



Organic matter accumulating in Aeh and Bh horizons of a Podzol — chemical characterization in primary organo-mineral associations

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Abstract

The chemical structure of soil organic matter from the eluvial (Aeh) and (illuvial) Bh horizon of a Podzol was studied in primary organo-mineral associations by ¹³C CPMAS NMR spectroscopy and acid hydrolysis. In Podzols, organic matter is leached from the forest floor and Aeh horizons into the Bh horizon, where it is intimately associated with the mineral phase. In the Aeh horizon the majority of the residual organic matter was present in methylene structures, contributing 42% to the organic matter associated with the clay fraction. In the Aeh horizon decreasing particle size was typically accompanied by increasing ratios alkyl C-to-O-alkyl C, suggesting increasing decomposition of polysaccharides for the residual organic matter. The illuvial Bh horizon, containing high proportions of iron- and aluminum-oxides and hydroxides, was higher in aromatic carbon, while polysaccharides were similar and methylene carbon were smaller than in the Aeh horizon. In the particle size separates proportions of polysaccharides were constant, resulting in decreasing ratios alkyl C/O-alkyl C. Proportions of hydrolyzable amino acids were larger in the Aeh horizon (52% of the total N) than in the Bh horizon (21%), both for bulk soils and size separates. Our results suggested that in Bh horizons of Podzols, aromatic structures and also presumably labile structures like polysaccharides can be stabilized by organo-mineral associations. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Soil organic matter; Stabilization; ¹³C CPMAS NMR; Particle size separates; Podzol

1. Introduction

Soil organic matter (SOM) comprises plant, animal and microbial residues in all stages of decay, intimately mixed with inorganic soil components (Kögel-Knabner, 1993). SOM can be studied in primary organo-mineral associations obtained after complete dispersion of aggregates. Previous investigations of SOM associated with primary organo-mineral associations revealed a distinct trend from plant derived coarse particles to microbial derived fine particles, suggesting an increasing extent of decomposition (Turchenek and Oades, 1979). Nitrogen enrichment in finer size separates, was reflected by consistently lower

C/N ratios, and supported the hypothesis of increasing degree of decomposition of plant residues (Christensen, 1996).

¹³C CPMAS NMR spectroscopy was applied to follow the extent of decomposition of SOM in particle size separates of A horizons from agricultural soils in Australia (Baldock et al., 1992, 1997). Sand size separates were dominated by O-alkyl C, probably mainly due to polysaccharides in plant residues. Clay fractions were dominated by alkyl C which is considered to relate to a gradual accumulation of plant derived waxes and microbial methylene type structures. Contributions from aromatic C reached a maximum in the silt size separate and decreased in the fine separate, whereas carboxyl C varied. Similar trends were found in a recent statistical analysis of published ¹³C CPMAS NMR of mainly surface soil (A) horizons (Mahieu et al., 1999). Baldock et al. (1992) have ascribed these textural differences to two simultaneous processes, whose net effects change with

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particle size. The first mechanism was the physical and chemical breakdown of sand sized plant material reflected by increasing loss of *O*-alkyl C structures. The resulting lignin-rich debris contributed to the aromatic carbon maximum in silt sized separates. Size reduction and lignin degradation continued, resulting in highly altered aromatic matter intimately associated with clay minerals. The second process was accumulation of nitrogen as polysaccharides and lignins are sequentially converted via microbial biomass to a highly aliphatic residue, including appreciable polysaccharides. The majority of NMR spectroscopical informations on particle size fractions originate from surface (A) horizons, mainly under agricultural use (Mahieu et al., 1999). We are not aware of a NMR spectroscopical study on particle size separates from subsoil (B) horizons under forest use.

In the present study, particle size separates from A and B horizons of a Podzol under forest have been examined. Podzols exhibit a characteristic sequence of horizons created by strong leaching and acidity (Duchaufour, 1998). Podzols are poor in available nutrients, and have low pH, which implies that biological homogenization is virtually absent. This results in well-defined profiles with thick organic horizons, overlying the mineral A horizon which is darkened in its upper portion by organic materials. The lower (eluvial) A horizon is almost white sand, resulting from acidic and organic-rich percolating water, which washes soluble and colloidal materials down the profile. The dark (illuvial) B horizon is a zone of net accumulation, where organic matter is intimately associated with metal oxyhydroxides. Podzols can act as chromatographic systems for percolating soluble and colloidal material, according to sorption intensity. Laboratory experiments showed that hydrophobic dissolved organic matter, mainly lignocellulose-derived, sorbs strongly, whereas the hydrophilic fraction, more labile structures, like free polysaccharides, sorb weakly (Guggenberger and Zech, 1993; Kaiser and Guggenberger, 2000; Kaiser and Zech, 1998). Strong sorptive interactions could stabilize organic matter against degradation.

The objective of the present study was to elucidate the chemical composition of the organic matter in the Aeh and Bh horizon of a Podzol in bulk soils and seven particle size separates. We used a suite of complementary methods, including elemental analysis, ¹³C CPMAS NMR spectroscopy and hydrolyzable α -amino acids.

2. Materials and methods

2.1. Sampling sites

The Podzol Flaesheim was situated in the southern Münsterland in Flaesheim, Germany, south of Haltern (coordinates: R ²⁵83575, H ⁵⁷32175, Topographical Sheet TK25 4209 Haltern). The height above sea level was

approximately 87 m, slope 2°, north aspect. The mean annual precipitation was 800 mm, and mean annual temperature 10°C. The vegetation has been forest since the beginning of the 20th century, and was *Pinus sylvestris* at the time of sampling, forming a mor humus type.

2.2. Sample pretreatment

Soil description, sampling and classification were performed according to established procedures (FAO, 1994). Horizons were designated according to the German Soil Survey Description (AG-Boden, 1994). Bulk densities of the mineral horizons were determined gravimetrically in standardized steel rings after drying at 105°C. Roots and visible plant remains were mechanically removed from mineral soil samples. After freeze-drying, the soil aggregates were crushed and particles > 2 mm were removed by dry sieving. For chemical analysis an aliquot of the soil sieved to < 2 mm was ground in a ball mill for 10 min. The pH was measured with a glass electrode in the supernatant of a mixture of 0.01 M CaCl₂ and soil (2.5:1 based on mass).

2.3. Particle size separation

Two particle size separation procedures were used. Mineral horizons were analyzed for the particle size distribution of the mineral phase (Table 1) following the standard pipette/sieve method (Gee and Bauder, 1986). From the Aeh and Bh horizons we isolated seven particle-size separates, obtained by a combination of wet sieving and sedimentation (Table 2) after complete dispersion 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 ml⁻¹ of suspension to

Table 1
Description of the Haplic Podzol

Horizon ^b	Depth cm	pH	C _{org}		Total N	C:N	Sand	Silt	Clay
			g kg ⁻¹						
L	+13	— ^a	482.0	13.0	37	—	—	—	
Of	+10	—	334.1	13.5	25	—	—	—	
Oh	+5	—	258.0	9.7	27	—	—	—	
Aeh	12	3.1	14.5	0.4	36	92.4	6.5	1.3	
Ae	25	3.3	3.1	0.2	16	92.2	8.5	0.3	
Bh	31	2.9	34.5	1.0	35	87.1	8.9	4.1	
Bs	35	3.4	13.6	0.6	23	85.0	11.4	3.7	
Bsv	140	4.0	1.2	0.1	—	95.9	2.3	1.8	
Bv	>140	4.2	0.5	0.1	—	96.1	1.2	0.6	

^a Not determined.

^b Horizon designations were according to AG-Boden (1994).

^c Results obtained by standard particle size analysis (Gee and Bauder, 1986).

obtain complete dispersion of the samples. By wet sieving, the suspension was separated into three sand separates (630–2000, 200–630 and 63–200 μm). The clay separate (<2 μm) and three silt separates (20–63, 6–20 and 2–6 μm) were obtained by gravity sedimentation, and were recovered from the suspensions by filtration (cellulose nitrate, 0.45 μm). This procedure yields organo-mineral particle size separates, and a soluble fraction. The fraction <0.45 μm was collected, concentrated with a rotatory evaporator, and freeze dried. Alterations of the chemical structure of organic matter due to ultrasonication and subsequent ball milling were expected to be small (Schmidt et al., 1997a).

2.4. Elemental analysis

Carbon and nitrogen content of the bulk soils and the various separates were determined in duplicate with a Leco CNS 2000. The content of dissolved organic carbon was determined with a Shimadzu TOC 5050 Analyzer.

Table 2
Mass distribution, concentration and distribution of carbon and nitrogen for the particle size separates of different horizons

Horizon ^b	Mass ^c % of bulk	C _{org}		N		C:N
		g kg ^{-1d}	% of bulk	g kg ^{-1d}	% of bulk	
<i>Aeh</i>						
Coarse sand	0.8	1.8	0.1	0.2	0.4	— ^a
Medium sand	33.1	0.4	0.9	<0.1	0.9	—
Fine sand	49.3	0.6	2.1	<0.1	1.3	—
Coarse silt	8.0	2.7	1.5	<0.1	2.1	—
Medium silt	2.2	102.5	16.1	2.7	15.3	38
Fine silt	2.0	263.8	37.6	5.7	29.4	46
Clay	1.5	393.7	42.0	10.9	42.2	36
DOM ^e	—	—	1.0	—	—	—
% Recovery	96.9	—	101.3	—	91.5	—
<i>Bh</i>						
Coarse sand	0.7	3.5	0.1	<0.1	<0.1	—
Medium sand	32.1	1.3	1.3	<0.1	0.3	—
Fine sand	42.8	1.8	2.4	<0.1	0.5	—
Coarse silt	7.1	4.6	1.0	<0.1	<0.1	—
Medium silt	3.7	196.3	22.4	5.5	21.6	36
Fine silt	3.7	285.1	32.5	8.4	33.0	34
Clay	3.8	272.7	31.9	10.4	42.0	26
DOM ^e	0.2	348.6	2.9	14.7	—	24
% Recovery	94.1	—	94.5	—	97.5	—

^a Not determined.

^b AG-Boden (1994).

^c Particle size separates obtained after ultrasonic dispersion as described in Section 2.

^d Expressed as g kg⁻¹ bulk soil or size separate.

^e Dissolved organic matter: after filtration the content of dissolved organic carbon was determined for the separate <0.45 μm with an aliquot of the solution, and from this data per cent of total organic carbon was calculated.

2.5. 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) (Schaefer and Stejskal, 1976) was applied with a spinning speed of 4 kHz. 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). Before Fourier transformation, we applied line broadenings between 20 and 100 Hz. ¹³C chemical shifts (Table 3) are reported relative to tetramethylsilane (=0 ppm). 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 higher deviations can occur for the regions 220–160 and 45 to –10 ppm (Knicker, 1993). To remove paramagnetic material, and to improve the resolution of the spectra, all bulk and particle size separates from mineral horizons were treated with hydrofluoric acid (HF 10%) prior to NMR analysis (Schmidt et al., 1997b). This study indicated that chemical alterations due to HF treatment were small.

2.6. Hydrolyzable α -amino acids

The content of hydrolyzable amino acid nitrogen was determined with the colorimetric ninhydrin method (Stevenson, 1982). Between 0.2 and 0.5 g of the sample was hydrolyzed (6 N HCl/formic acid) for 12 h at 110°C in duplicate. The relative standard deviation for the determination of amino acid-N was 4.2% ($n=33$) and for the colorimetric analysis 4.4% ($n=69$). The precision of the whole method was ± 0.080 g amino acid nitrogen/kg soil dry weight.

Table 3
Chemical shift assignment of peaks in the solid-state ¹³C CPMAS NMR spectra (referenced to tetramethylsilane=0 ppm) (Lüdemann and Nimz, 1973; Wilson, 1987)

ppm	Assignment
0–45	Alkyl-C
45–110	O-Alkyl-C
45–60	Aliphatic C-N, methoxyl
60–95	Alkyl-O (carbohydrates, alcohols)
95–110	Acetal and ketal carbon (carbohydrates)
110–160	Aromatic-C
110–140	Aryl-H and aryl-C carbons
140–160	Aryl-O and aryl-N carbons
160–220	Carbonylic-C/carboxylic-C
160–180	Carboxyl and amide carbons
180–220	Aldehyde and ketone carbons

3. Results and discussion

3.1. Physical characteristics and elemental composition

The characteristic features of Podzols are eluvial horizons (Aeh, Ae), overlying illuvial horizons where humic material (Bh) and sesquioxidic material (Bs) like iron- and aluminum-oxides and hydroxides accumulate. The Podzol studied here had a sandy texture (Table 1) throughout the profile (85–96% sand) with characteristic, elevated silt and clay contents in the Bh horizon. Organic carbon, and pH varied within the range expected for Podzols under forest (Rehfuss, 1990). In the organic layers (L, Of, Oh) carbon and nitrogen contents and C:N ratios decreased with depth (Table 1), indicating an increased degree of decomposition of organic matter. Compared to other Podzols (Rehfuss, 1990) carbon and nitrogen contents were slightly lower in the organic horizons. Mineral layers showed characteristic small carbon and nitrogen contents in the eluvial Aeh horizon (C: 14.5 g kg⁻¹ air dry soil, and N: 0.4 g kg⁻¹). The illuvial Bh horizon contained more organic carbon (Bh: 34.5 g carbon kg⁻¹).

Table 2 gives the mass, organic C and N concentration and content results for the particle size separates of the Aeh, and Bh horizons, obtained using ultrasonic dispersion. Mass proportions of the separates corresponded well with those obtained by standard particle size analysis (Table 1), except for slightly smaller yields for sand separates. This indicated a more effective dispersion of sand particles by ultrasound compared to standard analysis and was observed previously (Schmidt et al., 1999). The particle size distribution revealed patterns with only minor variations between A and B horizons. Sand size particles dominated, whereas <20 µm sized particles contributed little (Aeh: ≤ 5.7%, and Bh: ≤ 11.2%) to the total soil mass. Carbon and nitrogen concentrations showed opposite trends with largest concentrations in <20 µm separates. Despite their low mass, these three separates contributed the majority to the total soil organic matter, both for organic carbon (Aeh: 95.7%, Bh: 86.8%) and for nitrogen (Aeh: 86.9%, and Bh: 96.6%). Compared to previous studies on size separates summarized by Christensen (1992), the observed pattern could be considered typical of sandy soils, although carbon and nitrogen concentrations were higher than those reported for agricultural soils. In previous studies carbon-to-nitrogen ratios (C:N) typically decreased from coarse to fine separates, suggesting an increasing extent of decomposition. In the Podzol studied here, C:N ratios were lowest in the clay separates. C:N ratios for the >20 µm separates could not be calculated due to their low nitrogen contents.

3.2. ¹³C CPMAS NMR spectroscopy of bulk soils

¹³C CPMAS NMR spectra of the Aeh and Bh horizon were obtained for bulk soils (Fig. 1a and g). The

chemical shift assignments (ppm) are given in Table 3. Spectra of the bulk Aeh and Bh horizons (Fig. 1a and g) showed signals at 33 ppm in the alkyl-C region (45 to –10 ppm), which may be attributed to methylene carbon. Resonances in the O-alkyl region (60–110 ppm) indicated the presence of alcoholic or ether structures; the signal at 72 ppm corresponded to ring C-5, C-3 and C-2 carbons of polysaccharides. The resonance at 106 ppm was attributed to the anomeric carbon in polysaccharides. Signals in the sp²-hybridized carbon region (160–110 ppm) indicated the presence of protonated and C-substituted aryl carbon (110, 130 ppm) and O-substituted aromatic carbon (150 ppm), typically present in lignin, cutin and condensed tannin (Preston et al., 1997). In the carboxyl C region (220–160 ppm) broad signals attributable to carboxyl carbon, amide and ester structures were observed (Preston et al., 1982; Preston and Schnitzer, 1984). Differences in signal intensities between Aeh and Bh horizon in carbon species were largest for aromatic C and alkyl C. Relative contributions from aromatic C in the Aeh horizon (19% of the total signal intensity) were lower than in the Bh horizon (29%). The opposite was true for alkyl C, with higher signal intensities for the Aeh horizon (37%) than for the Bh horizon (28%). These results are consistent with the experimental evidence that hydrophobic aromatics sorb more strongly to metal oxides than hydrophilic fractions like polysaccharides (Kaiser and Guggenberger, 2000). For carboxyl C and O-alkyl C differences were less pronounced. The spectra generally were similar to those observed for other natural Podzols (Hempfling et al., 1987; Skjemstad et al., 1992).

3.3. ¹³C CPMAS NMR spectroscopy of particle size separates

Particle size separates <63 µm contained the majority of the total organic carbon present in the Aeh horizon (97.2%) and Bh horizon (87.8%), and were investigated by ¹³C CPMAS NMR spectroscopy (Fig. 1b–e, h–k). Sand sized separates, however, were too low in carbon content to obtain well resolved spectra, even after HF treatment.

The spectra of the coarse silt separates of the Aeh and Bh horizon (Fig. 1b, h) revealed signals for alkyl C (33 ppm), O-alkyl C (72, 89 and partly 106 ppm), carboxyl groups (175 ppm), and aromatic C, probably from lignin units. The silt separates of the Bh horizon (Fig. 1h, i, j) showed signals at 106, 130 and 116 ppm with weak shoulders at 150 ppm. The peak at 106 may partly derive from C-1 and C-6 of syringyl units, but may be overlapped by the 115–120 ppm resonances of the C-2 and C-6 in guaiacyl units and the anomeric carbon of polysaccharides. The resonance at 148 ppm could be related to C-3 carbon in guaiacyl units. The resonance at 135 ppm has contributions from C-1 and C-4 carbons in syringyl and C-1 in guaiacyl units. The shoulder at 153 may be due to C-3 and C-5 aryl-O carbons of syringyl units.

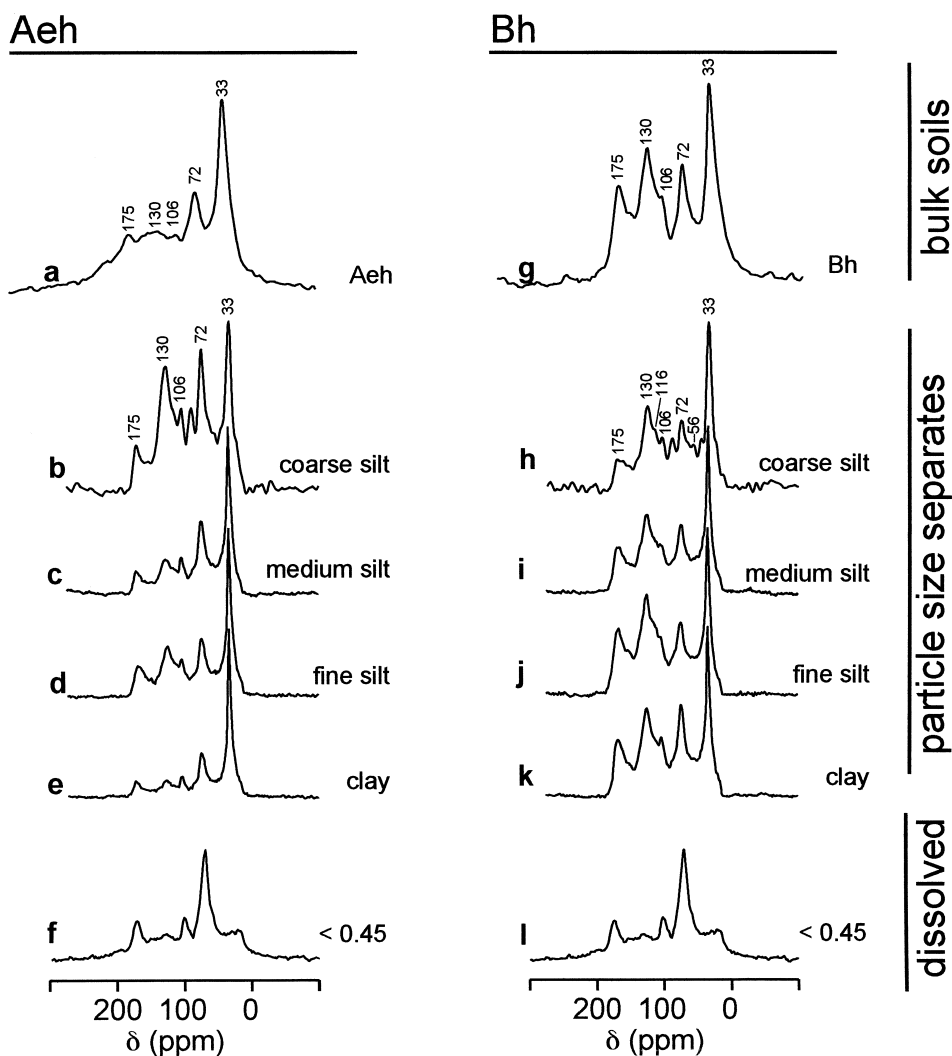


Fig. 1. ^{13}C CPMAS NMR spectra of the Aeh and Bh horizon and of the $< 63\ \mu\text{m}$ particle size separates from Podzol Flaesheim. The particle size separates were clay ($< 2\ \mu\text{m}$), fine silt ($2\text{--}6\ \mu\text{m}$), medium silt ($6\text{--}20\ \mu\text{m}$), coarse silt ($20\text{--}63\ \mu\text{m}$). The dissolved fraction ($< 0.45\ \mu\text{m}$) was organic matter released during the particle size fractionation procedure.

Previous work on peats, organic soil horizons, and particle size fractions of agricultural A horizons showed that the ratio alkyl C-to-O-alkyl C increased when decomposition proceeded (Baldock et al., 1992, 1997). The increasing ratio can be interpreted as an increasing degree of decomposition of polysaccharides from plants, and an enrichment of methylene structures by the microbial biomass. To visualize this trend, we calculated the ratio alkyl C-to-O-alkyl C in Table 4. In progressing from coarse silt (D : 0.64) to the clay sized separate (D : 1.53) the ratio D increased consistently, suggesting an increasing degree of decomposition in agreement with the decreasing C/N ratios (Table 2). Intensities for aromatic C peaked in the silt sized separate, while signals for carboxyl C varied (6–9%).

The organic matter associated with the size separates obtained from the illuvial Bh horizon, however, revealed a completely different pattern in two aspects. First, size separates from the Bh horizon had larger intensities in the chemical shift region of aromatic carbon (34–40% of the total signal intensity) than those from the Aeh horizon (18–35%), similar to spectra of the bulk soils. Second, in progressing from coarse to fine particle size separates, relative signal intensities of O-alkyl C were constant, whereas intensities from alkyl C decreased (Table 4). This trend was reflected by consistent decreases in the value of D from coarse silt (D : 0.97) to clay (D : 0.71) separates. The changes in D values were in striking contrast to trends observed for the Aeh horizon and those reported for previous studies, as summarized by Baldock et al. (1997).

Table 4
Relative areas of peaks in ^{13}C MAS NMR spectra of bulk soils and particle size separates from both Podzols

Sample	Carboxylic C	Aromatic C	O-alkyl C	Alkyl C	D^b
	% of total signal intensity ^a				
<i>Bulk soil</i>					
Aeh	15	19	30	37	1.23
Bh	14	29	29	28	0.97
<i>Aeh/size separates</i>					
20–63	6	35	36	23	0.64
6–20	6	24	36	34	0.94
2–6	8	31	28	33	1.18
0.45–2	6	18	30	46	1.53
<0.45	14	18	42	27	0.64
<i>Bh/size separates</i>					
20–63	6	34	31	30	0.97
6–20	10	36	29	25	0.86
2–6	12	40	27	21	0.87
0.45–2	10	37	31	22	0.71
<0.45	17	20	42	21	0.50

^a Data in per cent of total signal intensity (alkyl C 10 to 45 ppm, O-alkyl C 45 to 110 ppm, aromatic C 110 to 160 ppm, carboxyl C 160 to 220 ppm).

^b Ratio $D = I_{\text{alkyl C}} / I_{\text{O-alkyl C}}$ where $I_{\text{alkyl C}}$ is the relative signal intensity in the chemical shift region for alkyl C (45 to –10 ppm), and $I_{\text{O-alkyl C}}$ is the intensity in the O-alkyl C region (110 to 45 ppm).

In the clay sized separates, a major proportion of the O-alkyl C (31%) was probably present as polysaccharides, which was concluded from the distinct signal centered around 72 ppm in combination with the resonance at 106 ppm. Methylene structures, indicated by the distinct peak at 33 ppm, contributed less (22%) to the total signal intensity.

The fact that the clay fraction, containing most of the oxides, had a large proportion of polysaccharide C in addition to aromatic C, is not consistent with results from laboratory experiments, which suggest that aromatic dissolved organic matter in percolating water sorb stronger than other fractions (Guggenberger and Zech, 1993; Kaiser and Zech, 1998; Kaiser and Guggenberger, 2000). Sorption of dissolved organic matter to soils and oxides resulted in a preferential binding of aromatic structures and a discrimination of polysaccharides and methylene structures (Kaiser et al., 1997). Sorptive preservation of organic matter should result in a relative accumulation of aromatic structures in the clay fraction of the Bh horizon. This, however, was not the case. The fact that presumably labile polysaccharides were present in the clay sized separate could be attributed to microbial decay of sorbed organic matter and subsequent accumulation of microbial remains. Aliphatic compounds in Bh horizons of forest soils could also originate from root

input (Kögel-Knabner and Hatcher, 1989; Riederer et al., 1993; Nierop, 1998). Limited quantitative reliability of ^{13}C CPMAS NMR spectra was probably less important, because all samples were treated with hydrofluoric acid, effectively removing paramagnetic material (Schmidt et al., 1997b).

The organic matter from the Aeh and Bh horizon released a water-soluble fraction <0.45 μm during the particle size separation procedure (Table 2). The Aeh horizon produced less soluble organic matter (1.0% of the total organic carbon) than the Bh horizon (2.9%). Both fractions yielded almost identical ^{13}C NMR spectra (Fig. 1f and l) typical for polysaccharides. This was demonstrated by dominant peaks at 72 ppm accompanied by signals at 106 ppm. In both samples O-alkyl C contributed the majority (42%) to the total signal intensities, followed by alkyl C (Aeh: 27%, Bh: 21%). Distinct peaks were observed in the carboxyl region, whereas a broad set of signals contributing only 18–20% to the total signal intensity was observed in the aromatic region. The NMR spectra suggested that the water soluble organic matter released from the Aeh and Bh horizon was probably very similar in chemical structure, and showed little contribution from aromatic carbon. This was consistent with previous work that found preferential desorption of compounds with low aromaticity (Kaiser and Zech, 1998). The relative proportion of organic carbon released was larger in the Bh horizon than in the Aeh horizon. However, it is not clear if the organic matter released during the particle size separation procedure reflects solubility in situ directly.

3.4. Hydrolyzable α -amino acids

Hydrolyzable α -amino acids were characterized in bulk soils and in particle size separates (Table 5). Due to low nitrogen contents of the coarse separates only <20 μm separates were studied. To compare α -amino acid distribution in samples differing in total nitrogen content, we report results also as per cent of total N. In the bulk soils contributions of hydrolyzable α -amino acids to the total

Table 5
Hydrolyzable amino acids expressed as per cent of total nitrogen and (in parentheses) as concentration ($\text{g nitrogen kg}^{-1}$ size separate or bulk soil). The particle size separates were clay (<2 μm), fine silt (2–6 μm), medium silt (6–20 μm)

Horizon	Size separate			Bulk soil
	Medium silt	Fine silt	Clay	
Mass per cent of total nitrogen (in parentheses concentration/ g kg^{-1})				
Aeh	31 (2.7)	34 (5.7)	43 (8.4)	52 (0.4)
Bh	23 (5.5)	20 (8.4)	21 (10.4)	21 (1.0)

soil nitrogen were higher in the A horizon (52%) than in the B horizon (21%), which was similar to results reported for Canadian Podzols (Sowden, 1959). Yields in the particle size separates reflected results obtained for bulk soils. Proportions of hydrolyzable α -amino acids were larger in the separates obtained from the Aeh horizon (31–43% of the total N) than in those from the Bh horizon (20–23%), confirming previous results (Catroux and Schnitzer, 1987; Watson and Parsons, 1974). Standard acid hydrolysis releases only a part of the organic nitrogen in bulk soils and particle size separates. ^{15}N CPMAS NMR spectroscopy allowed the nature of nitrogen in the soil matrix to be investigated without chemical extraction. The particle size separates investigated here were previously characterized using ^{15}N NMR (Knicker et al., 1999). Between 60 and 90% of the identified nitrogen in the particle size separates was assigned to amides most probably derived from peptide-like structures. Differences between Aeh and Bh horizon were small for corresponding size fractions. Proportions of hydrolyzable α -amino acids were (i) generally smaller than those detectable with ^{15}N NMR, and (ii) smaller in the Bh horizon than in the Aeh horizon. It seemed that stabilization of organic matter against hydrolytic attack was largest in the Bh horizon.

4. Conclusions

A combination of elemental analysis, solid-state ^{13}C NMR spectroscopy and acid hydrolysis was used to study the distribution and chemical structure of organic matter in the Aeh and Bh horizon of a Podzol. In the Bh horizon organic matter and iron- and aluminum oxihydroxides accumulated. Stabilization of organic matter was supposed to occur by preferential binding of aromatic structures over labile structures like methylene and polysaccharide structures, as concluded from laboratory experiments (Kaiser and Guggenberger, 2000). In the Aeh horizon the majority of the organic matter was present in methylene structures, contributing almost one half to the organic matter associated with the clay fraction. In progressing from coarse to fine particle size separates, the Aeh horizon showed decreasing ratios carbon-to-nitrogen, and increasing ratios alkyl C/O-alkyl C. Both results supported the hypothesis that decomposition of soil organic matter increased with decreasing particle size. The illuvial Bh horizon contained higher proportions of iron- and aluminum-oxides and hydroxides, and aromatic carbon. Similar proportions of O-alkyl C, and smaller proportions of methylene carbon than the Aeh horizon. With decreasing particle size the C:N and alkyl C/O-alkyl C ratios decreased for the Aeh horizon but not for the the Bh horizon.

Our results suggested that in Bh horizons of Podzols stabilization of organic matter by organo-mineral

associations is not limited to aromatic structures; also, presumably, labile structures like polysaccharides can be stabilized.

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