

Organic matter in particle-size fractions from A and B horizons of a Haplic Alisol

M. W. I. SCHMIDT & I. KÖGEL-KNABNER

Lehrstuhl für Bodenkunde, Technische Universität München, 85350 Freising-Weihenstephan, Germany

Summary

The organic matter in soils may be stabilized by its interactions with minerals. We have studied such interactions in a Haplic Alisol under forest in which clay and organic matter have migrated from an eluvial A horizon to accumulate in an illuvial B horizon. We have tried to trace the fate of organic matter in these horizons (Ah and Bvt) by determining clay mineralogy, carbon and nitrogen content, hydrolysable amino acids, lignin signature by alkaline CuO oxidation and carbon species by ^{13}C CPMAS NMR of bulk soils and particle-size fractions. In both horizons, most of the organic matter was present in O-alkyl and methylene structures, each contributing one-third to the bulk organic matter. In the Ah horizon the ratios of carbon-to-nitrogen, and yields for lignin and hydrolysable amino acids decreased as the particle-size class decreased, but side-chain oxidation of lignin compounds increased with decreasing particle size. In contrast to previous observations, the proportions of O-alkyl carbon increased as particle size decreased, constituting a major proportion of the organic carbon in the clay-size fractions from both the Ah and Bvt horizons ($\geq 38\%$), while proportions of methylene carbon decreased. Illite was the dominant mineral in the fraction $\leq 6\ \mu\text{m}$, whereas the mobile fine clay fraction ($<0.2\ \mu\text{m}$) was rich in smectites – minerals with large surface areas. Our results support the hypothesis that potentially labile organic matter, such as O-alkyl carbon typically present in polysaccharides, may be stabilized against further degradation in organomineral complexes.

Introduction

Soil organic matter comprises plant, animal and microbial residues in all stages of decay. The degree of decomposition of plant material in soil is controlled by its bioavailability. The microbial availability of plant material can be limited by the latter's binding to minerals, such as clay particles (Oades, 1990; Oades *et al.*, 1988; Sollins *et al.*, 1996). Generally, clay contents are positively correlated with soil organic matter concentrations, when other factors such as vegetation, climate and hydrology are similar (Davidson, 1995). However, recent research indicates that there is a protective capacity, characteristic of individual soils (Hassink & Whitmore, 1997). Some volcanic soils may have a greater stabilizing influence on organic matter than would be predicted from their clay contents, probably due to the presence of allophane and ferrihydrite, both of which have a large specific surface capable of adsorbing organic molecules (Saggar *et al.*, 1996; Parfitt *et al.*, 1997; Torn *et al.*, 1997).

Mechanisms of interactions between organic materials and clay minerals are poorly understood from laboratory studies. We know even less about naturally occurring organomineral complexes, though we do know that they migrate down the profile in a broad range of soils; characteristically in Alisols, Luvisols, Acrisols and Lixisols in the FAO terminology. In such soils the A horizon is depleted of clay by percolating water, which transports dispersed colloids and fine clay down the profile to accumulate in a Bvt horizon, where clay and organic matter are intimately associated with metal oxyhydroxides typically forming clay skins or cutans. The clay contents in the argic B horizon are always greater than in the overlying A horizon, often by as much as 20%. Strong sorptive interactions between organic matter and minerals potentially stabilize organic matter against degradation. Thus, such soils may contain large proportions of natural organomineral complexes, providing a unique opportunity to study how organomineral interactions affect organic matter.

Materials and methods

We selected a much-studied Haplic Alisol from near Siggen, Schleswig-Holstein, Germany (Coordinates: R $^{44}398000$, H $^{60}6017630$, Topographical Sheet DGK5 Süssau). Previous

Correspondence: M. W. I. Schmidt, University of Zürich, Department of Geography, Winterthurerstrasse 190, 8057 Zürich, Switzerland.

E-mail: mschmidt@geo.unizh.ch

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studies of its pedogenesis and ecology are summarized by Schimming *et al.* (1993). Here we focus on the organic matter from the Ah and Bvt horizons present in primary organo-mineral complexes obtained after complete dispersion of aggregates. We carried out a comprehensive range of analyses (clay mineralogy, carbon and nitrogen content, hydrolysable amino acids, lignin signature and carbon species) to characterize the organic matter in the bulk soil and in various particle-size fractions.

The soil studied is formed in Weichselian boulder marl. It lies 12 m above sea level on a slope of 1°. The mean annual precipitation is 570 mm, and mean annual temperature 8°C. The vegetation is beech-oak woodland (*Melico-Fagetum*), mainly *Fagus sylvatica* L., *Carpinus betulus* L., and *Quercus robur* L. which forms a mull humus. The mean age of the stand is approximately 100 years, and documents show that the site has been forested since the 13th century (Schimming *et al.*, 1993). The soil was described and sampled according to FAO (1994).

Sample pretreatment

The soil is a Haplic Alisol (FAO, 1994), or Typische Parabraunerde (AG-Boden, 1994). We sampled it in triplicate and combined subsamples. From a pit 1.5 m × 2 m × 2 m deep we took three samples for both the A and B horizons and combined them on site to give a bulk sample for each horizon. Horizons were designated according to the German Soil Survey Description (AG-Boden, 1994). Roots and visible plant remains were mechanically removed from mineral soil samples. After freeze-drying, the soil was crushed and passed through a 2-mm sieve. For the chemical analyses an aliquot of the soil was ground in a ball mill for 10 minutes.

Particle-size separation

In previous work Schimming *et al.* (1993) analysed mineral horizons for the particle-size distribution (Table 2) of the mineral phase using the pipette-sieve method after oxidation of organic matter with hydrogen peroxide (Gee & Bauder, 1986). In this study we have determined seven particle-size fractions from the Ah and Bvt horizons, by combining wet sieving and sedimentation after dispersing the soil completely by ultrasound (Schmidt *et al.*, 1999). Briefly, we calibrated the ultrasonic energy of an ultrasonic titanium probe with a diameter of 19 mm (Labsonic U, Braun Melsungen, Germany), and applied 440 J per ml of suspension. By wet sieving, the suspension was separated into three sand fractions (630–2000 µm, 200–630 µm, 63–200 µm). The clay fraction (< 2 µm) and three silt fractions (20–63 µm, 6–20 µm, 2–6 µm) were obtained by gravity sedimentation, and were recovered from the suspensions by filtration (cellulose nitrate, 0.45 µm). This procedure yielded organomineral particle-size fractions, and a soluble fraction < 0.45 µm which was collected. Standard

deviation for the ultrasonic dispersion-particle-size fractionation procedure was determined earlier for 12 samples including those studied here, and varied for individual fractions between 1 and 6 g kg⁻¹ whole soil (Schmidt *et al.*, 1999).

Dissolved organic matter

The concentration of dissolved organic carbon in the filtrate < 0.45 µm was determined with an aliquot of the solution (Shimadzu TOC 5050), and from these data the percentage of the total organic carbon was calculated.

Clay mineralogy

Clay mineralogy was determined on three size fractions (i.e. 2–6 µm, 0.2–2 µm and < 0.2 µm) by X-ray diffraction on a Phillips PW 1830/1820 (40 kV, 40 mA) instrument combined with a Huber SMC 9000 stepping motor set to 0.02° per second. The wavelength of the cobalt α₂ was 1.79026 Å. Samples were treated with glycol prior to analysis, and their components were estimated quantitatively by established procedures as described by Stanjek (2000).

Elemental analysis

Carbon and nitrogen content of the bulk soils and the various fractions were determined in duplicate with a Leco CNS 2000, with a minimum detection of 0.1 g kg⁻¹ (standard deviation 0.3 g kg⁻¹). The content of dissolved organic carbon was determined in an aliquot of the < 0.45-µm fraction with a Shimadzu TOC 5050 Analyzer.

Hydrolysable α-amino acids

The content of hydrolysable α-amino acid nitrogen was determined by the colorimetric ninhydrin method (Stevenson, 1982; Kögel-Knabner, 1995). Between 0.2 and 0.5 g of sample were hydrolysed in duplicate (6 M HCl and formic acid) for 12 hours at 110°C. The relative standard deviation for the determination of amino acid-nitrogen was 4.2% (determined from 33 samples) and for the colorimetric analysis 4.4% (*n* = 69). The precision of the whole method was ±0.080 g amino acid nitrogen per kg dry soil.

Lignin signature from alkaline CuO oxidation

Alkaline CuO oxidation was carried out as described by Kögel-Knabner (1995). Subsamples containing *c.* 50 mg organic carbon were added to 2 M NaOH in Teflon vials with CuO in triplicate. Vials were sealed under N₂ and heated for 2.5 hours at 170°C. After oxidation, the samples were acidified, and precipitates were removed by centrifuging. Samples were extracted by using solid-phase-extraction columns filled with C18 material (Baker, Germany). Silyl derivatives were

separated and quantified by gas chromatography–flame ionization detection (GC–FID). The total yields were determined for vanillyl (vanillin + vanillic acid), syringyl (syringaldehyde + syringic acid) and cinnamyl units (*p*-coumaric acid + ferulic acid), respectively. By combining the yields of vanillyl (V), cinnamyl (C) and syringyl (S) CuO oxidation products we obtain VSC-lignin yields, and these were either normalized to soil mass, or normalized to organic carbon of the bulk soil, or of the size fraction. The *p*-hydroxyl compounds are not included in the sum of phenolic oxidation products because they can also be derived from non-lignin structures. Relative standard deviations for the detection of the phenolic products was 9% determined on a sample of $n=10$. The acid-to-aldehyde ratios of vanillyl (ac/al)_V and syringyl (ac/al)_S and the syringyl-to-vanillyl (S/V) ratio are calculated as mass ratios.

¹³C CPMAS NMR spectroscopy

The solid-state ¹³C NMR spectra were obtained on a Bruker MSL 100 (25.178 MHz). The cross-polarization and magic angle spinning technique (CPMAS) (Wilson, 1987) was applied with a spinning speed of 4 kHz. All ¹³C NMR experiments were carried out with a contact time of 1.0 ms, a 90°¹H-pulse width of 6.6 ms and a pulse delay of 100–600 ms (Knicker, 1993). We applied a line broadening of 100 Hz before doing the Fourier transformation. Chemical shifts (Table 1) are reported relative to tetramethylsilane (=0 p.p.m.). For the relative intensity distribution of the solid-state ¹³C CPMAS NMR, the precision is approximately 10% of the signal intensity, depending on the signal-to-noise ratio. However, for spectra with low signal-to-noise ratios larger deviations can occur for the regions 220 to 160 p.p.m. and 45 to –10 p.p.m. (Knicker, 1993). We treated the samples with hydrofluoric acid (HF 10%) prior to NMR analysis to remove paramagnetic material, and to improve the resolution of the spectra. Schmidt *et al.* (1997) show that chemical alterations due to HF treatment are small.

Results and discussion

Although we tried to obtain as much information on the organic matter as possible, carbon and nitrogen concentra-

tions were too small for some of the organic geochemical analyses, especially in the bulk soils and mainly in the larger particle-size fractions. To circumvent these analytical limitations, we used two strategies. First, we selected the Ah horizon believed to contain organic matter similar to the underlying Al horizon but containing more carbon and nitrogen. Second, for both horizons, we analysed the smaller fractions ($\leq 63 \mu\text{m}$) with larger carbon and nitrogen concentrations, which nevertheless represent most (up to 74.8–91.4% of the bulk) of the organic carbon and nitrogen. Using this approach we could generate a set of data on the organic matter in the A and B horizon, which is – to the best of our knowledge – the first of this kind.

Physical characteristics, carbon and nitrogen content and mineralogy

The soil selected has features typical of a broad range of illuvial soils and additionally has brown oxyhydroxides in the illuvial horizon, so we designated it Bvt (v for 'Verbraunung').

Overall, the texture of the soil is dominated by silt and clay (53–61 mass %) throughout the profile (Schimming *et al.*, 1993). The clay content in the Bvt horizon (28.8 mass %) is characteristically larger than in the Ah horizon (15.7%), indicating clay accumulation (Table 2). For both horizons we determined clay mineralogy in the fine silt (2–6 μm) and clay (<2 μm) fractions, the latter separated into a coarse clay (0.2–2 μm) and fine clay (<0.2 μm) fraction (Table 3). The clay mineralogy is similar in both horizons. All three size fractions were dominated by illite ($\geq 48\%$), with smaller amounts of vermiculite and kaolinite (5–28%). The smaller size fractions contained more smectites (20–42%), which are typical constituents of clays accumulating in argillic horizons (Duchaufour, 1998). Iron and aluminium oxyhydroxides were also present (Table 2; Schimming *et al.*, 1993), as indicated by oxalate and dithionite-extractable Fe and Al, with rather similar concentrations in all the horizons we investigated. The Ah horizon typically contained more carbon (19.4 g carbon kg⁻¹ air-dry soil) and nitrogen than the underlying argillic Bvt horizon (4.2 g carbon kg⁻¹).

Mass proportions of the particle-size fractions determined by ultrasonic dispersion (Table 4) correspond well with those

Table 1 Chemical shift assignment of peaks in the solid-state ¹³C CPMAS NMR spectra (referenced to tetramethylsilane = 0 p.p.m.) (Wilson, 1987)

Chemical shift range /p.p.m.	Assignment
220–160	Carboxyl/carbonyl/amide carbons
160–140	Aromatic COR or CNR groups
140–120	Aromatic C–H carbons, guaiacyl C-2, C-6 in lignin, olefinic carbons
120–100	Anomeric carbon of carbohydrates, C-2, C-6 of syringyl units in lignin
100–60	O-alkyl of carbohydrates and alcohols, C- of some amino acids
60–45	Methoxyl groups, C- of most amino acids
45 to –10	2°, 3° and 4° carbons in alkyl structures

Table 2 Description of the soil

Horizon ^a	Depth /cm	pH (CaCl ₂)	Inorganic C	Organic C	Total N	C:N	Texture ^{b,c}			Iron extractable by ^b	
							Sand	Silt	Clay	Oxalate	Dithionite
			_____	/g kg ⁻¹	_____		_____	/mass %	_____	_____	/g kg ⁻¹
Ah	0–14	3.7	< 0.1	19.4	1.6	12	46.9	37.4	15.7	2.9	6.2
Alv	14–47	3.8	< 0.1	5.2	0.6	9	41.2	34.0	24.7	2.2	9.5
Bvt	47–91	4.3	< 0.1	4.2	0.5	8	39.5	32.1	28.8	1.9	10.4

^aHorizon designations (AG-Boden, 1994).

^bData from Schimming *et al.* (1993).

^cResults obtained by standard particle-size analysis (Gee & Bauder, 1986).

obtained by standard particle-size analysis, except for slightly smaller yields for sand fractions. This indicates a more effective dispersion of sand particles by ultrasound than by standard analysis, as Schmidt *et al.* (1999) have observed. The particle-size distribution of the Ah and Bvt horizons is similar, except for the larger clay contents in the Bvt horizon. The < 63- μm -sized particles contributed 43.4% (Ah) and 57.9% (Bvt) to the total soil mass. Carbon and nitrogen concentrations were largest in < 20- μm fractions, with larger values in the Ah horizon than in the Bvt horizon. To exclude the effect of different absolute carbon and nitrogen concentrations, we calculated the relative contributions of individual fractions to the total soil organic matter. Clay-sized fractions contributed most of the total soil organic matter, both for organic carbon (Ah: 55.9% of total carbon, Bvt: 66.4%) and for nitrogen (Ah: 71.8% of total nitrogen, Bvt: 78.0%), with considerably smaller contributions ($\leq 8.3\%$ of total carbon and nitrogen) from the silt- and sand-sized fractions. The observed distribution seems typical of clayey and loamy soils when compared with previous studies, summarized by Christensen (1996). Previous studies have shown that carbon-to-nitrogen ratios (C:N) decreased from coarse to fine fractions, suggesting an increasing decomposition. In the soil studied here, C:N ratios were smallest in the clay fractions, except for the coarse silt fraction of the Ah horizon. The C:N ratios for the sand fractions could not be calculated because they contained too little nitrogen for

accurate measurement. In the following investigations, we focus on the $\leq 63\text{-}\mu\text{m}$ fractions, representing most (between 74.8 and 91.4%) of the total organic carbon or nitrogen present in both the Ah and Bvt horizons.

Hydrolysable α -amino acids

We measured the hydrolysable α -amino acids in bulk soils and in particle-size fractions (Table 5). The coarse fractions contained too little nitrogen for analysis, so we examined only bulk soils (Ah, Bvt) and the fine fractions < 20 μm of the Ah horizon. So we can compare the α -amino acid distributions in samples differing in total nitrogen content, we report our results as proportions of total nitrogen. In the bulk soils, contributions of hydrolysable α -amino acids to the total soil nitrogen were larger in the Ah horizon (46 mass % of the total soil nitrogen) than in the Bvt horizon (39%). The total mass of the particle-size fractions was small (40.8% of the total mass recovered), but they held 83.3% of the total nitrogen (Table 4) and 87% of the total hydrolysable amino acid nitrogen (Table 5). Proportions of hydrolysable α -amino acids decreased consistently from medium silt (66%) and fine silt (57%) to the clay fraction (39%). These trends are similar to results summarized by Christensen (1996). Overall, it seemed that larger proportions of the nitrogen resisted hydrolytic attack (i) in smaller particle-size fractions than in larger ones, and (ii) in the Bvt horizon than in the Ah horizon.

Table 3 Clay mineralogy of the particle-size fractions

Fraction / μm	Smectite	Vermiculite	Illite	Kaolinite
	/ % of total			
Ah				
2–6	0	19	61	19
0.2–2	20	5	63	11
< 0.2	30	8	54	8
Bvt				
2–6	0	28	57	15
0.2–2	20	15	52	14
< 0.2	42	5	48	5

Lignin signature

Phenols derived from lignin by alkaline CuO oxidation (Table 6, Figure 1) provide two types of information. First, tentative lignin sources can be assigned, since phenolic oxidation products depend on the vegetation from which the soil organic matter was formed (Ertel & Hedges, 1984). Lignin from gymnosperm wood produces mainly vanillyl type (V) oxidation products, whereas lignin from angiosperm wood produces syringyl units (S) in addition to vanillyl units. Large yields of cinnamyl units (C) are characteristic of non-woody tissues of both types of plants. Here, angiosperms dominate, as indicated

Table 4 Mass distribution, concentration and distribution of carbon and nitrogen in the particle-size fractions of Ah and Bvt horizons

Horizon ^a and size fraction / μm	Mass /% of bulk ^b	Organic C		N		C:N
		/g kg ^{-1c}	/% of bulk	/g kg ^{-1c}	/% of bulk	
Ah						
Coarse sand 630–2000	4.3	1.3	0.4	< 0.1	0.4	–
Medium sand 200–630	16.3	1.5	1.4	< 0.1	1.0	– ^d
Fine sand 63–200	23.5	5.7	8.3	< 0.1	1.6	–
Coarse silt 20–63	11.9	4.9	3.4	0.8	6.0	6
Medium silt 6–20	10.6	13.3	8.2	0.8	5.3	17
Fine silt 2–6	5.2	24.1	7.3	1.9	6.2	13
Clay <2	15.7	56.6	55.9	6.8	71.8	8
DOM ^e	–	–	5.4	–	–	–
% recovery	87.5		90.4		92.1	
Bvt						
Coarse sand 630–2000	3.9	1.0	0.9	< 0.1	–	–
Medium sand 200–630	12.9	0.6	1.8	< 0.1	–	–
Fine sand 63–200	19.7	0.7	3.3	< 0.1	–	–
Coarse silt 20–63	10.9	0.8	2.1	< 0.1	–	–
Medium silt 6–20	9.1	2.7	5.9	0.3	5.5	9
Fine silt 2–6	7.9	3.8	7.1	0.5	7.9	8
Clay <2	30.0	9.3	66.4	1.3	78.0	7
% recovery	94.4		87.6		91.4	

^aAG-Boden (1994).^bParticle-size fractions obtained after ultrasonic dispersion. For analytical details and standard deviation see Materials and methods.^cExpressed as g kg⁻¹ size fraction.^dNitrogen concentrations too small to calculate.^eDissolved organic matter. For analytical details and standard deviation see Materials and methods.

by a S/V ratio of 0.90 for the bulk soil – typical for a forest soil under deciduous trees (Guggenberger *et al.*, 1994). Second, lignin degradation can be assessed by calculating ratios for individual units. We used the ratios acid-to-aldehyde of the vanillyl or syringyl units, (ac/al)_V and (ac/al)_S, respectively, and the ratio S/V. Increasing ratios of (ac/al) would indicate increasing side-chain oxidation of the phenylpropane unit, for example by microbial degradation (Hedges *et al.*, 1988).

For the bulk Ah horizon (Table 6) the yield of lignin-derived phenols (8.5 g VSC kg⁻¹ C) varied within the range reported for other A horizons under forest (Zech *et al.*, 1992). The size fractions < 63 μm hold the majority (> 74.8%) of the organic carbon and an even larger percentage (93%) of the bulk lignin. Lignin yields generally decreased from coarse to fine fractions, suggesting correspondingly increased lignin decomposition. This was supported by increasing ratios of (ac/al)_V and

Table 5 Hydrolysable α -amino acids for the bulk soils and size fractions. Yields (mass amino-nitrogen) are expressed in two ways: (i) as proportions of the total nitrogen present in the bulk soil or fraction, and (ii) as proportions of the total nitrogen present in the bulk soil

	Hydrolysable α -amino acid-nitrogen ^a expressed as mass percentage of total nitrogen	
	Present in the bulk soil or fraction, respectively	Present in the bulk soil
Ah bulk	46	100
20–2000 μm	– ^b	
Medium silt	66	8
Fine silt	57	8
Clay	39	71
Sum	Not applicable	87
Bvt bulk	39	100

^aFor analytical details and standard deviation see Materials and methods.^bNitrogen concentrations too small for analysis.

Table 6 Lignin yields from the bulk soil of the Ah horizon and from separate size fractions. VSC-lignin is the combined yield of vanillyl (V), cinnamyl (C) and syringyl (S) units (g VSC kg^{-1}), determined as CuO oxidation products. VSC-lignin was normalized to organic carbon (bulk soil, respectively, size fraction)

	Yields ^a /g VSC kg^{-1} C
Bulk soil	8.5
20–2000 μm	– ^b
Coarse silt	9.7
Medium silt	9.6
Fine silt	7.2
Clay	8.1
Sum	7.9
% of bulk	93

^aFor analytical details and standard deviation see Materials and methods.

^bCarbon concentrations too small for analysis.

$(\text{ac/al})_s$ with decreasing particle size (Figure 1), indicative for an increasing oxidative decomposition of lignin (Hedges *et al.*, 1988), confirming previous observations (Guggenberger *et al.*, 1994). Declining S/V ratios demonstrated that syringyl units were degraded preferentially to vanillyl units. Overall, the lignin signature suggested progressive lignin decomposition (i.e. oxidation) from coarse to fine size fractions.

¹³C CPMAS NMR spectroscopy

Figure 2 shows the ¹³C CPMAS NMR spectrum obtained from the bulk Ah horizon, and Table 7 lists the integrated

areas. Chemical shift assignments (p.p.m.) are given in Table 1. Two sets of signals dominated the spectrum. First, resonances in the alkyl-C region (45 to –10 p.p.m.) contributed 31% of the total signal intensity. The major signal centred at 33 p.p.m. may be attributed to methylene carbon. Second, resonances (34%) in the O-alkyl region (60–110 p.p.m.) indicate the presence of alcohol or ether structures, the signal at 72 p.p.m. corresponding to ring C-5, C-3 and C-2 carbons and that at 106 p.p.m. corresponding to the anomeric carbon in polysaccharides. Smaller signals in the sp^2 -hybridized carbon region (160–110 p.p.m.) indicate the presence of protonated and C-substituted aryl carbon (110, 130 p.p.m.) and O-substituted aromatic carbon (150 p.p.m.), typically present in lignin and condensed tannins (Preston *et al.*, 1997). In the carboxyl C region (220–160 p.p.m.) we observed broad signals attributable by Preston & Schnitzer (1984) to carboxyl carbon, amide and ester structures.

Particle-size fractions larger than 63 μm from the Ah horizon contained too little carbon to provide well-resolved spectra, even after HF treatment. The smaller fractions, however, comprised 74.8% of the total organic matter, and produced reasonable spectra (Figure 2b–f) which can be taken as typical of most of the total organic carbon. Spectra obtained from the size fractions (Figure 2b–f), very similar to those of the bulk soil, revealed signals for alkyl C (33 p.p.m.), O-alkyl C (72, 89 and 106 p.p.m.), carboxyl groups (175 p.p.m.), and aromatic C, probably from lignin units, as could be seen from signals at 106, 130 and 116 p.p.m. with weak shoulders at 150 p.p.m. In the bulk argillic Bvt horizon, the carbon concentration was too small for us to obtain reasonably resolved spectra. The clay-sized fraction, however, contained

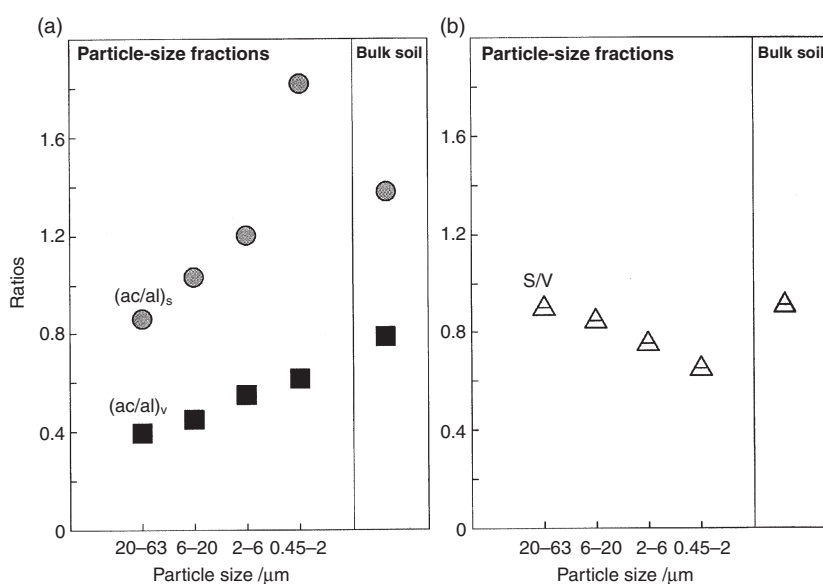


Figure 1 Lignin parameters of the Ah horizon, both bulk soil and particle-size fractions, all calculated on a mass basis. (a) Acid-to-aldehyde ratios of vanillyl $(\text{ac/al})_v$ and syringyl $(\text{ac/al})_s$ units. (b) Ratio of syringyl-to-vanillyl units (S/V).

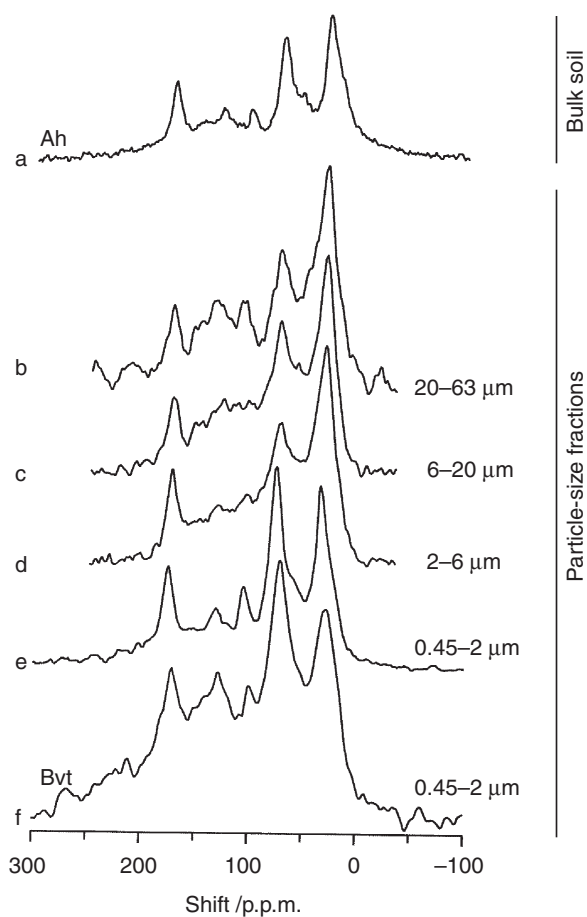


Figure 2 ^{13}C cross-polarization solid-state NMR spectra of the bulk Ah horizon (a) and its size fractions (b–e), and of the clay fraction from the Bvt horizon (f). The spectra were obtained from samples treated with hydrofluoric acid, as described in Materials and methods.

66.4% of the total organic carbon and produced a good spectrum, basically very similar to that of the clay-sized fraction from the Ah horizon.

The results obtained from ^{13}C CPMAS spectroscopy were surprising in two aspects. First, previous work on peats, organic soil horizons, and particle-size fractions of agricultural A horizons showed that the ratio of alkyl C-to-O-alkyl C increased when decomposition proceeded (Baldock *et al.*, 1997). An increasing ratio can be interpreted as an increasing degree of decomposition of polysaccharides (indicated by signals in the O-alkyl C region) from plants, and an enrichment of methylene structures (signals in the alkyl C region) by the microbial biomass. To visualize potential trends better, we calculated the ratio of alkyl C-to-O-alkyl C (Table 7), and designated it as the decomposition index D . In progressing from coarse to fine particle-size fractions, relative signal intensities of O-alkyl C increased, whereas intensities from alkyl C decreased. This trend was reflected by a decrease in the value of D from coarse silt (D : 0.92) to clay (D : 0.58) fractions, suggesting a decreasing degree of decomposition. This decrease, however, does not match the decreasing C:N ratios, and the progressive lignin decomposition. It is in striking contrast to trends observed in various previous studies, including that by Oades *et al.* (1987) on an Australian soil. Intensities for aromatic C peaked in the coarse silt-sized fraction, while signals for carboxyl C varied (9–17%), as found in many soils (Mahieu *et al.*, 1999). Second, in the clay-sized fractions, a major proportion of the O-alkyl C (31%) was probably present as polysaccharides, which we concluded from the distinct signal centred around 72 p.p.m. in combination with the resonance at 106 p.p.m. Methylene structures, indicated by the peak at 33 p.p.m., contributed less (26%) to the total signal intensity. Schmidt *et al.* (2000) found that the clay-sized

Table 7 Relative areas of peaks in ^{13}C CPMAS NMR spectra from bulk soils and size fractions

Sample	Carboxylic C	Aromatic C	O-alkyl C	Alkyl C	Decomposition index D^b
	160–220 p.p.m.	110–160 p.p.m.	45–110 p.p.m.	–10–45 p.p.m.	
	% of total signal intensity ^a				
Ah bulk	18	17	34	31	0.91
Ah size fractions					
63–2000 μm	– ^c	–	–	–	–
Coarse silt	10	21	36	33	0.92
Medium silt	9	18	42	31	0.74
Fine silt	10	16	41	34	0.83
Clay	14	15	45	26	0.58
Bvt bulk	–	–	–	–	–
Bvt size fractions					
2–2000 μm	–	–	–	–	–
Clay	18	21	38	23	0.60

^aFor analytical details and standard deviation see Materials and methods.

^bDecomposition index D = alkyl C-to-O-alkyl C.

^cCarbon concentrations too small for analysis.

fractions of a Podzol, containing most of the oxides and clay minerals, also held large proportions of potentially labile polysaccharide C and small proportions of aromatic C. These findings accord with results from laboratory experiments which suggest that sorption of dissolved organic matter to soils and oxides result in a preferential binding of aromatic structures and a discrimination against polysaccharides and methylene structures (Kaiser *et al.*, 1997). Limited quantitative reliability of ^{13}C CPMAS NMR spectra due to the presence of interfering paramagnetic material was probably less important, because all samples were treated with hydrofluoric acid, which effectively removed paramagnetic minerals (Schmidt *et al.*, 1997).

The origin of the polysaccharides in the clay-sized fractions from the Ah and Bvt horizon remains unclear, since soil polysaccharides can originate from plants or microorganisms. We have not analysed individual sugars, but there are three reasons which favour a microbial origin for the polysaccharides in the clay fraction. First, the lignin signature suggests that decomposition of plant material is advanced. The most abundant biopolymer in plants is cellulose, a polysaccharide intimately associated with the chemically more resistant lignin. Thus, it is very likely that cellulose is also degraded. Second, the clay fractions have small C:N ratios (≤ 10), which are typical of microbial biomass. Third, Guggenberger *et al.* (1994) and Oades *et al.* (1987) show that polysaccharides in clay-sized fractions are mainly of microbial origin.

Obviously there must be mechanisms stabilizing potentially labile polysaccharides against further degradation. These mechanisms may be related to the migration of intimately interacting organic and mineral matter in clay-sized organomineral complexes. In many soils similar to the one we have studied, smectites dominate in the mobile fine clay fraction and provide large internal and external surface areas, though we are not yet sure how accessible internal sorption sites are to most organic matter. In the fine clay of the soil studied we found large proportions of both polysaccharides and smectites. Sorptive interactions could explain stabilization of polysaccharides against decomposition, as discussed by Baldock & Skjemstad (2000).

Conclusions

We have used a combination of clay mineralogical and elemental analysis, solid-state ^{13}C NMR spectroscopy, acid hydrolysis and alkaline CuO oxidation to study the distribution and chemical structure of organic matter in naturally occurring organomineral complexes from the Ah and Bvt horizon of a Haplic Alisol.

In the Ah horizon most of the organic matter was present in polysaccharide and methylene structures, each contributing one-third to the bulk organic matter. Data for the size fractions support the hypothesis that decomposition of soil organic matter increases with decreasing particle size. Progressing from coarse to fine particle-size fractions, the Ah horizon shows decreasing C:N ratios, decreasing yields for

VSC-lignin and hydrolysable amino acids, and increasing side-chain oxidation of VSC-lignin. However, ^{13}C CPMAS spectroscopy shows that the proportions of methylene carbon decrease while contributions from O-alkyl C – very likely polysaccharides – increase, contributing a dominant proportion to the organic matter in the clay fractions from the Ah (45%) and the Bvt horizon (38%). The increasing proportions of potentially labile polysaccharides from coarse to fine fractions in Ah and Bvt horizons was unexpected and described here for the first time for such a soil. Small, microbial-like C:N ratios and analogies with previous published work suggest that plant material was decomposed, thus favouring a microbial origin for the polysaccharides. The fact that potentially labile polysaccharides accumulated in the clay-sized fractions may be related to the close association of clay and organic matter in the soil studied. Sorptive organomineral interactions with clay-sized minerals with large surface areas could protect these polysaccharides against further degradation. In fact, smectite – a clay mineral with large surface area – dominated the mobile, fine clay fractions of our soil.

Our results suggest that in clay-sized fractions of soils similar to the one we studied, potentially labile structures such as polysaccharides and other organic matter can be protected against further degradation, probably through organomineral interactions.

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