988, 1989; *Hindell* et al., 1997; *Marhan* and *Scheu*, 2006). By ingesting and processing large amounts of mineral soil, endogeic earthworms in particular affect mobilization and stabilization processes of OM (*Kubiena,* 1948*; Shaw* and *Pawluk,* 1986*; Scheu,* 1987). By the addition of large amounts of mucus and water in the foregut, aggregates are broken up and clay minerals are dispersed, thereby liberating physically protected OM and making it accessible for digestion in the midgut of the animal (*Barois*, 1992; *Schmidt* et al., 1999d). On the contrary, by resorption of water and mucus in the hindgut, earthworms stimulate the re-association and new formation of organo-mineral complexes thereby contributing to the stabilization of OM. Desorption and resorption processes during the gut passage result in the frequently observed pattern of increased decomposition of OM in fresh cast materials but reduced decomposition in aging casts (*Martin,* 1991; *Lavelle* et al*.,* 2004; *Tiunov* and *Scheu*, 2000; *Marhan* and *Scheu*, 2006).

Although the gut passage through earthworms strongly affects soil structure, microbial activity, and decomposition processes, the composition of the microbial community appears to be little affected. Fluorescence microscopy, genetic fingerprinting (T-RFLP analysis), and phospholipid fatty analysis suggests that the general structure of microbial communities remains little affected by the gut passage through earthworms (*Schönholzer* et al., 1999; *Egert* et al., 2004; *Marhan* et al., 2007). However, by feeding on both OM-rich patches and mineral soil and intimately mixing them during the gut passage, the structure and activity of microbial communities are strongly affected ("nutrient-enrichment effects"; *Devliegher* and *Vertraete*, 1995). Further, despite the structure of microbial communities may be little affected, their activity may change markedly, *e.g.*, it has been documented recently that the acitivity of denitrifiers is strongly increased in earthworm guts and fresh cast materials (*Horn* et al., 2006; *Drake* and *Horn*, 2006). Further, although little information is available, the few studies undertaken suggest that the structure of microbial communities associated with particle-size fractions in soil is little modified by the gut passage through earthworms (*Marhan* et al., 2007; *Sampedro* et al., 2006; Fig. 4). Rather than changing the associations of microorganisms with certain particle-size fractions, earthworms alter the distribution of microorganisms associated with soil macro- and micro-aggregates (*Mummey* et al., 2006), and this likely is responsible for modifying the decomposition of OM enclosed in earthworm casts in the long term (*Tiunov* and *Scheu*, 2000; *Marhan* and *Scheu*, 2006). The formation of micro-aggregates within macro-aggregates and an associated microbial community in the micro-aggregates which is relatively inactive compared to macro-aggregate populations likely contributes to the long-term stabilization of OM in earthworm casts (*Mummey* et al., 2006).

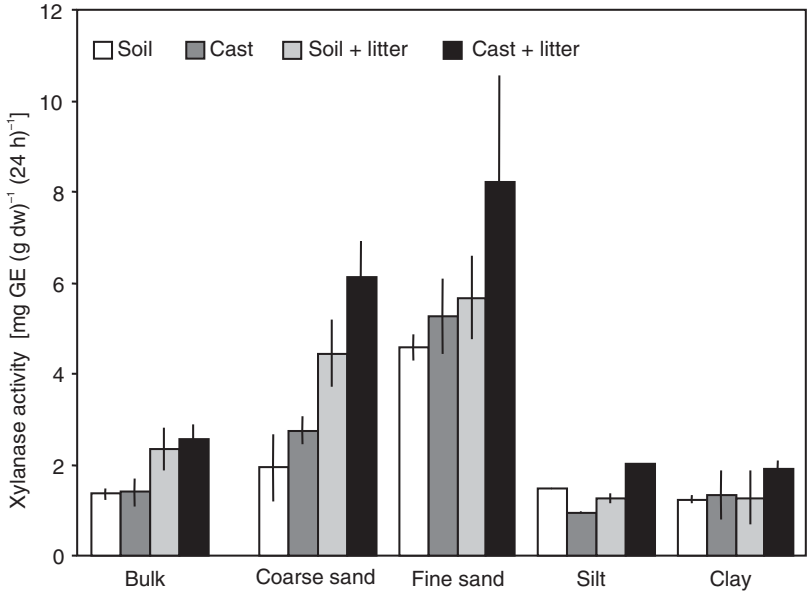

Figure 4: Xylanase activity in bulk soil and particle-size fractions of soil and casts of *Lumbricus terrestris* without (– Litter) and with addition of beech litter (+ Litter). Means of two or three replicates ±1 S.D. GE: glucose equivalents (from *Marhan* et al., 2006).

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5 Amount and distribution of mineral-bound OM in soils

The amount and relative percentage of OC that is found in organo-mineral fractions is highly variable between soil types, but also between horizons within the same soil. Only a limited number of studies exist that give a complete data set for the OC concentration as well as the distribution of OC between fractions. Table 1 gives a summary from studies with available data from a number of different soils using the dispersion and density fractionation approach. The relative proportion of the OC that is associated with the heavy fraction is highly variable. Organic C concentrations in the heavy fraction vary between <1 and >100 g OC kg^{-1}.This may be due to a number of reasons. Organic C distribution might be affected by different procedures for aggregate disruption as mentioned above. Likewise, different densities of 1.6 to 2.2 g cm^{-3} for separation of the clay-bound OC are used. As pointed out by *Christensen* (1992), *Vanyushina* and *Travnikova* (2003), and *Sollins* et al. (2006), this strongly affects the mass ratio of organic to mineral phase of these particles. The differences in proportion of OC in the heavy fractions may be due to variations in mineralogy (amount and type of minerals) and OC loading of the minerals (*Oades*, 1988; *Christensen*, 1996; *Hedges* and *Oades*, 1997). So, a soil richer in short-range-ordered Fe oxyhydroxides and Al silicates shows larger concentrations in mineral-bound fractions than that richer in clay minerals (*Rasmussen* et al., 2005). In the comparison of different density separates obtained without dispersion, *Sollins* et al. (2006) showed that primary minerals with a low affinity to OM predominate in the fractions >2.28 g cm^{-3}. Carbon-14 data show that the OC associated with these seemingly unreactive minerals has longer turnover times than the OC in the lighter fractions which are supposed to include the more reactive mineral surfaces.

Generally, the relative proportion of OC bound in organo-mineral fractions is lower in forest topsoils compared to soils under agriculture (*e.g.*, *John* et al., 2005; *Eusterhues* et al., 2005a, b). In agricultural soils, the lower input of OM and the frequent disruption of aggregates (*Six* et al., 1998) leads to a preferential loss of OC from free and occluded particulate OM fractions. This is seen in the data for the Rottalmünster site with different land use in Tab. 1. Similar data with a preferential loss of free OC in the clay-sized fraction are described by *Chenu* and *Plante* (2006). Table 1 also shows that the relative importance of clay-bound OC increases with soil depth. It becomes especially high in subsoil horizons with high contents of clay-sized minerals, such as Bt or spodic horizons. In the subsoil horizons, in particular different OC loading of the mineral phase may be responsible for the different OC concentrations in the heavy fractions, in addition to the variable mineralogy (Tab. 1). But only limited data are available for the OC loading of the mineral phase, *i.e.*, the ratio of OC to the specific surface area of the minerals.

6 Specific surface area of the mineral phase and loading with OM

Particle-size fractionation of the PP1090 soils exhibits highest C concentrations in the fine fractions <6.3 μm (*Rumpel* et al., 2004). This observation matches the widely accepted assumption that minerals in the clay fraction stimulate C storage and protection from degradation. It is also in accord with the idea that the available inorganic surface area controls OM sorption. Organic C loadings in the range of 0.5 to 1 mg OC m^{-2} were reported in sediments and soil (*Keil* et al., 1994; *Mayer*, 1994b and references therein) and suggested to represent a monolayer coverage over all mineral surfaces. Later, *Mayer* and *Xing* (2001) found the extent of surface loading in acid soils to depend on pH, with higher loadings (close to 1 mg OC m^{-2}) at low pH, and interpreted this as evidence for a patchy distribution of OM on mineral surfaces. However, detailed investigations of the silt- and clay-sized minerals in the PP1090 soils led us to different conclusions:

Kahle et al. (2003b) and *Kleber* et al. (2004) found higher Fe oxide densities in coarse-clay *versus* fine-clay fractions to correspond with higher OC loading in coarse- *versus* fine-clay fractions. This indicates the ability of minerals to preserve OC results from the combined influence of reactive surface sites and a large specific surface area. Figure 5 shows the OC loading for a number of soils investigated in the course of the SPP differentiated into (1) A horizons from acidic forest soils (Waldstein, Steinkreuz, Borsberg, Zeughaus), (2) in B and C horizons of these same soils, and (3) in A horizons from circumneutral, mainly illitic arable soils (Halle, Rotthalmünster). It shows a decline of OC loadings with depth, obviously mirroring OC contents and thus suggesting a strong coupling of OC loadings to OM input (*Kaiser* and *Guggenberger*, 2003). Only the A horizons have a loading higher than the so-called monolayer equivalent. Scanning

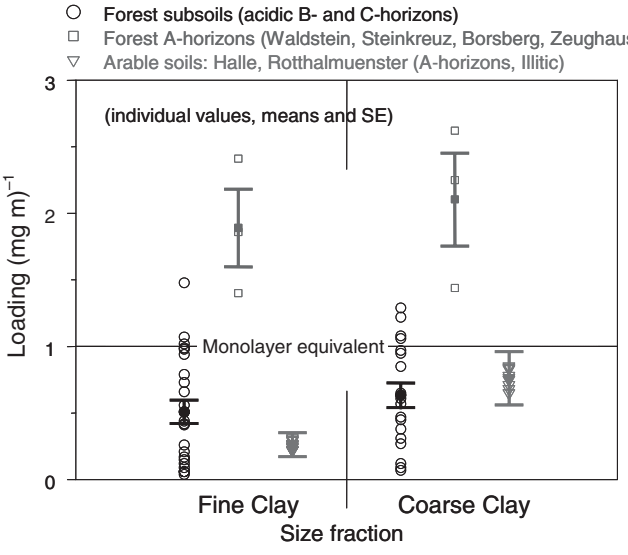

Figure 5: Organic matter loadings for fine-clay and coarse-clay particle-size fractions. Error bars represent mean values and the standard error (SE) of the means. The dotted horizontal line indicates the loading level commonly taken to represent a monolayer equivalent of organic matter over all surfaces and was termed the monolayer-equivalent (ME) level (*Mayer*, 1994a). The term monolayer-equivalent is stressed, because it is reasonably well established that organic matter covers mineral surfaces in a discontinuous manner (*Ransom* et al. 1998; *Arnarson* and *Keil,* 2001; *Mayer* and *Xing*, 2001; *Kahle* et al., 2002).

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Table 1: Amount and distribution of light and heavy OM fractions in different soils.

Reference	Comments	Soil type	Description	Depth or horizon	fPOM (C in fraction % of SOC)	oPOM (C in fraction % of SOC)	Mineral (C in fraction % of SOC)	Free ($g\,kg^{-1}$)	Occluded ($g\,kg^{-1}$)	Mineral ($g\,kg^{-1}$)
Roscoe and *Buurman* (2003)	D = 1.7 $g\,cm^{-3}$	Typic Haplustox	Cerrado	0–7.5 cm	25.9	40.5	33.7	25.8	40.4	33.6
		Typic Haplustox	after conversion into cropland, no tillage	0–7.5 cm	23.9	45.8	30.3	21.2	40.7	26.9
		Typic Haplustox	after conversion into cropland, plow tillage	0–7.5 cm	25.1	45.5	29.4	24.0	43.6	28.2
		Typic Haplustox	Cerrado	7.5–15 cm	29.8	41.6	28.6	28.3	39.5	27.1
		Typic Haplustox	after conversion into cropland, no tillage	7.5–15 cm	29.4	42.7	27.8	27.3	39.6	25.8
		Typic Haplustox	after conversion into cropland, plow tillage	7.5–15 cm	26.7	43.5	29.8	25.2	41.1	28.2
Rasmussen et al. (2005),	D = 1.6 $g\,cm^{-3}$, ^{14}C analyses	Ultic Haploxeralfs	Granite soil	A2	37.6	24.7	37.6	14.0	9.2	14.0
		Ultic Haploxeralfs	Granite soil	Bt	5.4	6.3	88.2	4.8	5.6	78.0
		Ultic Haploxeralfs	Granite soil	BC	18.8	47.8	33.3	1.3	3.3	2.3
		Ultic Haploxeralfs	Andesite-Granite soil	A2	30.6	18.6	50.8	22.5	13.7	37.4
		Ultic Haploxeralfs	Andesite-Granite soil	Bt	17.0	24.4	58.7	4.8	6.9	16.6
		Ultic Haploxeralfs	Andesite-Granite soil	BC	17.9	50.0	32.1	3.0	8.4	5.4
John et al. (2005)	D = 2.0 $g\,cm^{-3}$, oPOM originally determined for two density fractions	Luvisol (Rottalmünster)	Wheat	0–30 cm	3.3	10.1	86.6	0.4	1.2	10.3
		Luvisol (Rottalmünster)	Maize	0–30 cm	4.1	9.1	86.8	0.5	1.2	11.2
		Luvisol (Rottalmünster)	Grass	0–10 cm	3.7	10.3	85.9	0.9	2.6	21.5
		Luvisol (Rottalmünster)	Grass	10–20 cm	3.6	5.4	91.0	0.4	0.6	10.6
		Luvisol (Rottalmünster)	Grass	20–30 cm	5.9	6.2	87.9	0.5	0.5	7.5
		Luvisol (Rottalmünster)	Forest	0–7 cm	33.6	18.0	48.4	13.5	7.3	19.5
		Luvisol (Rottalmünster)	Forest	7–25 cm	20.4	16.8	62.8	1.9	1.6	6.0
		Luvisol (Rottalmünster)	Forest	25–40 cm	14.6	8.2	77.2	0.5	0.3	2.5
Swanston et al. (2005)	D = 1.6 $g\,cm^{-3}$, 14C analyses	Ultisol	Forest	0–15cm	25.5	16.7	49.7			
		Ultisol	Forest	15–30cm	23.8	14.9	51.9			
Echeverria et al. (2004)	D = 2 $g\,cm^{-3}$	Hapludults, Kanhapludults	Eatonton	0–10 cm or A horizon	10.2		89.8			
		Kanhapludults	Athens	0–10 cm or A horizon	4.8		95.2			
		Paleudults, Haplohumods	Waycross	0–10 cm or A horizon	12.1		87.9			
		Haplothords	N. Florida	0–10 cm or A horizon	14.7		85.3			
Rovira and *Vallejo* (2003)				mediteranean calcareous forest soils	H	89.2	4.7	6.1		

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Reference	Comments	Soil type	Description	Depth or horizon	fPOM (C in fraction % of SOC)	oPOM (C in fraction % of SOC)	Mineral (C in fraction % of SOC)	Free ($g\,kg^{-1}$)	Occluded ($g\,kg^{-1}$)	Mineral ($g\,kg^{-1}$)
				mediteranean calcareous soils	A	57.8	16.8	25.4		
				mediteranean calcareous soils	B	26.1	17.2	56.8		
Golchin et al. (1994)	D = 1.6 $g\,cm^{-3}$, NMR	Solodic (Natrixeralf)	mixed forest with grass ground cover	0–10 cm	19.3	9.2	71.5			
		Noncalcic brown earth	(Hapludalf)	Poplar box forest with blue and wallaby grasses	0–10 cm	14.7	16.6	68.7		
			Grey Clay (Chromustert)	Brigalow forest	0–10 cm	31.3	17.5	51.2		
			Red-brown earth (Rhodoxeralf)	Old pasture (>60 yrs)	0–10 cm	7.7	9.7	82.6		
			Black earth (Pellustert)	Native grassland	0–10 cm	6.9	15.6	77.5		
Eusterhues and Kögel-Knabner (unpublished)	D = 2 $g\,cm^{-3}$	Cambisol (Steinkreuz)	acid forest under beech	0–5 cm or Ah horizon			10.0			8.3
		Cambisol (Steinkreuz)	acid forest under beech	5–24 cm or Bv horizon			38.0			3.7
		Cambisol (Steinkreuz)	acid forest under beech	24–50 cm or SdBv1 horizon			42.0			3.4
		Cambisol (Steinkreuz)	acid forest under beech	50–80 cm or SdBv2 horizon			68.0			2.0
		Cambisol (Steinkreuz)	acid forest under beech	85–115 cm or IIICv horizon			89.0			1.0
		Cambisol (Steinkreuz)	acid forest under beech	115–140 cm or IVCv1 horizon			73.0			0.8
Eusterhues and Kögel-Knabner (unpublished)	D = 2 $g\,cm^{-3}$	Podzol (Waldstein)	acid forest under spruce	0–6 cm or Aeh horizon			19.0			7.2
		Podzol (Waldstein)	acid forest under spruce	6–9 cm or Bsh horizon			27.0			25.1
		Podzol (Waldstein)	acid forest under spruce	9–10 cm or Bs horizon			33.0			17.2
		Podzol (Waldstein)	acid forest under spruce	10–47 cm or Bv1 horizon			98.0			7.8
		Podzol (Waldstein)	acid forest under spruce	47–60 cm or Bv2 horizon			75.0			1.5
		Podzol (Waldstein)	acid forest under spruce	60–100 cm or Cv horizon			73.0			1.5
Kaiser and Guggenberger (unpublished)	D = 1.6 $g\,cm^{-3}$	Cambisol (Steinkreuz)	acid forest under beech	0–5 cm or Ah horizon	65.0	12.0	23.0	45.3	8.4	16.0
		Cambisol (Steinkreuz)	acid forest under beech	5–24 cm or Bw1 horizon	40.0	10.0	50.0	4.4	1.1	5.5
		Cambisol (Steinkreuz)	acid forest under beech	24–50 cm or Bw2 horizon	15.0	8.0	77.0	0.5	0.3	2.7
		Cambisol (Steinkreuz)	acid forest under beech	50–80 cm or Bw3 horizon	10.0	9.0	81.0	0.2	0.1	1.3
		Cambisol (Steinkreuz)	acid forest under beech	80–85 cm or 2C horizon	5.0	7.0	88.0	0.1	0.1	1.3
		Cambisol (Steinkreuz)	acid forest under beech	85–115 cm or 3C horizon	10.0	6.0	84.0	0.1	0.1	1.2

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Reference	Comments	Soil type	Description	Depth or horizon	fPOM	oPOM	Mineral	Free	Occluded	Mineral
					(C in fraction % of SOC)	(C in fraction % of SOC)	(C in fraction % of SOC)	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})
		Cambisol (Steinkreuz)	acid forest under beech	115–140 cm or 4C horizon	10.0	5.0	85.0	0.1	0.0	0.7
	D = 1.6 g cm^{-3}	Podzol (Waldstein)	acid forest under spruce	0–10cm or Aeh horizon	67.0	8.0	25.0	24.6	3.0	9.5
		Podzol (Waldstein)	acid forest under spruce	10–12 cm or Bh horizon	16.0	11.0	73.0	15.0	10.3	68.1
		Podzol (Waldstein)	acid forest under spruce	12–30 cm or Bs horizon	9.0	12.0	79.0	4.7	6.2	41.0
		Podzol (Waldstein)	acid forest under spruce	30–55 cm or Bw horizon	7.0	5.0	88.0	0.5	0.4	6.7
		Podzol (Waldstein)	acid forest under spruce	55–70 cm or C1 horizon	5.0	5.0	90.0	0.1	0.1	2.1
		Podzol (Waldstein)	acid forest under spruce	70–80 cm or C2 horizon	5.0	3.0	92.0	0.1	0.0	1.4
Baisden et al. (2002)	fPOM oPOM < 1.6–2.2 g cm^{-3} DF > 2.22 g cm^{-3}	Sierra Nevada	annual grassland	0–2.5 cm	45.0	40.0	8.0	26.6	23.6	4.7
				2.5–9 cm	27.0	54.0	17.0	7.8	15.7	4.9
				9–22 cm	3.0	49.0	57.0	0.4	5.9	6.8
		Sierra Nevada	annual grassland	0–2 cm	27.0	34.0	20.0	4.1	5.2	3.1
				2–10 cm	10.0	35.0	43.0	0.8	2.6	3.2
				10–29 cm	7.0	35.0	51.0	0.2	0.9	1.3
		Sierra Nevada	annual grassland	0–2 cm	54.0	30.0	6.0	10.8	6.0	1.2
				2–12 cm	11.0	50.0	24.0	6.2	28.0	13.4
				12–24 cm	7.0	27.0	56.0	0.1	0.5	1.1
		Sierra Nevada	annual grassland	0–2 cm	21.0	43.0	26.0	12.0	24.5	14.8
				2–11 cm	9.0	24.0	66.0	0.9	2.3	6.3
				11–40 cm	2.0	9.0	88.0	0.1	0.4	3.9
Chenu and *Plante* (2006)	tPOM 1 .6 g cm^{-3} organo-mineral: >1.6 g cm^{-3} and <2.2 g cm^{-3}	France	forest under pine	0–30 cm	2.3		86–83	66.0		104.0
		France	maize since 35 years, converted from forest	0–30 cm	1.4		84–76	16.0		41–42

electron microscopy of the fine fractions shows that the OM is not uniformly distributed over all clay-mineral surfaces: whereas most phyllosilicate surfaces are free of OM, some platelets are continuously coated across their basal surface (Fig. 6, Eusterhues et al., unpublished, *cf.*, also *Bachmann* et al., 2008, this issue, pp. 14–26). Accordingly, we could not find strong correlations between the mineral-bound organic C and the total specific mineral surface (Fig. 7a, *Eusterhues* et al., 2005b). On the other hand, strong correlations exist between the mineral-bound OC and the Fe oxide content for the subsoils (Fig. 7b; *Eusterhues* et al., 2005b; unpublished). This suggests that Fe oxides are the dominant carrier for mineral-bound OM in the subsoils. In contrast to subsoil horizons, A horizons of all soils contain too much C or insufficient

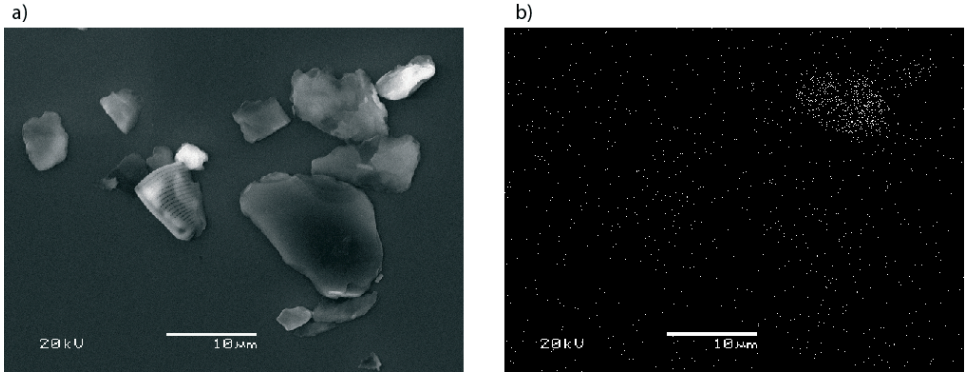

Figure 6: SEM micrograph of silt and clay particles from the topsoil of the Rottalmünster Luvisol: (a) secondary electron image; (b) EDX dot map of C distribution of image a.

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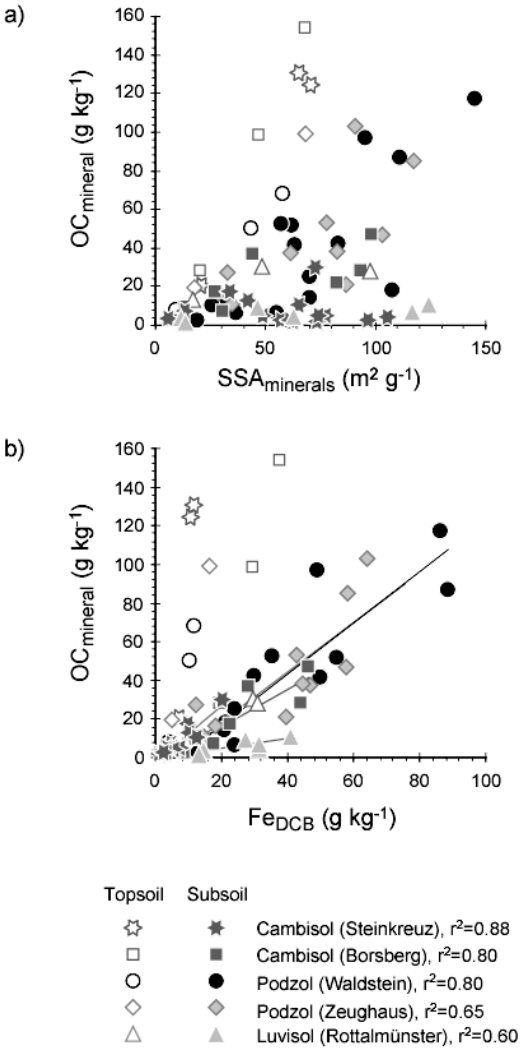

Figure 7: Relation between mineral-bound OM and (a) specific surface area or (b) Fe oxide content. $OC_{mineral}$ is the organic-C concentration of the density fraction >2 g cm^{-3}, $SSA_{minerals}$ the BET-N_2 surface area measured after destruction of the organic matter with $Na_2S_2O_8$, Fe_{DCB} is the concentration of dithionite-citrate-bicarbonate-extractable Fe.

Fe oxides to follow the observed trend. In the topsoil, we either assume a larger C loading on Fe oxides or a greater importance of other mineral surfaces. As can be deduced from the sorption experiments of OM on goethite and ferrihydrite, the capacity of these minerals to protect sorbed OM is smaller than their capacity to bind it (*Kaiser* and *Guggenberger*, 2007a; *Kaiser* et al., 2007). Different C-to-FeDCB ratios between topsoils and subsoils or between the acid forest subsoils and the neutral arable subsoil can be caused by differences in (1) the sorbate composition, (2) the amount of protonated hydroxyl groups at different pH, (3) the OM input, and (4) the specific Fe oxide-surface area (*Kaiser* and *Guggenberger*, 2003; *Eusterhues* et al., 2005b; *Kleber* et al., 2005). Mass balance calculations, trying to reconstruct the measured total mineral-surface area by summing up assumed specific surface areas of the individual mineral phases (*cf.*, *Eusterhues* et al., 2005b for details) indeed yield varying specific surface areas for ferrihydrite (300–800 m^2 g^{-1}) and goethite (25–300 m^2 g^{-1}) in different soils. In the Luvisol, the lowest specific surface areas of 300 m^2 g^{-1} for ferrihydrite and 25 m^2 g^{-1} for goethite were sufficient to reconstruct the measured specific surface area. In Fig. 8, the total mineral-bound OC is related to these calculated surface areas of ferrihydrite and goethite. Regression lines for the subsoils of all soils show similar slopes, quantifying the OM loading within the range of 1.1 to 1.9 mg C per m^2 oxide surface. These loadings must be regarded as maximum values since a certain fraction is additionally fixed on the phyllosilicates and probably also on poorly crystalline allophane-like phases. However, these loadings are higher but in the same magnitude than compared to maximum loadings found for sorption of the so-called hydrophilic fraction of DOM on goethite (0.22–0.50 mg C m^{-2} goethite) and the so-called hydrophobic fraction of DOM (1.13–1.28 mg C m^{-2} goethite; *Kaiser* and *Guggenberger*, 2003). The mono-layer-equivalent coating is assumed to be approx. 1 mg C m^{-2} (*Keil* et al., 1994; *Mayer*, 1994a), while *Bergamaschi* et al. (1997) found typical loadings of 2–5 mg m^{-2} for sediments underlying high-productivity zones. *Murphy* et al. (1990) report a Langmuir-type isotherm for the sorption of peat humic acid on hematite, showing its sorption maximum at 1.1 mg C m^{-2} hematite. However, at high concentrations of humic acid (approx. 5–20 μmol mL^{-1}), the sorption plateau passes into a rising slope again, corre-

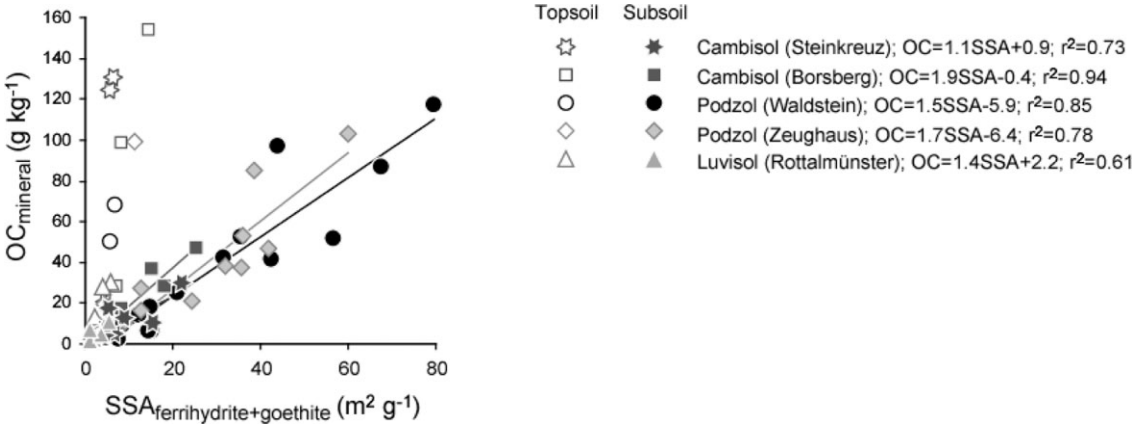

Figure 8: Relation between mineral-bound OM and specific surface area of Fe oxides.

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sponding to, *e.g.*, 2.8 mg C m^{-2} hematite at 20 μmol mL^{-1}. This was interpreted as reflecting multiple layering of humic acids on the mineral surface.

The fact that all subsoils, though different in Fe oxide content, pH, and pedogenesis, show similar C loadings suggests that 1–2 mg C m^{-2} might be a typical OC loading for Fe oxides in soil. We concluded that Fe oxides are the most important sorbents for the formation of organo-mineral associations in the subsoil, while phyllosilicate surfaces are comparatively less important.

7 Composition of OM in organo-mineral associations

Methods that can be applied to solid samples, such as analytical pyrolysis, (thermo)chemolysis, and ^{13}C-NMR spectroscopy are particularly useful to characterize the composition of the OM associated with the minerals. Generally differences of bulk-soil OM from different soils are not very pronounced (*Mahieu* et al., 1999), and bulk soil OM does also not reflect the different plant input. To identify the contribution of plant litter input, the particulate OM fractions are most useful. Figure 9 for the fractions from Rotthalmünster shows a plant debris composition, dominated by high proportions of O-alkyl C (mainly cellulose and hemicelluloses), followed by alkyl C, and smaller contributions of aromatic C, resembling the composition of the respective site-specific plant residues. Compared to that, the mineral-bound OM is generally depleted in lignin and phenolic components (*Guggenberger* et al., 1994; *Kiem* and *Kögel-Knabner*, 2003; *Schöning* et al., 2005a). This is also seen in the ^{13}C-NMR spectra for all soils investigated here (Fig. 9), as the signal intensity in the aromatic region (110–160 ppm) and especially the aryl-O signal at 150 ppm characteristic for phenolic compounds is low. *Baldock* et al. (1992) and *Mahieu* et al. (1999) already pointed out that clay-size fractions generally show a higher content of alkyl C than the whole soils and in particular the coarser fractions, as revealed by ^{13}C-NMR spectroscopy. Generally the fine or heavy fractions show higher proportions of carboxyl C than plant residues, indicative of the more oxidized stage of the stabilized OM. The fine fractions isolated from loamy soils are dominated by high proportions of O/N alkyl C, as demon-

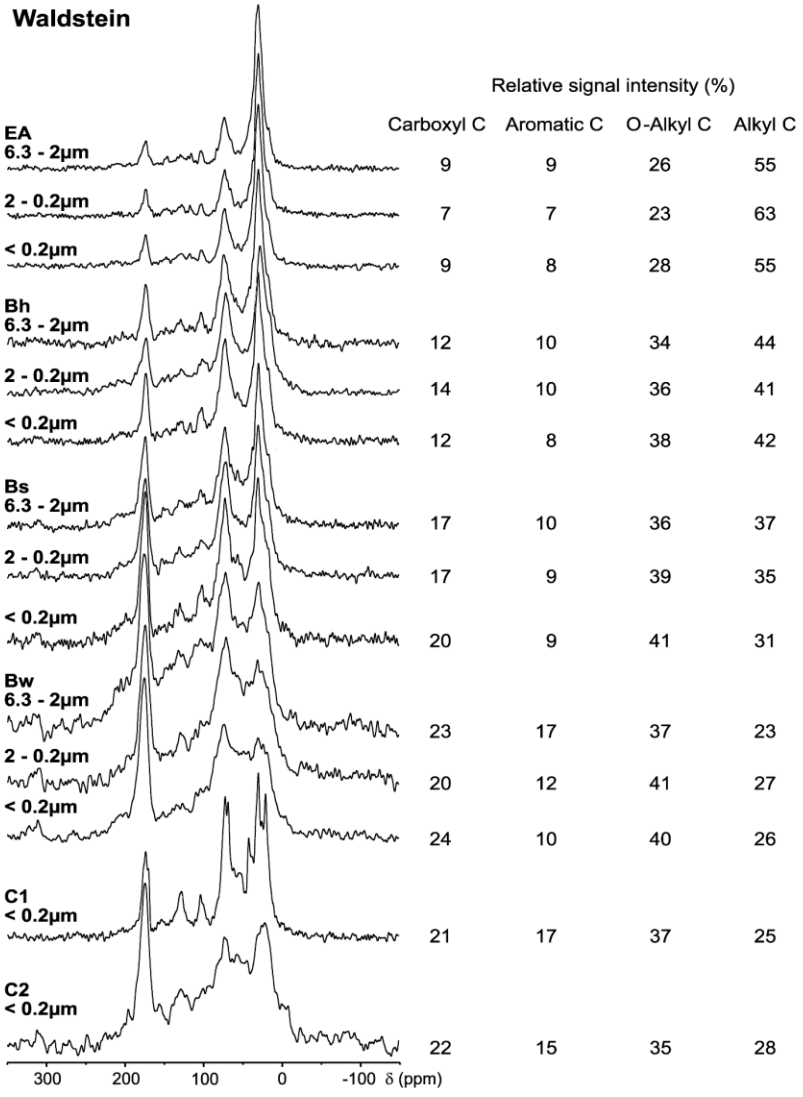

Figure 9: Composition of light and heavy fractions of organic matter isolated from the sites Bad Lauchstädt, Waldstein, Steinkreuz, and Rotthalmünster as obtained from the analysis by ^{13}C-NMR spectroscopy (from *Kiem* et al., 2003; *Rumpel* et al., 2004; *Helfrich* et al., 2006).

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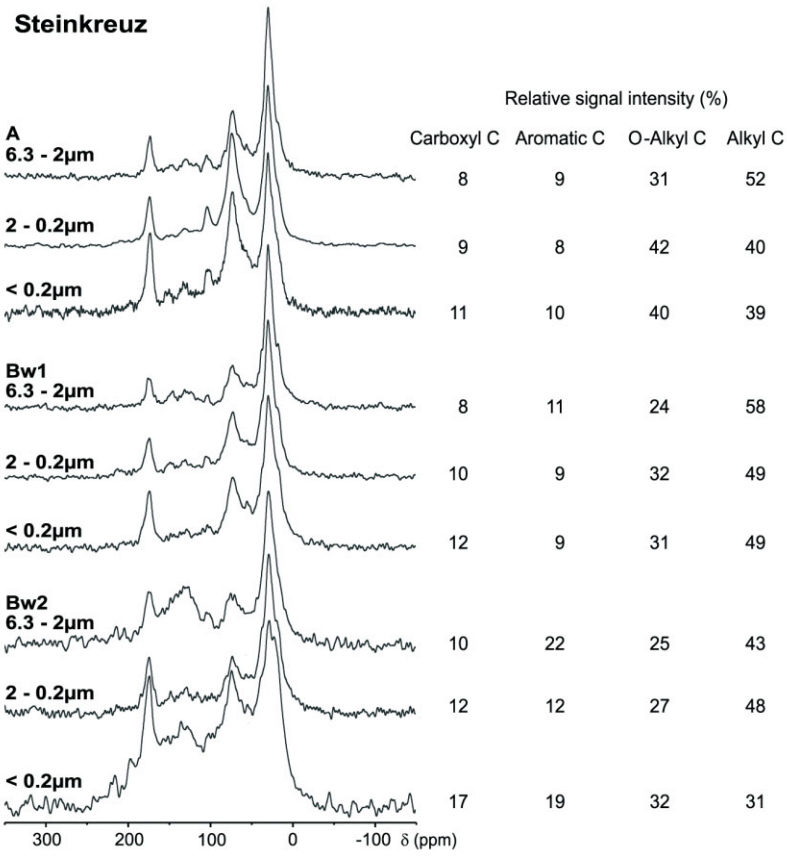

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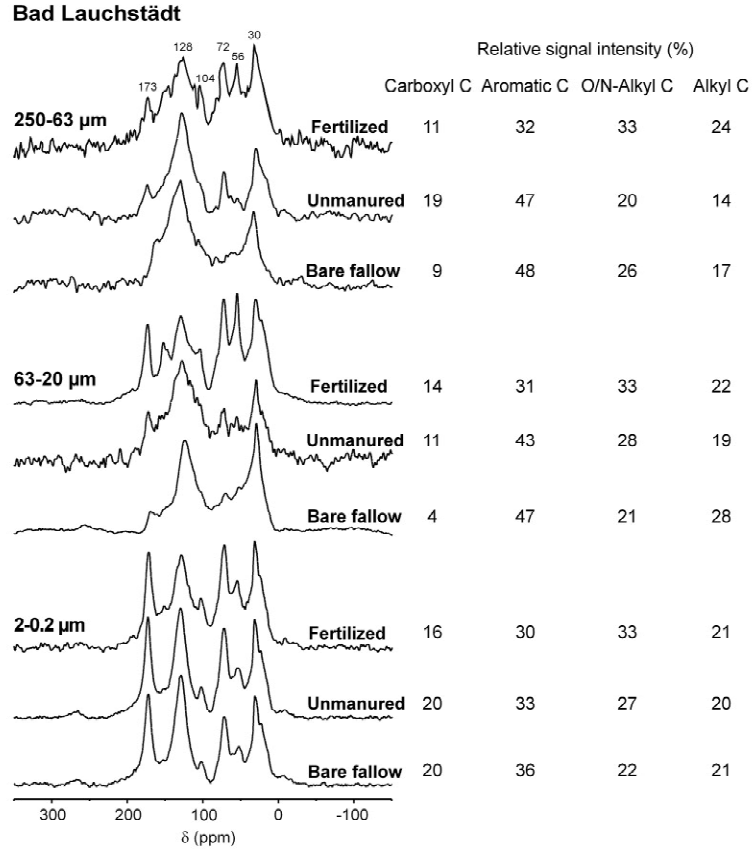

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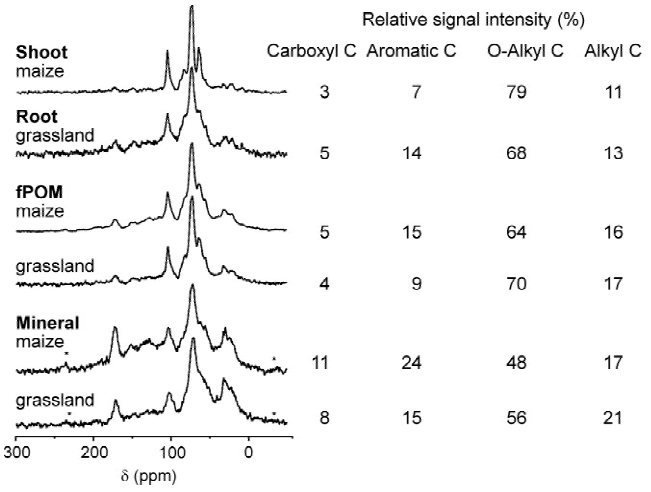

Figure 9: continued

strated for the Rotthalmünster soils. They are mostly assigned to polysaccharides and proteins, with large contributions of microbial polysaccharides (*Guggenberger* et al., 1994; *Kiem* and *Kögel-Knabner*, 2003). In contrast, the OM in the fine fractions of sandy soils are dominated by long-chain aliphatic molecules in the topsoil horizons, illustrated for both acid sandy soils Waldstein and Steinkreuz by the signal at 30 ppm. At present, it is not clear, what the mechanism underlying these differences in the composition of sandy and loamy soils is. In the subsoil, the fine fraction of the sandy acid forest soils is composed of low molecular weight organic acids, as indicated by the dominance of alkyl C in combination with the high carboxylic signal intensity around 175 ppm in the ^{13}C-NMR spectra. This reflects the formation of the acid subsoil horizons *via* a chromatographic process that accumulates small, highly oxidized organic acids in the deeper horizons (*Rumpel* et al., 2004).

In soils containing charred OM, a part of this char is found in association with the mineral phase, as can be delineated from HNO_3 oxidation and SEM (*Brodowski* et al, 2005, 2007). This leads to higher proportions of aromatic C with a high signal intensity around 130 ppm in the ^{13}C-NMR spectra as evident in the ^{13}C-NMR spectra from Bad Lauchstädt (Fig. 9), while other analytical techniques, such as pyrolysis or themochemolysis, are often not able to detect the charred OM.

8 Stability of OM in organo-mineral associations

8.1 Stabilizing effect of binding of OM to mineral surfaces: evidence from sorption experiments

Sorption to minerals generally reduces the susceptibility of OM towards oxidative attack (*Kaiser* and *Guggenberger*, 2003) and the bioavailability (*Kalbitz* et al., 2005). Interactions between soil minerals and DOM result in a distinct stabilization as indicated by increased mean residence times after sorption to a mineral subsoil (*Kalbitz* et al., 2005), soil minerals (*Mikutta* et al., 2007), and after precipitation by Al (*Scheel* et al., 2007) (Fig. 10a). So, the mineralization rate constants of a stable C pool (k2 of a double-exponential model) increased by a factor between 3 and 25 comparing DOM and sorbed OM (Fig. 10a; *Kalbitz* et al., 2005). This increase in stability against microbial decay was accompanied by an increase in thermal stability as shown by pyrolysis–field ionization mass spectrometry (Py-FIMS). The major volatilization peak of bulk DOM shifted by 200 K, 255 K, and 125 K after sorption of DOM from maize straw, Oi and Oa horizons, respectively. The volatilization curves of main compound classes (carbohydrates, phenols and lignin monomers, lipids, alkylaromatics, heterocyclic N-containing compounds, peptides) showed a thermal behavior similar to the bulk DOM (Fig. 10b). This indicates that inherently labile organic compounds of DOM such as carbohydrates are stabilized by soil minerals to a similar extent as more stable components like phenols and lignin monomers. *Kalbitz* et al. (2005) found that labile DOM from maize straw or Oi horizons was stabilized by soil minerals to a larger extent than already stable DOM from Oa horizons. This observation coincides with a larger shift in the Py-FIMS volatilization peak after sorption of this labile

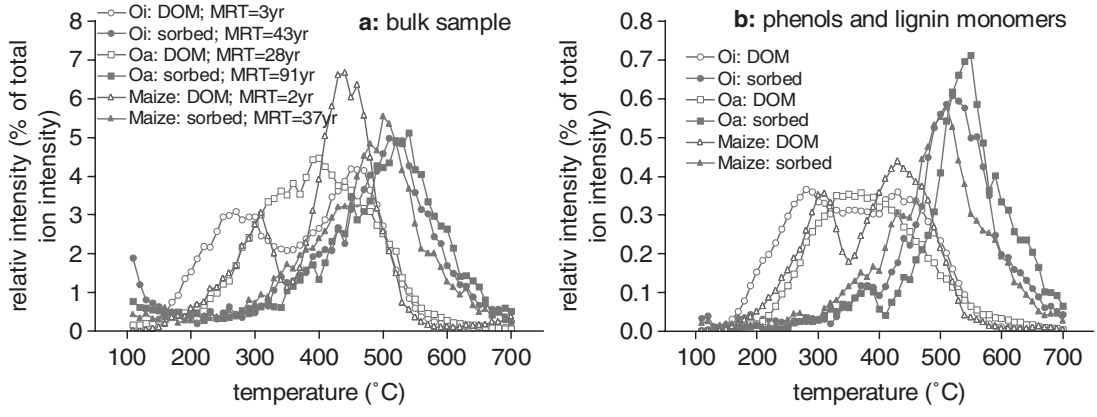

Figure 10: Thermograms of bulk samples (a) and of phenols and lignin monomers (b) of dissolved organic matter (DOM) from the Oi and Oa horizon of a podzolic soil and from maize straw before and after sorption to a mineral soil. Data for DOM before sorption are derived from *Kalbitz* et al. (2003) whereas data after sorption are unpublished. These data were obtained in a similar way like in *Kalbitz* et al. (2003). The mean residence times (MRT) of the bulk samples were published by *Kalbitz* et al. (2005).

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DOM (maize, Oi: +200 to +255 K) in comparison to sorption of stable DOM (Oa: +125 K). That means, the extent of stabilization against microbial decomposition was indicated by the extent in the shift towards higher temperatures after sorption. This is in line with *Buurman* et al. (2002) who explained high oxidation temperatures of OM from strongly developed profiles with high C-to-Fe and C-to-sesquioxide ratios by the stabilization of OM in OM-metal precipitates and in differences in organic chemistry. Coincidence of thermal stability and resistance against microbial decomposition was also reported in other studies by Py-FIMS (*Schulten* and *Leinweber*, 1999, 2000). This is explained by a correlation between the activation energy required for the thermal bond cleavage in Py-FIMS and the chemical energy required for the enzymatic cleavage of chemical bonds in microbial-decomposition processes. From these results, we can conclude that stabilization of DOM by organo-mineral interactions can be traced by two independent methods, measurements of C mineralization in incubation experiments and analysis of the thermal behavior using Py-FIMS. More studies are required to get deeper insight into the energetic details of OM bonds, mineral associations, and microbial decomposition. *Mikutta* et al. (2007) showed that OM bound to minerals mainly by ligand exchange was more resistant against mineralization than OM held by noncoulombic interactions (van der Waals forces). Furthermore, Ca bridges enhanced the stability of sorbed OM, but less than the binding *via* ligand exchange (*Mikutta* et al., 2007).

8.2 Stabilizing effect of binding of OM to mineral surfaces: age and turnover of OM in organo-mineral associations isolated from soils

When comparing the ^{14}C ages of the density fractions <1.6 g cm^{-3} with those of the fractions >1.6 g cm^{-3} (Tab. 2), it is obvious that the ^{14}C age of the latter is generally higher, with the exception of the three uppermost horizons at Wald-

Table 2: Organic carbon concentrations and storage in different fractions of the soils Steinkreuz and Waldstein, along with the ^{14}C activity in percent modern carbon and ^{14}C age in years before 1950 (*Eusterhues* et al., 2003, 2005; Kaiser and Guggenberger, unpublished); numbers in brackets indicate OC percentage in the fractions related to bulk soil OC.

Horizon	Depth	OC concentration	OC storage	^{14}C activity bulk soil	^{14}C age bulk soil	^{14}C activity of H_2O_2-resistant fraction	^{14}C age of H_2O_2-resistant fraction	^{14}C activity of fraction oxidized by H_2O_2[a]	^{14}C age of fraction oxidized by H_2O_2 [a]	^{14}C activity of fraction <1.6 g cm^{-3} [a]	^{14}C age of fraction <1.6 g cm^{-3} [a]	^{14}C activity of fraction >1.6 g cm^{-3}	^{14}C age of fraction >1.6 g cm^{-3}	^{14}C activity of fraction >1.6 g cm^{-3} and resistant to NaOCl treatment[a]	^{14}C age of fraction >1.6 g cm^{-3} and resistant to NaOCl treatment[a]
	(cm)	(g kg^{-1})	(kg m^{-2})	(pm C)	(y BP)	(pm C)	(y BP)	(pm C)	(y BP)	(pm C)	(y BP)	(pm C)	(y BP)	(pm C)	(y BP)
Steinkreuz (Cambisol)															
A	0–5	82.6	4.29	112.3	modern	104.5	modern (11)	112.9	modern (89)	112.4	modern (77)	111.9	modern (23)	99.3	60±30 (1)
Bw1	5–24	9.8	2.41	101.3	modern	86.6	1150±22 (5)	101.8	modern (95)	104.8	modern (50)	98.0	160±25 (50)	72.3	2606±35 (5)
Bw2	24–50	3.0	0.79	92.1	655±25	71.8	2665±35 (16)	95.9	340 (84)	119.0	modern (23)	84.2	1375±30 (77)	36.2	8170±80 (12)
Bw3	50–80	1.4	0.40	80.9	1700±30	51.5	5325±45 (27)	92.0	672 (73)	122.8	modern (19)	69.8	2890±30 (81)	31.0	9400±240 (24)
3C	85–115	1.1	0.14	80.6	1758±56	49.3	5675±45 (30)	93.1	571 (70)	117.2	modern (16)	70.8	2780±45 (84)	21.6	12310±170 (31)
4C1	115–140	0.5	0.07	76.3	2165±30	66.8	3245±40 (58)	88.7	959 (42)	116.4	modern (15)	69.1	2960±30 (85)	21.4	12390±190 (34)
Waldstein (Podzol)															
EA	0–10	38.1	2.90	93.6	525±30	88.1	1015±25 (3)	94.2	479 (97)	92.0	655 (75)	98.5	120±25 (25)	90.7	835±25 (3)
Bh	10–12	92.8	1.04	98.5	120±25	91.4	725±20 (6)	99.5	40 (94)	95.8	435 (27)	99.5	30±20 (73)	85.7	1290±25 (15)
Bs	12–30	52.0	5.47	91.1	745±40	72.6	2575±25 (9)	92.8	597 (91)	87.0	1010 (21)	92.2	700±25 (79)	81.1	1715±20 (24)
Bw	30–55	7.7	2.09	82.2	1570±25	73.1	2515±30 (13)	83.3	1471 (87)	87.3	980 (12)	81.5	1640±20 (88)	70.5	2805±35 (38)
C1	55–70	1.7	0.25	62.0	3840±70	55.7	4700±35 (25)	64.1	3577 (75)	90.8	730 (10)	58.8	4265±30 (90)	43.7	6650±90 (47)
C2	70–80	1.9	0.19	62.0	3840±70	52.0	5260±45 (25)	65.4	3412 (75)	112.8	modern (8)	56.5	4580±30 (92)	42.3	6910±110 (52)

[a] calculated by difference

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stein. *Rasmussen* et al. (2005) also reported higher ^{14}C ages for mineral-associated OM than for free particulate OM. Investigating six density fractions from <1.65 g cm^{-3} to >2.55 g cm^{-3}, *Sollins* et al. (2006) found too much charcoal in the two lightest fractions to allow for a reliable turnover estimate, but a continuous increase in turnover time from 150 to >980 y with increasing density in the subsequent density fractions. The relatively low ^{14}C activity in the two lightest density fractions is probably caused by charcoal from a forest fire (*Sollins* et al., 2006). Black C particles were frequently observed in the light fraction from the Waldstein surface-soil horizons and likely contribute to the relatively high ^{14}C age in this fraction (*Marschner* et al., 2008, this issue, pp. 91–110). In the PP1090 forest soils, the proportion of total OC and ^{14}C age within the heavy fraction (>1.6 g cm^{-3}) increased, concurrent with the increase of the ^{14}C age of the oxidation-resistant fraction with soil depth. Such results were reported by a number of studies (*e.g.*, *Torn* et al., 1997; *Paul* et al., 2001; *Swanston* et al., 2005) and are indicative of increased mineral association and protection of OM at greater depths.

As noted above, extraction of OM provides no unambiguous results due to its unspecific nature. Nevertheless, combined information from various extracts together with information of the mineral assemblage may provide some useful information on the nature of the organic-mineral association. Sodium pyrophosphate has been applied sequentially after preliminary extraction of soil with water (*Ellerbrock* et al., 2005) and NaOH (*Wattel-Koekkoek* and *Buurman*, 2004) in order to obtain more stable OM fractions. For instance, the pyrophosphate-soluble OM from two agricultural soils (Rotthalmünster and Halle) has been found to exhibit a slower turnover than the water-extractable OM (*Ellerbrock* et al., 2005). Similarly, pyrophosphate-extractable OM from Mozambiquan clay fractions was found to contain older and probably more recalcitrant organic constituents than the OM extracted by NaOH (*Wattel-Koekkoek* and *Buurman*, 2004). Pyrophosphate has also been used to study the impact of metal-organic complexes on the stabilization of OM. For a set of terrace soils including Alfisols, Inceptisols, and Mollisols, *Masiello* et al. (2004) observed close correlations of the ^{14}C inventories with both, pyrophosphate-extractable Al and Fe (r = 0.98 and 0.95, respectively). These correlations have been taken as evidence that complexation of OM by Al and Fe is an important mechanism for the stabilization of OM.

Helfrich et al. (2007) applied stepwise hydrolysis with trifluoroacetic acid to two agricultural soils (Rotthalmünster and Halle) after removal of particulate and humified OM and found also an enrichment of old OM in the sample residuum as reflected by ^{13}C and ^{14}C isotope patterns. The increase in the apparent ^{14}C age of OM following hydrolysis was comparable to those observed after oxidative treatments using H_2O_2, $Na_2S_2O_8$, and NaOCl (>1000 y). These results fit quite well to earlier data from a Saskatchewan arable soil (Canada), showing that OM being resistant to 6 *N* HCl hydrolysis is oldest and concurs with the ^{14}C age of coarse clay and fine silt (*Anderson* and *Paul*, 1984). According to *Paul* et al. (2006), the nonhydrolyzable fraction shows an average of 1,200 y greater mean residence times than total SOC.

Chemical degradation with H_2O_2 (Tab. 2) was used in two acid sandy soils under forest to isolate a stable, residual OM fraction (*Eusterhues* et al., 2003, 2005a). Generally, the ^{14}C activity was smaller for the H_2O_2-resistant fraction than for the fraction oxidized by H_2O_2, meaning that in the resistant fraction, OC has a noticeably higher mean age. With increasing soil depth, the contribution of the H_2O_2-resistant fraction increased, as did the mean ^{14}C age. This could indicate a more effective stabilization mechanism in the subsoil horizons.

Treatment of the >1.6 g cm^{-3} fraction with NaOCl left a fraction behind that is oldest amongst all fractions isolated by the different techniques (Kaiser and Guggenberger, unpublished; Tab. 2). The proportion of this old C fraction on total OC increased from <5% in the surface soil to approx. 50% in the deepest horizons, again showing the pronounced protection of OM in the subsoil. However, a ^{14}C age of >12,000 y in the NaOCl-resistant fraction of the 3C and 4C1 horizons at Steinkreuz may indicate the presence of geogenic OC in the Triassic sedimentary parent material. Such high ^{14}C ages of NaOCl-resistant mineral-associated OM from subsoil horizons confirms the conclusion of *Kaiser* and *Guggenberger* (2003) that potential OM stabilization at mineral surfaces is limited to those OM molecules that sorb to surfaces with small surface loading. Turnover rates of OM are probably related to the time when the organic molecules are sorbed; the molecules that are sorbed first are best stabilized. Also *Mikutta* et al. (2006) reported ^{14}C ages in a range of millenia for the NaOCl-resistant fraction of 12 bulk soils, while those of the NaOCl-removable fraction is mostly modern. Such differences indicate that mineral-associated OM may not just represent a stable, passive C pool but also a more active cycling one. This is corroborated by *Swanston* et al. (2005) who made use of an inadvertant ^{14}C-pulse label to a whole forest system, caused by the incineration of medical waste. While in an uncontaminated control soil, ^{14}C activity in the dense fraction was much less than in the free and occluded light fractions, ^{14}C from the pulse label was rapidly incorporated into the dense fraction, showing that this organo-mineral fraction comprises highly stable material as well as more recent inputs (*Swanston* et al., 2005). This is also obvious by mean residence time estimates for mineral-bound OM employing the ^{13}C–natural abundance approach which is usually in the range of decades to a few centuries (*Skjemstad* et al., 1990; *Balesdent* et al., 1998). In contrast to the ^{14}C method, the ^{13}C–natural abundance approach gives an estimate of turnover dominated by relatively recent inputs and OC fractions that cycle within the time frame of the experiment (*Six* and *Jastrow*, 2002).

8.3 Stabilizing effect of binding of OM to surfaces: conceptual models accounting for varying degrees of sorptive preservation

The formation of stable OM with turnover times of up to several millenia by strong sorptive bondings to specific mineral surfaces is well-accepted, but the nature of the more rapidly cycling mineral-bound OM fraction is currently under discussion. According to *Kaiser* and *Guggenberger* (2003, 2007a),

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at higher OM loadings at the mineral surface, less organic ligands per organic molecule are involved in the bonding, making the organic molecules more flexible and extending more into the soil solution, thus being more susceptible for microbial decomposition. *Ellerbrock* et al. (2005) assume that mainly the interaction between hydrophilic functional groups within OM and polyvalent cations located at mineral surfaces result in OM-mineral complexes, which may protect OM against decomposition. Therefore, the OM-to-clay ratio, the presence (or absence) of polyvalent cations, and the OM composition may influence distribution and conformation of OM on mineral surfaces. *Sollins* et al. (2006) and *Kleber* et al. (2007) provide an alternative explanation stating a zonal structure for OM associated with mineral surfaces, with the outer layers of the OM having a more rapid turnover than the inner ones. And finally, microbial colonization of mineral particles must be considered. *Swanston* et al. (2005) argue that to the extent that the heavy fraction acts as a sink for microbial biomass and products, it must include an OC component that can cycle rapidly.

9 Relevance of organo-mineral interactions for C stabilization in soils—conclusions and future research perspectives

We are still far away from agreed protocols for the quantitative/complete isolation or extraction of the mineral-bound OM from soils. Conventional physical fractionation protocols have to be tested for more soils and compared with the results from the newly emerging chemical approaches. Approaches combining physical-fractionation procedures with chemical treatments might allow for isolating mineral-bound fractions more specifically.

There is a clear improvement in our understanding of how soil microorganisms and their food sources are distributed within microhabitats. Clay-sized organo-mineral associations are preferred habitat for a complex microbial community offering substrates as well as high surface area for adsorption and protection of soil microorganisms. The richness of the soil microbial community in these fractions is due to the spatial isolation of organisms and substrates of different quality. Future studies should not only consider microbial-mediated decomposition of OM in different fractions, but also microbial-mediated changes of mineral surfaces which contribute to C stabilization of OM. Surface conditioning of mineral surfaces by microorganisms, which actively excrete polysaccharides and proteins warrants more attention for the creation of stable organo-mineral associations, as suggested by high contributions of microbial-derived organic compounds and low C : N ratios in the fine fractions. Since current studies in soil ecology focused mainly on soil fractions destroying the natural micro-environment of particles and soil microorganisms, a further challenge is to understand the distribution, structure, and function of soil biota in the three-dimensional soil system.

The proportion of OC bound to minerals and stored in the clay fraction increases with increasing depth and is generally higher in the subsoil compared to the topsoil. Likewise, the strength of the bonding increases with soil depth, indicating the important role of subsoil OM in the OM stabilization. Clear differences are found between different soil types with respect to the type of OM in organo-mineral associations, although the underlying principles are not yet fully understood. Major components of the mineral-bound OM are of the alkyl and O/N-alkyl C type, whereas aromatic C is of minor importance, except for the presence of charred OM. In sandy acid soils, there is clear evidence for the stabilization of mainly alkyl C, which becomes more acidic and of lower molecular weight with increasing depth. Here, the stabilization seems to be mediated mainly *via* interaction with Fe oxides and short–range order Al silicates. In loamy soils, we find a much larger contribution of O/N alkyl C compounds, most probably microbial polysaccharides and proteins. This shows the importance of soil genesis on OM stabilization by formation of organo-mineral associations. At present, we have only a small data basis on the amount of OC in organo-mineral associations in selected soil types and horizons. A major task for future work is to enlarge our knowledge to more soil types, which also means a larger range in mineralogy.

Pedogenic oxides dominate the reactive surface area for organo-mineral interactions in all temperate soils investigated here. They seem to be especially important for the stabilization of OM in the subsoil. In arable topsoils developed from illitic loess, permanently charged siloxane surfaces of phyllosilicates are of additional relevance, although their specific surface area is generally much lower, compared to pedogenic Fe oxides. Total specific surface area, micropores, and mesopores could not be successfully used to predict OM accumulation by organo-mineral associations. This is most probably due to the fact that only a part of the mineral surfaces comes in contact with OM and that OM may not enter into small pore spaces. However, not the whole mineral-bound OM fraction shows the same (radiocarbon age, turnover, or) resistance against microbial degradation, so that we assume that effective stabilization only takes place for a certain fraction of the attached OM. It has also become clear that dissolved Al is a strong protective agent in acid soils. This mechanism is rather specific as restricted to acidic environments, but here, precipitation of OM by free Al is a significant control on OM preservation.

Major difficulties in the understanding and prediction of OM dynamics originate from the simultaneous operation of several mechanisms in addition to the protection by association of OM with mineral surfaces. This may be especially the case in A horizons, whereas we can often find the organo-mineral associations to be the dominating mechanism in subsoil horizons. Much more research is needed on the match between organic phase and mineral phase in relation to different soil types and conditions prevailing in these soils. Also, factors that control surface loading and coverage of minerals in different soil types need more attention in future work. We expect that identification and quantitation of the actual bonding mechanisms might explain a great deal of our data. For soil material this, however, is extremely challenging due to the heterogeneity and “imperfection” of the natural mineral surfaces as well as due to the small size of clays and oxides.

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Acknowledgments

We thank the *"Deutsche Forschungsgemeinschaft" (DFG)* for financial support of this work in the framework of the *Priority Program 1090 "Soils as source and sink of CO_2 – mechanisms and regulation of organic matter stabilisation in soils".* We further would like to express our thanks for the possibility to work at several long-term experimental sites, which were provided by the *Bayreuth Center of Ecology and Environmental Research, University of Bayreuth (BayCEER), the University of Halle-Wittenberg, The Helmholtz Centre for Environmental Research Leipzig-Halle (UFZ),* and the *"Höhere Landbauschule Rotthalmünster".* We would also like to express our thanks to *Margit von Lützow* and three anonymous reviewers for helpful comments and suggestions.

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