

## O/N-alkyl and alkyl C are stabilised in fine particle size fractions of forest soils

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**Abstract.** Carbon stocks and organic matter composition in bulk soils and particle size fractions of Ah horizons from Luvisols, Leptosols and Phaeozems under European beech (*Fagus sylvatica* L.) forest were investigated by elemental analysis, solid state <sup>13</sup>C nuclear magnetic resonance (<sup>13</sup>C CPMAS NMR) spectroscopy and lignin analysis (CuO-oxidation). Radiocarbon age was used as an indicator for C turnover. The SOM of bulk soils and particle size fractions is dominated by O/N-alkyl C and alkyl C. Compared to sand and silt fractions, clay fractions had lower C/N ratios and <sup>14</sup>C abundances. Aryl C and more specifically phenolic components (O-aryl C) decreased from sand to clay fractions. The concomitant decrease of lignin, determined by CuO oxidation, suggests that a major proportion of O-aryl C can be attributed to lignin. Positive nonlinear relations between the O-aryl C and the C/N ratio reveal the trend of decreasing O-aryl C proportions with increasing decomposition. Although lignin is believed to be highly recalcitrant, only low amounts of lignin are found in the stable clay fractions. In contrast to O-aryl C, the O/N-alkyl C contribution decreased from sand to silt fractions, but increased again in the clay fractions, whereas alkyl C contents exhibited lowest values in the sand fractions. These results are indicative of stabilisation processes operating specifically on polysaccharides and alkyl C, but not on aryl C, through association with the clay fraction.

### Introduction

Soil organic matter (SOM) is a heterogeneous mixture of compounds with varying turnover rates (Wang and Hsieh 2002). The stability of SOM is controlled by (1) the chemical structure of SOM and (2) the existence of various mechanisms of protection offered by the soil matrix and soil minerals (Baldock and Skjemstad 2000). It has been shown, that soil texture exerts a significant control over soil organic carbon (SOC) and nitrogen (N) dynamics and storage. Clay content, base status, and Fe and Al hydrous oxides strongly influence the preservation of SOM (Oades 1988). Thus, physical fractionation approaches considering interactions between SOM and mineral particles of different size are used to separate structurally and functionally different carbon pools (Christensen 2001). They help to elucidate soil processes and mechanisms

of SOC storage (Six et al. 2002). Approaches to isolate primary organo-mineral complexes rely on a complete dispersion and subsequent particle-size or density separation; combined aggregate and density fractionation procedures allow for the study of the effect of soil aggregation on C sequestration in soils (Christensen 2001). The majority of studies on the characterisation of SOM in particle-size fractions have been performed on agricultural soils.

Seventy to eighty percent of the plant input in soils consists of polysaccharides and lignin (Kögel-Knabner 2002). These are usually isolated as a light or coarse fraction (often called particulate organic matter (POM)), depending on the isolation procedure. Using results from  $^{13}\text{C}$  CPMAS NMR spectroscopy Baldock et al. (1992) found increasing amounts of alkyl C from large (2000–250  $\mu\text{m}$ ) to small particle sizes ( $< 2 \mu\text{m}$ ) in arable soils, in peat and in soils under tropical forest. They suggested a selective preservation mechanism or an *in situ* synthesis of alkyl C in the clay fraction. SOM in the sand-sized separates is primarily composed of plant residues, while microbial biomass is supposed to be concentrated in clay-sized separates (Zech and Guggenberger 1996; Christensen 2001). This is usually associated with a decrease in the C/N ratio from the coarse to the fine fraction (Spycher et al. 1983; Kögel-Knabner and Ziegler 1993).

Different isotope methodologies are employed to estimate the average turnover of SOM in physical fractions. Only few studies address  $^{14}\text{C}$  contents in particle size fractions. Applying this technique, Anderson and Paul (1984) found the oldest carbon of two arable soils in the coarse clay and the fine silt fraction, respectively. Similar results obtained from two forest soils are presented by Quideau et al. (2001). In contrast  $^{14}\text{C}$ -data of particle size fractions from two Mollisols under different land use show age maxima in the medium clay and fine clay fractions (Scharpenseel et al. 1986). However, in laboratory experiments with separated fractions no difference between the respiration, when expressed per gram of initial C, could be found between light and heavy fractions from forest soils suggesting a similar recalcitrance of organic carbon (OC) in light and heavy fractions and supporting the importance of microbial accessibility and physical protection of OC resources (Swanston et al. 2002).

Reports combining results of chemical composition with results of carbon turnover are rare. Gleixner et al. (2002) performed studies on the molecular residence time of SOM from bulk soils and found a very high life time for N-containing and polysaccharide-derived pyrolysis products. Quideau et al. (2000) combined  $^{14}\text{C}$  radiocarbon dating,  $^{13}\text{C}$  CPMAS NMR and particle size fractionation to investigate soils under oak and pine forest. The turnover rates of SOM under pine corresponded to an increase in the alkyl C/ O/N-alkyl C ratio from the fresh litter to the clay fraction (Quideau et al. 2000). Some studies related the carbon turnover assessed by the  $^{13}\text{C}$  abundance with chemical characteristics. Data on the degree of microbial alteration fit well with the increasing age in POM fractions and density fractions of fine silt associated carbon (Six et al. 2001). Combining  $^{13}\text{C}$  CPMAS NMR and  $^{13}\text{C}$  abundance data of occluded organic materials, Golchin et al. (1995) found that the O-alkyl

C content of occluded organic materials was inversely related to their stabilities whereas their aromatic C content was directly related to their stabilities.

The focus of this study is on the radiocarbon content and the chemical composition of SOM in soils under European beech, which is one of the most common tree species in Western and Central Europe (Köble and Seufert 2001). We compared Ah horizons from four managed forest sites distributed from North to South Europe. The sites cover different climates as well as soil textures and include a forest chronosequence. Using particle size fractionation we intended to separate carbon pools with specific chemical characteristics and contrasting turnover rates. By relating results from  $^{13}\text{C}$  CPMAS NMR spectroscopy and lignin analysis to the abundance of  $^{14}\text{C}$  we aim to fill the gap between studies on the dynamics and on the chemical composition of SOM.

## Materials and methods

### *Soil description and sampling*

Soil samples originated from managed beech (*Fagus sylvatica* L.) forest sites in Collelongo (Italy), Hesse (France), Sorø (Denmark) and Leinefelde (Germany) (Table 1). In Leinefelde samples were collected from a chronosequence including stands with an average tree age of 30, 62, 111 and 153 + 16 years (153 + 16 refers to a mixture of 153 years old trees with 16 years old trees) (Table 1). Samples were taken from one representative soil pit at each site. Only at the 30 years old stand in Leinefelde samples were taken from two profiles because of the great soil heterogeneity (designated as Leinefelde 30/1 and Leinefelde 30/2). All soil pits were sampled by horizons. Samples were always taken from the complete litter and Ah horizons. The thickness of the Ah horizons varied between 0.07 and 0.25 m. The humus form at all sites is mull. Soil types comprise Rendzic Leptosols on lime stone in Collelongo and Leinefelde (30/1), Stagnic Luvisols on shell limestone in Hesse and on loess in Leinefelde (30/2, 62 and 153 + 16), a Haplic Luvisol on loess in Leinefelde (111) and a Stagnic Phaeozem on calcareous glacial material in Sorø (FAO 1998). In all figures, the results of the chronosequence are presented separately ('Chronosequence Leinefelde'). For comparison of 'European Sites' the sites in Collelongo, Hesse, Sorø and the 62 years old stand from Leinefelde are selected. The 62 years old stand from Leinefelde versus the other Leinefelde sites was chosen to be included in the 'European Sites comparison', because it represents a stand with neither very young nor very old trees.

### *Basic soil parameters*

Chemical analyses of mineral soil samples were performed using air dried < 2 mm samples. The pH was determined with a glass electrode in the

Table 1. Site characteristics.

Site	Latitude/ Longitude	Climate	Stand age (years)	Soil classification (FAO)	Thickness of Ah horizon (m)	pH	Texture (g kg <sup>-1</sup> )			
							200–2000 $\mu\text{m}$	20–200 $\mu\text{m}$	2–20 $\mu\text{m}$	< 2 $\mu\text{m}$
Collelongo/ Italy	41°52'N/ 13°38'E	Mediterranean/montane	109	Rendzic Leptosol	0.19	6.9	15	68	323	594
Hesse/ France	48°40'N/ 07°05'E	Temperate/suboceanic	34	Stagnic Luvisol	0.08	4.1	14	279	402	305
Sorø/ Denmark	55°29'N/ 11°38'E	Temperate/oceanic	60	Stagnic Phaeozem	0.25	4.1	230	393	141	236
Leinefelde/ Germany	51°20'N/ 10°22'E	Temperate/continental	30 <sup>a</sup>	Rendzic Leptosol	0.15–0.20	5.5	1	162	279	558
			30 <sup>a</sup>	Stagnic Luvisol	0.08	5.1	1	272	352	375
			62	Stagnic Luvisol	0.08	4.7	1	235	453	311
			111	Haplic Luvisol	0.07	4.5	6	233	399	362
			153 + 16 <sup>b</sup>	Stagnic Luvisol	0.08	5.0	1	147	428	424

<sup>a</sup> Two profiles representing the main soil types were sampled and designated as Leinefelde 30/1 and 30/2.

<sup>b</sup> 153 years old trees mixed with 16 years old trees. The proportion between old and young trees is about 20:80.

supernatant of a soil suspension using 1:2.5 mixtures of soil and 0.01 M CaCl<sub>2</sub>. Determination of soil texture was carried out according to Schlichting and Blume (1966). The proportions of fractions < 63 μm were measured with a Sedigraph 5100 (Micromeritics Corp., Norcross, USA). According to the Soil Science Society of America nomenclature (SSSA 2001) the soil texture in the Ah horizons corresponds to sandy clay loam in Sorø, silt clay loam in Hesse and Leinefelde (30/2, 62 and 111), silty clay in Leinefelde (30/1 and 153 + 16) and clay in Collelongo. Prior to C and N analyses, <sup>13</sup>C CPMAS NMR spectroscopy and alkaline CuO oxidation aliquots of bulk samples were ground to a size of < 100 μm with a ball mill. Determination of total carbon (TC) and total nitrogen concentrations in bulk soils and particle size fractions was performed in duplicate by dry combustion using a 'Vario EL' elemental analyser (Elementar Analysensysteme GmbH, Hanau, Germany). The coefficient of variation of replicated analyses was below 4%. The TC of samples from Hesse, Leinefelde and Sorø equal the OC concentrations due to low pH values (below 5.5). Inorganic carbon (IC) concentrations of samples from Collelongo were determined after ignition of samples for 3 h at 550 °C. The OC concentrations of the samples from Collelongo are calculated as the difference between TC and IC.

#### *Particle size fractionation*

Thirty gram of air dried soil was suspended in distilled water (soil to water ratio 1:5). In order to avoid a redistribution of coarse POM to finer size classes, ultrasonic dispersion was carried out in two steps with a Branson Sonifier 250 (Branson Ultrasonics Corp., Danbury, USA) as described by Amelung and Zech (1999). Macroaggregates were destroyed with an energy input of 60 J/ml and particles > 200 μm – including POM – were removed by wet sieving. Complete dispersion of microaggregates (< 200 μm) was achieved with a second ultrasonic treatment using an energy of 440 J/ml (soil to water ratio 1:10). Afterwards, the 20–200 μm fraction was isolated by wet sieving. The remaining part of the sample was further separated in a 2–20 μm and < 2 μm fraction by repeated gravitational sedimentation using Atterberg cylinders filled with distilled water. The clay fraction was recovered from the solution by pressure filtration (0.2 μm polypropylene filter) and freeze dried. All size separates > 2 μm were oven dried at 40 °C. Aliquots of particle size fractions taken for C and N analyses, radiocarbon dating, <sup>13</sup>C CPMAS NMR spectroscopy and alkaline CuO-oxidation were homogenised and ground with agate mortar and pestle to a size of < 100 μm.

#### *<sup>13</sup>C CPMAS NMR spectroscopy*

<sup>13</sup>C CPMAS NMR spectra were obtained from bulk soils and particle size fractions. Due to low OC concentrations it was not possible to obtain spectra from the 20–200 μm fractions. Ground 200–2000 μm fractions containing negligible concentrations of paramagnetics were taken for <sup>13</sup>C CPMAS NMR

spectroscopy without pre-treatment. All other samples were treated with 10% hydrofluoric acid (HF) (v/v) to remove mineral material including paramagnetic compounds such as iron and to concentrate the SOM (Schmidt et al. 1997). In detail, 5 g of bulk samples or 1–2 g of particle size fractions were thoroughly mixed with 50 ml 10% HF and shaken horizontally for more than 8 h. After centrifugation, the supernatant was removed. This procedure was repeated four times. Afterwards, the solid part of the suspension was washed five times with distilled water, freeze dried and homogenised with agate mortar and pestle. Solid state  $^{13}\text{C}$  CPMAS NMR spectra were acquired using a Bruker DSX 200 spectrometer (Bruker BioSpin GmbH, Karlsruhe, Germany) at a  $^{13}\text{C}$  resonance frequency of 50.3 MHz. Samples were filled into zirconium dioxide rotors and spun in a magic angle spinning probe at a rotation speed of 6.8 kHz to minimise chemical anisotropy. A ramped  $^1\text{H}$  pulse was used during a contact time of 1 ms to prevent Hartmann–Hahn mismatches. The total number of scans ranged from  $18 \times 10^3$  to  $89 \times 10^3$  with a constant recycle delay of 400 ms. Prior to Fourier transformation and phasing the spectra were processed with a line broadening of 50 Hz. Chemical shifts are referenced to tetramethylsilane (TMS = 0 ppm).  $^{13}\text{C}$  CPMAS NMR spectra were divided into five chemical shift regions, representing alkyl C (0–45 ppm), O/N-alkyl C (45–110 ppm), C/H-aryl C (110–140 ppm), O-aryl C (140–160 ppm) and carbonyl C (160–220 ppm). The relative intensities of the chemical shift regions were determined by integration of  $^{13}\text{C}$  CPMAS NMR spectra over given chemical shift ranges after phase and baseline correction. The variation of the relative intensities due to Fourier transformation, phase and baseline correction is lower than 5% (Knicker 1993).

#### *Alkaline CuO oxidation*

Lignin derived phenols in bulk soils and particle size fractions were determined in duplicate using a modification of the CuO oxidation method developed by Hedges and Ertel (1982). Samples were not treated with HF before alkaline CuO oxidation. The extraction by ethyl ether was replaced by an extraction with  $\text{C}_{18}$  columns introduced by Kögel and Bochter (1985). Depending on OC concentrations between 50 and 500 mg of ground soil, 250 mg CuO, 50 mg  $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$  and 50 mg  $\text{C}_6\text{H}_{12}\text{O}_6$  were weighed into teflon vessels and mixed with 15 ml of 2 M NaOH. Subsequently, lignin was digested in a pressure bomb (Groteklaes GmbH, Jülich, Germany) under a nitrogen atmosphere at 172 °C for 2 h. After cooling of the pressure bomb, samples were acidified to pH 2 with 6 M HCl and allowed to stand in the dark overnight. During this time humic acids precipitated and were separated by centrifugation. Aliquots of the supernatant, containing the lignin derived phenols, were extracted with  $\text{C}_{18}$  reversed phase columns. After drying of columns in a nitrogen stream for 1 h, the lignin derived phenols were extracted with five times 0.5 ml ethyl acetate. Ethyl acetate was evaporated in a nitrogen atmosphere, CuO oxidation products were dissolved in pyridine and derivatised

with bis(trimethylsilyl)trifluoroacetamide. The lignin derived phenols were analysed on an Agilent 6890 gas chromatograph with a flame ionisation detector equipped with a HP5 fused silica capillary column (30 m length, 0.25 mm ID, 0.25  $\mu\text{m}$  film thickness). 1  $\mu\text{l}$  of samples was injected using the split mode (split ratio 1:50) at an injector temperature of 300 °C. The initial column temperature was set to 100 °C and the oven was programmed to 200 °C at 5 °C/min and to 300 °C at 10 °C/min. The final temperature was kept for 15 min. The detector temperature was 300 °C. During the whole analysis a constant helium flow of 1.0 ml/min was maintained. The sum of vanillyl, syringyl and cinnamyl (VSC) units was taken to represent the lignin content. The coefficient of variation for the sum of VSC is usually 7% (Kiem and Kögel-Knabner 2003). The acid-to-aldehyde ratio of syringyl units is taken as an indicator for the degree of lignin modification (Ertel and Hedges 1984).

#### *Radiocarbon dating*

Radiocarbon activity of the particle size fractions was determined by accelerator mass spectroscopy (AMS) according to Kretschmer et al. (1997). Briefly, after dry combustion of the samples, a small aliquot of the arising carbon dioxide and nitrogen is bled into a mass spectrometer where the stable isotope ratios  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were measured. The remaining part of  $\text{CO}_2$  is reduced to graphite by heating a mixture of  $\text{H}_2$  and  $\text{CO}_2$  with iron powder at 625 °C. The graphite is pressed into targets and measured with the AMS. The amount of radiocarbon enrichment is expressed in percent modern carbon (pmC) that refers to the 1950  $^{14}\text{C}$  activity. The average pmC error was 0.5 pmC. All values are corrected for isotope fractionation using the  $\delta^{13}\text{C}$  values. Samples from Collelongo were treated with 4% HCl during 24 h to remove  $\text{CaCO}_3$  prior to radiocarbon analysis (Mook and Streurman 1983).

#### *Statistics*

Calculation of correlation coefficients was performed with the software package Statistica 6.1 (Statsoft Inc., Tulsa, USA). We used the Pearson's correlation coefficient ( $r$ ) and the Spearman rank correlation coefficient ( $r_s$ ), which is a distribution-free analog of correlation analysis.

## **Results**

#### *OC concentrations, OC stocks and C/N ratios*

The OC concentrations in the Ah horizons range from 31 to 96  $\text{g kg}^{-1}$  in the bulk soils and from 8 to 406  $\text{g kg}^{-1}$  in the particle size fractions (Table 2). To

Table 2. Organic carbon (OC) concentrations, OC enrichment ratios ( $E_{OC}$ ), OC stocks and C/N ratios in bulk soils and particle size fractions for the Ah horizons of 8 forest sites.

Site (stand age)	Fraction ( $\mu\text{m}$ )	OC ( $\text{g kg}^{-1}$ )	$E_{OC}$	OC stock ( $\text{kg m}^{-2}$ )	OC stock ( $\text{kg m}^{-3}$ )	Percentage of bulk soil OC stock (%)	C/N
Collelongo (109)	Bulk soil	96		7.7	41		11.8
	200–2000	154	1.7	0.4	2	5	29.4
	20–200	102	1.1	0.7	4	9	20.1
	2–20	88	0.9	2.8	15	36	12.4
	<2	88	0.9	3.8	20	50	9.7
Hesse (34)	Bulk soil	31		1.8	22		13.4
	200–2000	109	3.5	0.3	5	18	27.8
	20–200	9	0.3	0.1	3	8	16.5
	2–20	14	0.4	0.4	7	21	12.0
	<2	51	1.6	0.9	17	53	6.7
Sorø (60)	Bulk soil	45		8.1	32		12.8
	200–2000	17	0.4	0.8	3	10	15.4
	20–200	8	0.2	0.7	3	8	11.7
	2–20	84	1.9	2.5	10	31	13.4
	<2	163	3.6	4.1	17	51	10.7
Leinefelde (30/1)	Bulk soil	47		4.9	29		11.1
	200–2000	114	2.8	0.8	4	16	18.4
	20–200	30	0.6	0.5	3	11	20.7
	2–20	43	0.9	1.1	7	23	16.3
	<2	62	1.3	2.4	15	50	9.5
Leinefelde (30/2)	Bulk soil	48		3.0	38		14.4
	200–2000	406	8.4	0.7	9	22	30.2
	20–200	15	0.3	0.3	3	9	18.7
	2–20	26	0.5	0.7	9	23	17.3
	<2	74	1.5	1.4	17	46	10.9
Leinefelde (62)	Bulk soil	49		3.1	38		13.8
	200–2000	203	4.1	0.3	4	11	20.7
	20–200	20	0.4	0.3	4	10	19.1
	2–20	42	0.9	1.2	15	40	17.0
	<2	75	1.5	1.2	15	39	8.6
Leinefelde (111)	Bulk soil	32		1.9	27		12.9
	200–2000	214	6.7	0.3	4	13	22.3
	20–200	15	0.5	0.3	4	14	20.4
	2–20	24	0.7	0.6	8	30	17.5
	<2	60	1.9	0.8	12	43	8.9
Leinefelde (153 + 16)	Bulk soil	34		2.1	27		13.1
	200–2000	245	7.2	0.4	4	16	25.6
	20–200	9	0.3	0.1	1	5	24.3
	2–20	23	0.7	0.6	8	30	21.4
	<2	45	1.3	1.0	13	49	8.7

facilitate a comparison of carbon enrichments in fractions of soils with different OC levels in the bulk soils, enrichment factors for OC ( $E_{OC}$ ) were calculated ( $\text{g kg}^{-1}$  of OC in fraction/ $\text{g kg}^{-1}$  of OC in bulk soil) (Table 2). Most of the 200–2000  $\mu\text{m}$  and <2  $\mu\text{m}$  fractions are enriched, whereas most of the 20–200



and 2–20  $\mu\text{m}$  fractions are depleted in OC. The OC stocks in the total Ah horizons range from 1.8 to 8.1  $\text{kg m}^{-2}$  (Table 2). The Ah horizons at Collelongo, Leinefelde (30/1) and Sorø which are thicker than 15 cm exhibit the highest carbon stocks (more than 4.9  $\text{kg m}^{-2}$ ). The variability of carbon stocks on volume basis (22–41  $\text{kg m}^{-3}$ ) is lower than on horizon basis. The highest proportions of the total OC stock of the respective Ah horizons are accumulated in the <2 and 2–20  $\mu\text{m}$  fractions. The contribution of the 200–2000  $\mu\text{m}$  fractions to the total OC stocks are higher under the young stands in Leinefelde (30/1, 30/2 and 153 + 16) and in Hesse. In almost all investigated samples the C/N ratios are decreasing in finer particle size separates (Table 2). Only the C/N ratios of the 200–2000  $\mu\text{m}$  fractions under the 30 years old stand in Leinefelde (30/1) and of the 20–200  $\mu\text{m}$  fraction of Sorø do not fit in this trend.

*Structural characterisation by  $^{13}\text{C}$  CPMAS NMR spectroscopy*

$^{13}\text{C}$  CPMAS NMR spectra of particle size fractions of the Ah horizons from four European beech forest sites are presented in Figure 1. Main resonances occur at 30, 56, 72, 105, 130, 150 and 173 ppm. Resonances at 72 and 105 ppm are most probably assigned to C2, C3, C5 and C1 in cellulose and hemicellulose (Preston et al. 1998). The resonance at 30 ppm is typical for methylene C in long chain aliphatic compounds, the features around 56, 130 and 150 ppm are attributed to methoxyl, H-/C-substituted and phenolic C in lignin and tannins (Kögel-Knabner 1997). N-alkyl of amino acids may also contribute to the signal at 56 ppm. High signal intensities at 130 ppm often arise from the presence of condensed aromatic C structures, such as in charred organic matter, but may also be explained by C in C–C double bonds. Carbonyl C in carboxyl, amide, and ester groups of various compounds contributes to the peak at 173 ppm.

Table 3 presents the relative intensities of the chemical shift areas. Integration of the chemical shift regions shows high relative intensities of O/N-alkyl C (37–53%) and alkyl C (22–34%) in the bulk soils as well as in the particle size fractions. Lower intensities are found for aryl C (9–22%) and carbonyl C (7–17%). The highest variations between the relative intensities of particle size fractions were detected for O/N-alkyl C and aryl C (Table 3). The contribution of O/N-alkyl C in all Ah horizons decreases from the 200–2000  $\mu\text{m}$  fraction (42–52%) to the 2–20  $\mu\text{m}$  fraction (37–45%) and increases from the 2–20  $\mu\text{m}$  fraction to the <2  $\mu\text{m}$  fraction (42–53%). Relative intensities of aryl C decline from 19–21% in the 200–2000  $\mu\text{m}$  fractions to 9–12% in the <2  $\mu\text{m}$  fractions. The relative proportion of O-aryl C is correlating with the C/N ratios at the Chronosequence Leinefelde as well as at the European Sites (Figure 2a, b). The relation between O-aryl C and C/N ratios in particle size fractions is nonlinear. Similar to the relative intensity of O-aryl C the proportion of H/C-aryl C is decreasing with progressing turnover at the Chronosequence Leinefelde as well as at the European Sites. This leads to nearly linear relations between O-aryl C and H/C-aryl C (Figure 3a, b).

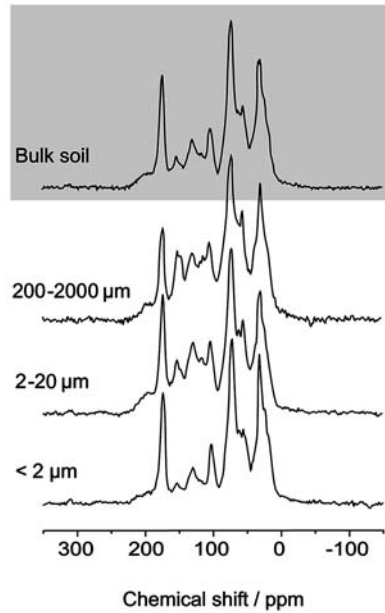
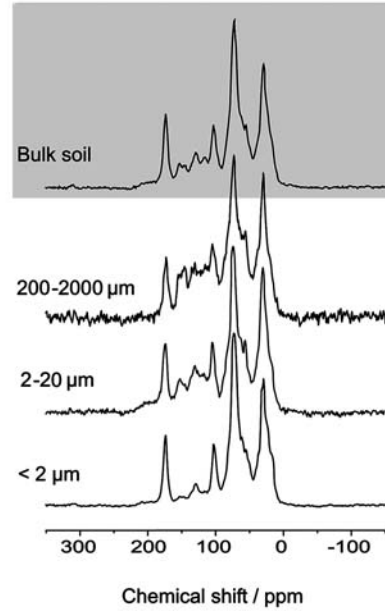
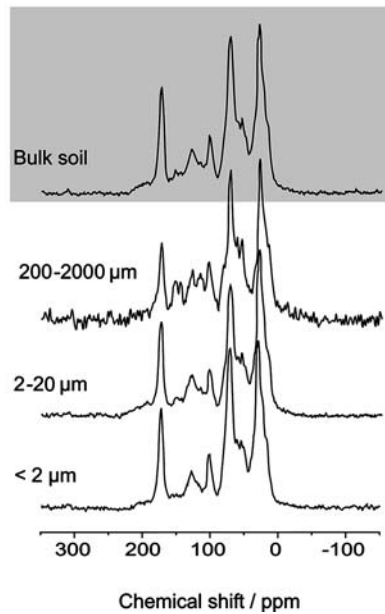
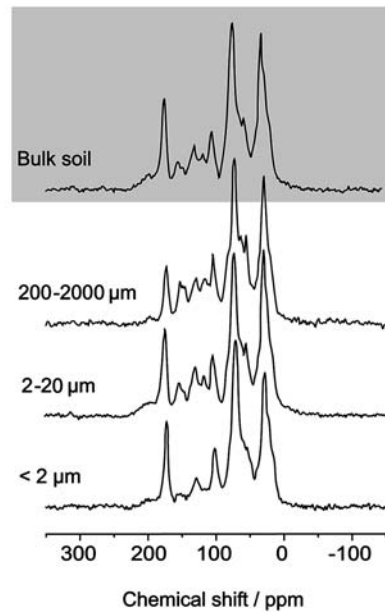
**(a) Collelongo****(b) Hesse****(c) Sorø****(d) Leinefelde (62)**

Figure 1.  $^{13}\text{C}$  CPMAS NMR spectra of particle size fractions of the Ah horizons from sites in Collelongo (a), Hesse (b), Sorø (c) and Leinefelde (62 years old stand) (d).

Table 3. Relative signal distributions of OC species in bulk soils and particle size fractions for the Ah horizons of 8 forest sites determined by  $^{13}\text{C}$  CPMAS NMR spectroscopy.

Site (stand age)	Fraction ( $\mu\text{m}$ )	% OC			
		Alkyl C (0–45 ppm)	O/N-alkyl C (45–110 ppm)	Aryl C (110–160 ppm)	Carbonyl C (160–220 ppm)
Collelongo (109)	Bulk soil	26.1	42.9	15.4	15.5
	200–2000	22.1	43.2	18.1	16.5
	2–20	21.6	40.5	21.5	16.4
	< 2	28.8	45.6	11.6	14.0
Hesse (34)	Bulk soil	26.4	48.6	14.5	10.5
	200–2000	24.7	47.6	21.3	7.6
	2–20	26.7	44.3	17.7	11.3
	< 2	29.4	53.2	8.9	8.5
Sorø (60)	Bulk soil	31.3	41.3	13.6	13.7
	200–2000	29.1	41.9	19.2	9.8
	2–20	33.3	37.4	13.8	15.4
	< 2	33.6	42.3	10.8	13.3
Leinefelde (30/1)	Bulk soil	24.9	47.9	13.5	13.7
	200–2000	21.6	46.1	20.8	11.4
	2–20	26.6	41.6	18.1	13.6
	< 2	22.2	52.6	11.1	14.1
Leinefelde (30/2)	Bulk soil	26.0	47.8	14.2	12.0
	200–2000	23.0	50.9	18.7	7.4
	2–20	27.3	43.1	18.5	11.2
	< 2	27.1	49.6	11.8	11.5
Leinefelde (62)	Bulk soil	29.9	42.1	14.3	13.7
	200–2000	26.6	47.1	19.1	7.2
	2–20	31.0	40.4	15.6	13.0
	< 2	27.1	51.5	11.0	10.8
Leinefelde (111)	Bulk soil	31.5	43.9	13.1	11.5
	200–2000	27.9	43.3	19.2	9.6
	2–20	31.9	40.2	15.2	12.7
	< 2	29.7	49.9	9.0	11.4
Leinefelde (153 + 16)	Bulk soil	27.2	47.2	13.9	11.7
	200–2000	23.7	51.9	17.0	7.4
	2–20	26.0	45.4	17.4	11.1
	< 2	25.2	52.3	11.3	12.5

#### *CuO oxidation products (lignin)*

The contents of CuO oxidation products normalized to OC are given in Table 4. The sum of CuO oxidation products VSC in the bulk soils ranges between 10 and 22  $\text{g kg}^{-1}$  OC. The highest concentrations of CuO oxidation products are present in the fractions from 2 to 2000  $\mu\text{m}$ . The SOM of the 200–2000  $\mu\text{m}$  fractions has VSC concentrations between 15 and 49  $\text{g kg}^{-1}$  OC, the 20–200  $\mu\text{m}$  fractions between 12 and 40  $\text{g kg}^{-1}$  OC and the 2–20  $\mu\text{m}$  fractions between 6 and 28  $\text{g kg}^{-1}$  OC. In contrast only 2–7  $\text{g kg}^{-1}$  OC can be determined in the < 2  $\mu\text{m}$  particle size class. The highest lignin stocks occur

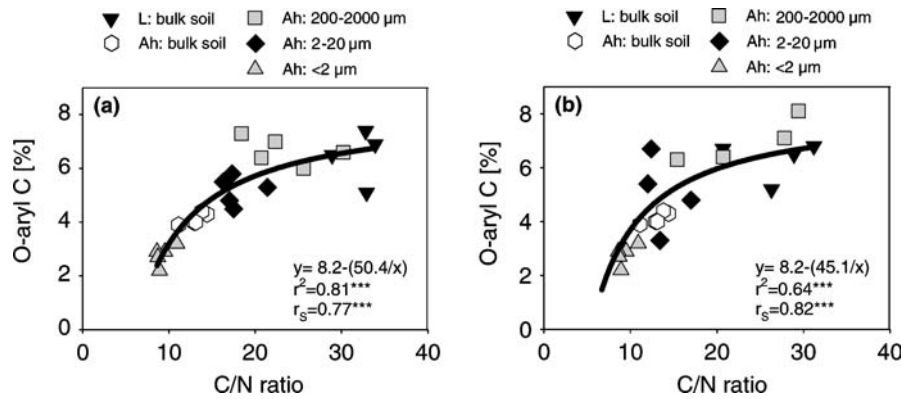


Figure 2. Relative intensity of O-aryl C in relation to C/N ratios in the L horizons (L), Ah horizons (Ah), and the particle size fractions of the Ah horizons (a) from the Chronosequence Leinefelde and (b) from the sites in Collelongo, Hesse, Sorø and Leinefelde (62 years old stand) ( $r_s$  = spearman correlation coefficient).

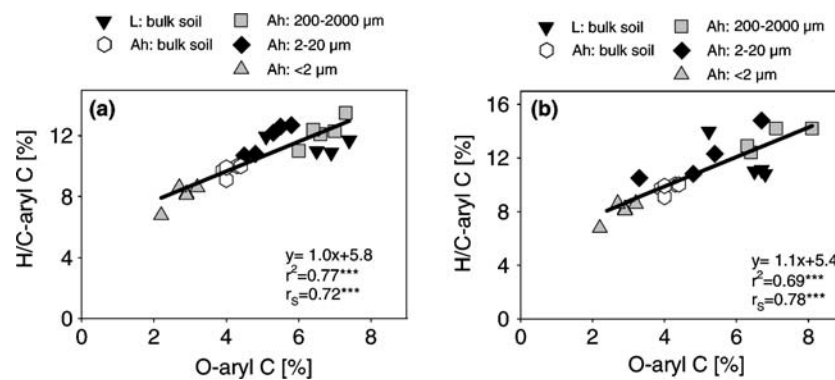


Figure 3. Relative intensity of H/C-aryl C in relation to relative intensity of O-aryl C in the L horizons (L), Ah horizons (Ah), and the particle size fractions of the Ah horizons (a) from the Chronosequence Leinefelde and (b) from the sites in Collelongo, Hesse, Sorø and Leinefelde (62 years old stand) ( $r_s$  = spearman correlation coefficient).

either in the 2–20  $\mu\text{m}$  fractions or the 200–2000  $\mu\text{m}$  fractions, the lowest either in the <2  $\mu\text{m}$  fractions (due to low concentrations of VSC components) or in the 20–200  $\mu\text{m}$  fractions (due to the low carbon stocks). At Hesse and Sorø, the contents of CuO products in the 20–200  $\mu\text{m}$  fractions are higher than in the 200–2000  $\mu\text{m}$  fractions. With the exception of the 2–20  $\mu\text{m}$  fraction in Sorø, enrichment factors of the coarse fractions are > 1, whereas the clay fractions show enrichment factors < 1. With the exception of Collelongo and Leinefelde 30/2, the acid-to-aldehyde ratios are lowest in the 200–2000  $\mu\text{m}$  fraction and highest in the <2  $\mu\text{m}$  fractions. The VSC concentrations, as determined by alkaline CuO oxidation, correlate with the signal intensities of O-aryl C

Table 4. Concentrations, enrichment ratios and stocks of phenolic CuO-oxidation products and acid-to-aldehyde ratios of syringyl units for the Ah horizons of 8 forest sites.

Site (stand age)	Fraction ( $\mu\text{m}$ )	VSC ( $\text{g kg}^{-1}$ OC)	$E_{\text{VSC}}$	VSC stock ( $10^{-3}$ $\text{kg m}^{-2}$ )	Percentage of bulk soil VSC stock (%)	(Ac/Al) <sub>s</sub> ( $10^{-1}$ )
<b>Collelongo</b>	<b>Bulk soil</b>	<b>10</b>		<b>53</b>		<b>3.3</b>
(109)	200–2000	27	2.8	7	13	2.6
	20–200	12	1.2	6	11	4.2
	2–20	14	1.6	27	51	3.6
	< 2	5	0.6	14	25	3.7
<b>Hesse</b>	<b>Bulk soil</b>	<b>17</b>		<b>32</b>		<b>2.3</b>
(34)	200–2000	30	1.8	10	32	1.9
	20–200	34	2.0	5	17	2.5
	2–20	25	1.5	10	31	2.6
	< 2	6	0.4	6	20	5.2
<b>Sorø</b>	<b>Bulk soil</b>	<b>8</b>		<b>83</b>		<b>5.1</b>
(60)	200–2000	15	1.8	20	25	3.3
	20–200	20	2.4	23	28	5.3
	2–20	6	0.7	26	32	6.5
	< 2	2	0.2	13	16	9.1
<b>Leinefelde</b>	<b>Bulk soil</b>	<b>13</b>		<b>73</b>		<b>2.9</b>
(30/1)	200–2000	29	2.2	20	27	2.8
	20–200	36	2.7	20	28	2.3
	2–20	19	1.4	21	29	2.8
	< 2	5	0.4	12	16	3.3
<b>Leinefelde</b>	<b>Bulk soil</b>	<b>18</b>		<b>55</b>		<b>2.3</b>
(30/2)	200–2000	39	2.2	24	44	1.4
	20–200	37	2.0	7	16	2.6
	2–20	21	1.2	11	25	2.4
	< 2	7	0.4	6	15	2.5
<b>Leinefelde</b>	<b>Bulk soil</b>	<b>19</b>		<b>59</b>		<b>2.7</b>
(62)	200–2000	49	2.6	17	28	2.4
	20–200	38	2.0	12	20	2.5
	2–20	19	1.0	23	40	3.0
	< 2	6	0.3	7	12	3.2
<b>Leinefelde</b>	<b>Bulk soil</b>	<b>22</b>		<b>42</b>		<b>3.2</b>
(111)	200–2000	42	1.9	11	25	2.8
	20–200	40	1.8	11	26	3.5
	2–20	28	1.3	16	38	3.7
	< 2	6	0.2	5	11	4.5
<b>Leinefelde</b>	<b>Bulk soil</b>	<b>15</b>		<b>32</b>		<b>2.9</b>
(153 + 16)	200–2000	32	2.1	11	34	2.5
	20–200	26	1.7	2	7	3.4
	2–20	22	1.5	13	42	3.2
	< 2	6	0.4	6	17	4.1

(140–160 ppm) (Figure 4a, b). At Leinefelde the amounts of lignin in the particle size fractions increase with increasing OC concentrations in the respective fractions (Figure 5). Similar relations are expected at the other sites, but the number of data pairs was not sufficient to compile similar plots.

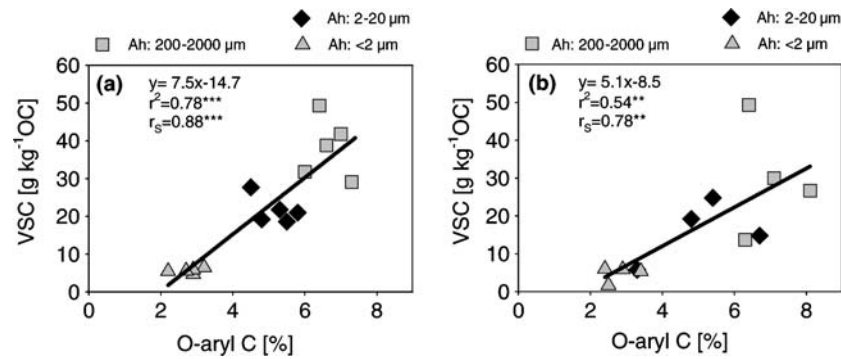


Figure 4. Relative intensity of O-aryl C in relation to concentrations of alkaline CuO oxidation products (VSC) in the particle size fractions of the Ah horizons (a) from the Chronosequence Leinefelde and (b) from the sites in Collelongo, Hesse, Sorø and Leinefelde (62 years old stand).

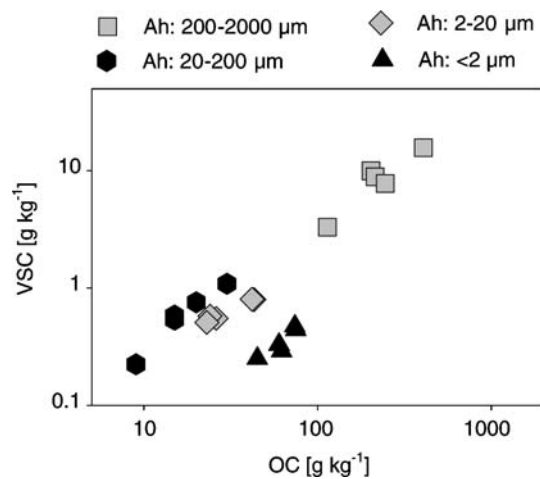


Figure 5. Alkaline CuO oxidation products (VSC) in relation to OC concentrations in the particle size fractions of the Ah horizons from the Chronosequence Leinefelde.

#### Radiocarbon content

The pmC values in the particle size fractions of the Ah horizons range from 91 to 113 pmC (Table 5). Comparing the pmC values of the clay fractions with the pmC values of the coarse fractions of the same sample we found lower pmC values in the clay fraction. The SOM of most particle size fractions shows pmC values higher than 100 and therefore the SOM in these fractions is younger than 50 years on average. Only the SOM of clay fractions in Collelongo and Sorø is clearly older with pmC values of 91 and 99, which is equivalent to 734 and 70 years before present, respectively. Positive, site specific relations are found between the O-aryl C and the pmC values at all sites (Figure 6a).

Table 5. Percent modern carbon in particle size fractions for the Ah horizons of four forest soils.

Site (stand age)	Fraction ( $\mu\text{m}$ )	pmC (%)
Collelongo (109)	200–2000	108.2
	20–200	112.1
	2–20	100.6
	< 2	91.3
Hesse (34)	200–2000	112.5
	20–200	110.3
	2–20	110.0
	< 2	105.9
Sorø (60)	200–2000	111.9
	20–200	110.1
	2–20	104.7
	< 2	99.2
Leinefelde (62)	200–2000	113.2
	20–200	110.0
	2–20	111.2
	< 2	109.4

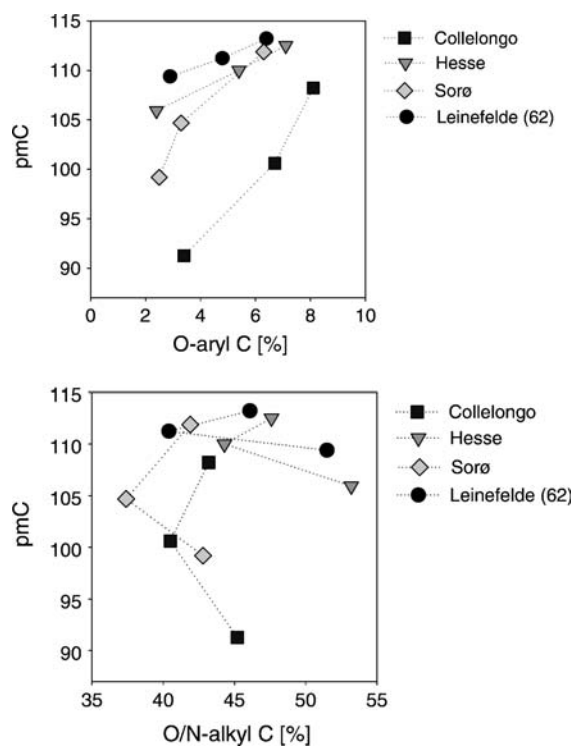


Figure 6. (a) Relative intensity of O-aryl C in relation to pmC in the particle size fractions (200–2000, 2–20 and < 2  $\mu\text{m}$ ) of the Ah horizons. (b) Relative intensity of O/N-alkyl C in relation to pmC in the particle size fractions (200–2000, 2–20 and < 2  $\mu\text{m}$ ) of the Ah horizons of joint forest soils.

Fractions containing the youngest carbon are enriched in O-aryl C, whereas the fractions with the oldest carbon are depleted in O-aryl C. A completely different pattern is obtained for the relation between the O/N-alkyl C and the percentage of modern carbon (Figure 6b), with a strong increase of O/N-alkyl C content in the fine fractions with long turnover times.

## Discussion

### *Carbon pools and stocks*

The wide range of  $E_{OCs}$  (Table 2), also shown by Christensen (1996) and Amelung et al. (1998), suggests that carbon pools of different structure and function were separated by particle size fractionation. The distribution of carbon stocks between the particle size fractions is similar to that described for acid forest soils by Kaiser et al. (2002). A major proportion of OC is stored in the clay fractions (Table 2), but the contribution of the clay fraction to the total OC stock in Ah horizons is lower than in arable soils, where contributions of 50% and more are common (Christensen 1996). This is most probably due to a comparatively high proportion of plant residues in forest soils, which are mainly associated with the 200–2000  $\mu\text{m}$  fraction (Balesdent et al. 1998). The greater relative proportions of OC in the coarse fractions of the young stands in Leinefelde and Hesse suggest that a high tree density in young stands affects the soil OC stock by providing higher litter inputs to the coarse fraction. Effects on absolute OC stocks associated with different stand age are not observed (Table 2). The comparison of OC stocks per square meter and per cubic meter shows that the main factor controlling the magnitudes of the total OC stocks in the bulk soil is the thickness of the Ah horizon. As previously described, for example by Baldock et al. (1992), Balesdent et al. (1998) and Guggenberger et al. (1994), the C/N ratios decrease in the fine fractions of forest soils (Table 2). The decrease of C/N ratios in the fine fractions is usually ascribed to a higher contribution of microbial derived compounds to SOM (Amelung et al. 1998; Haider 1999; Schmidt and Kögel-Knabner 2002).

### *Chemical composition*

The O/N-alkyl C contribution decreases from sand to silt fractions, but increases again in the clay fractions. This trend of O/N-alkyl C proportions in the particle size fractions (Table 3, Figure 1) is consistent with results from Beudert et al. (1989), Preston et al. (1994) and Skjemstad et al. (1996), but contrary to various other  $^{13}\text{C}$  CPMAS NMR studies showing clay fractions to consist predominantly of alkyl C (Baldock et al. 1992; Zech and Guggenberger 1996; Quideau et al. 2000). Preston et al. (1994) discussed that higher O/N-alkyl C proportions in clay fractions might be attributed to a lower



decomposition. However, this falls short of explaining the differences in O/N-alkyl C proportions between the  $<2 \mu\text{m}$  fractions and 2–20  $\mu\text{m}$  fractions of our soils; all  $<2 \mu\text{m}$  fractions of this study are characterised by higher O/N-alkyl C proportions in comparison to the 2–20  $\mu\text{m}$  fractions, whereas, at the same time, low C/N ratios indicate an increased decomposition stage. Most of the carbon assimilated by fungi and bacteria during decomposition ends up with microbial structures containing a significant proportion of O/N-alkyl C (Baldock et al. 1990; Golchin et al. 1996). Therefore the increasing proportion of O/N-alkyl C in the  $<2 \mu\text{m}$  fraction may arise from an increasing proportion of microbial sugars (Baldock et al. 1997; Schmidt and Kögel-Knabner 2002). Higher mass ratios of (galactose + mannose)-to-(arabinose + xylose) in clay fractions of different origin support this assumption (Guggenberger et al. 1994; Guggenberger and Zech 1999; Kiem and Kögel-Knabner 2003).

The aryl C signals, mainly ascribed to lignin and tannin, decrease with decreasing particle size. This is indicative of either a complete decomposition or transformation with a loss of the phenolic and aromatic structures (Table 3, Figure 1). The clear decrease is more apparent as no or only little charred OC is incorporated into the soils that would result in overlapping  $^{13}\text{C}$  CPMAS NMR signal intensities in the aryl C region. In contrast to many other studies (Skjemstad et al. 1996; Mahieu et al. 1999; Kiem et al. 2000) low aryl C signal intensities around 130 ppm were found, particularly in the 2–20 and  $<2 \mu\text{m}$  fractions. This agrees with results of Skjemstad et al. (1997). They detected high amounts of charred organic matter in Australian grassland soils which were under aboriginal fire management presumably for thousands of years, whereas adjacent forested soils which were not subjected to regularly burning, contain little charred OC. Schmidt et al. (1999) also report a high variability of charred OC content in German soils, ranging from 0 to 45% of the total OC in the A horizons.

Lignin and tannin contain O-aryl C as well as H/C-aryl C as structural units. The narrowing C/N ratios together with decreasing O-aryl C proportions (Tables 2 and 3, Figure 2a, b) confirm the decreasing proportion of O-aryl C with progressing degree of decomposition. The O-aryl C intensities in the 200–2000  $\mu\text{m}$  fractions and the L horizons are similar (around 6%). This demonstrates that SOM in 200–2000  $\mu\text{m}$  fractions is strongly influenced by relatively fresh and only slightly decomposed plant material. The C/N ratios and the O-aryl C intensities in the  $<2 \mu\text{m}$  fractions decreased when compared to coarse fractions. In spite of different climate, stand ages, soil types and textures at the sampling sites, similar O-aryl C intensities at specific stages of decomposition were detected. This indicates that site specific conditions do not affect the relation between O-aryl C intensity and C/N ratio. The decrease of O-aryl C is accompanied by a decrease of H/C-aryl C. At all sites the O-aryl C is positively correlated with the H/C-aryl C intensity (Figure 3a, b) indicating that the H/C-aryl C signal can in the main part be assigned to phenolic compounds like lignin and tannin. In addition to phenolic compounds, alkenes are assumed to contribute to the H/C-aryl C signal (Kögel-Knabner 1997). They likely explain

the higher H/C-aryl C intensities in comparison to the respective O-aryl C intensities.

The VSC values point out a high range of lignin concentrations in each particle size class (Table 4). The contents of VSC and the corresponding enrichment factors underline that lignin oxidation products are accumulated in coarse particle size fractions, whereas the clay fractions are depleted in lignin oxidation products. Similar trends for the VSC contents from coarse to fine fractions were previously described by Beudert et al. (1989), Kögel-Knabner and Ziegler (1993) and Guggenberger et al. (1994). The increasing degree of lignin degradation from coarse to fine fractions is supported by the acid-to-aldehyde ratios (Table 4). There is no correlation between age of the forest stand and the acid-to-aldehyde ratios across the chronosequence suggesting that the stand age does not affect the degradation status of lignin. The results of the wet chemical lignin analysis confirm the trend for O-aryl C found by  $^{13}\text{C}$  CPMAS NMR spectroscopy (Figure 4a, b). Thus, we assume that the signal at 150 ppm can almost entirely be assigned to lignin and/or tannin. However, the correlation is stronger if taking only values from the Chronosequence Leinefelde indicating that other O-aryl C components like tannins contribute in different extent to the O-aryl C intensities at the European Sites. The correlations between the alkaline CuO and  $^{13}\text{C}$  CPMAS NMR spectroscopy data underline that the removal of aromatic carbon due to HF treatment was negligible or at least did not affect the bulk functional group characterisation by  $^{13}\text{C}$  CPMAS NMR spectroscopy. Distinct relations between the VSC concentration and the OC concentration exist for the particle size fractions of Leinefelde, the increase of lignin with increasing OC concentrations being lowest in the clay fraction (Figure 5). A similar trend was already reported for a number of bulk forest soils by Kögel-Knabner (2000). The relative contribution of lignin to SOM in the particle size fractions appears to be independent from the OC concentration in the specific particle size fractions. The higher the magnitude of the OC stocks in the individual particle size fractions, the higher is the magnitude of the VSC stocks. The magnitudes of the lignin stocks in bulk soil samples are mainly controlled by the particle size distribution.

#### *Turnover in particle size fractions*

The pmC values of the particle size fractions (Table 5) are in the same magnitude as pmC values measured in particle size fractions under oak forest in southern California (Quideau et al. 2000). PmC values higher than 100 reflect the enhancement of  $^{14}\text{C}$  concentrations in the atmosphere after 1950 by bomb carbon. Lower pmC values in the clay fraction indicate higher proportions of components originating from plant material grown before 1950, whereas the coarse fractions (2000–20  $\mu\text{m}$ ) have a higher uptake of bomb carbon  $^{14}\text{C}$  owing to the high contribution of recent material from leaves and roots (Table 5). In contrast to results of Trumbore and Zheng (1996), we find a consistent trend

for the radiocarbon contents of particle size fractions. The lowest amounts of active material are always in the fine fractions. This is in line with results reported by Anderson and Paul (1984) who separated SOM from arable soils in pools with different turnover using a physical fractionation approach. But in contrast to their results the SOM of the clay fractions obtained in our study (Table 5) is consistently older than in the silt fractions. This was also found by Scharpenseel et al. (1986). The disagreement in the location of the most resistant SOC may be due to different stabilisation mechanisms in the different soil and vegetation types investigated (Guggenberger et al. 1994; Six et al. 2001), but may also be ascribed to distinctions in the isolation procedure for silt and clay. Scharpenseel et al. (1986) reported differences in the radiocarbon age of the clay fraction depending on the fractionation procedure. Lower radiocarbon contents were obtained, when clay fractions were isolated first after dispersion, whereas higher radiocarbon contents were found, when the clay fractions was isolated after the other fractions. Amelung and Zech (1999) exhibit that high energy inputs in the ultrasonication may lead to a redistribution of C from the coarse to the fine fractions, which is avoided in the two-step procedure (Amelung and Zech 1999) applied here. These results point out the importance of optimising the currently used fractionation procedures and the need for information on turnover of fractions obtained for a larger number of soils.

#### *Chemical composition and turnover*

The relations between O-aryl C and pmC as well as O/N-alkyl C and pmC (Figure 6a, b) emphasise the different behaviour of lignin and polysaccharides during humification (Kiem and Kögel-Knabner 2003) and point to a specific stabilisation mechanism of O/N-alkyl C in the clay fraction. OC pools with a higher degree of turnover are characterised by an accumulation of O/N-alkyl C and are depleted in aryl C, although aromatic compounds such as lignin are considered highly recalcitrant. Lignin is recalcitrant in early stages of decomposition and is decomposed at slower rates than plant polysaccharides (Haider 1999), but in the long term lignin is completely decomposed in soils. In some soils it may accumulate in the silt-sized fractions (Baldock et al. 1992), due to retarded decomposition. However, there seems to be no stabilisation of lignin or other phenolic compounds as a result of interactions with clay minerals confirming results of Kiem and Kögel-Knabner (2003). Plant-derived polysaccharides are also degraded in soils, but polysaccharides of most likely microbial origin accumulate in the fine fractions of some soils. Kiem and Kögel-Knabner (2003) showed positive relations between the polysaccharide C and the surface area and proposed interactions of sugars with mineral surfaces in fine separates as a possible mechanism for the long term stabilisation of polysaccharide-type carbon. The stabilisation of O/N-alkyl C compounds in the clay fraction is supported by results of Gleixner

et al. (2002) who detected old polysaccharide components in SOM from agricultural soils. However, clay fractions from other soils have a chemical composition dominated by alkyl C (Baldock et al. 1992). These differences remain to be investigated, to better understand the C stabilization mechanisms in different soils.

### Conclusions

The magnitude of OC stocks in the A horizons under European beech forest is mainly controlled by soil texture and horizon thickness, whereas stand age does not affect the OC stock. The major components of the bulk soil OC stocks are polysaccharide-type (O/N-alkyl C) materials and alkyl C compounds. Using particle size fractionation, carbon pools with distinct chemical composition and turnover times are separated, indicative of specific stabilization mechanisms. The SOM in sand fractions (2000–200  $\mu\text{m}$ ) is characterised by short turnover times and a plant-like chemical composition, whereas the SOM in clay fractions ( $<2 \mu\text{m}$ ) is older and has a different compositional signature. Although lignin may have an important role in the control of biodegradation rates at early stages of litter decay it is not undergoing long-term stabilization by association with the clay minerals. The O-aryl C and H/C aryl C is decreasing with increasing C/N ratios. In spite of different site conditions (climate, parent material), the relations between O-aryl C and C/N as well as between O-aryl C and H/C aryl C are similar at all sites indicating typical aryl contents for certain stages of decomposition. In contrast site specific relations exist for the radiocarbon age and the functional groups, probably because of the different mineral composition of the parent material and the resultant possibilities of chemical and physical preservation. However, the major components of the stable C pool in the clay fractions are always – similar to the bulk soil – O/N-alkyl C and alkyl C compounds. The O/N-alkyl C and alkyl C of the clay fractions account in each sample for more than 30% of the bulk soil OC stock. The detailed chemical composition of O/N-alkyl C and alkyl C in clay fractions remains to be studied to elucidate the long term turnover of OC.

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