

# Improvement of $^{13}\text{C}$ and $^{15}\text{N}$ CPMAS NMR spectra of bulk soils, particle size fractions and organic material by treatment with 10% hydrofluoric acid

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## Summary

The small organic matter content of mineral soils makes it difficult to obtain  $^{13}\text{C}$  and  $^{15}\text{N}$  nuclear magnetic resonance (NMR) spectra with acceptable signal-to-noise ratios. Subjecting such samples to hydrofluoric acid removes mineral matter and leads to a relative increase in organic material. The effect of treatment with 10% hydrofluoric acid on bulk chemical composition and resolution of solid-state  $^{13}\text{C}$  NMR spectra was investigated with six soils, some associated particle size fractions, plant litter and compost. The treatment enhanced the signal-to-noise ratio of the solid-state  $^{13}\text{C}$  NMR spectra. The improvement in spectrum quality was greatest in the clay fraction of soil contaminated with coal ash. The removal of paramagnetic compounds associated with the ash may be the main reason for the improvement. Based on total C, total N, C/N ratio and intensity distribution of the solid-state  $^{13}\text{C}$  NMR spectra, no changes in organic matter composition could be detected, except for a possible loss of carbohydrates. After treatment with HF, solid-state  $^{15}\text{N}$  NMR spectra of particle size fractions were obtained and indicated that the observable nitrogen is present mostly as peptides and free amino groups. Extraction with hydrofluoric acid is recommended as a routine treatment prior to solid-state  $^{13}\text{C}$  and  $^{15}\text{N}$  NMR on soil containing little C or N and soil samples containing paramagnetic compounds from natural or anthropogenic sources.

## Introduction

Solid-state  $^{13}\text{C}$  and  $^{15}\text{N}$  nuclear magnetic resonance (NMR) spectroscopy is proving to be powerful for characterization of organic matter in soils and sediments (Wilson, 1987; Fründ *et al.*, 1994; Hatcher *et al.*, 1994). In contrast to many other analytical methods, solid-state NMR spectroscopy does not depend on the solubility of the sample, thereby allowing the examination of insoluble soil fractions as well as bulk soil samples. Unfortunately, such samples often contain little organic matter and considerable amounts of minerals and paramagnetic compounds, which decrease the sensitivity of solid-state  $^{13}\text{C}$  and  $^{15}\text{N}$  NMR. The resulting spectra typically suffer from poor resolution and low signal-to-noise ratios. In many cases it is impossible to interpret such NMR spectra quantitatively. This is particularly true for solid-state  $^{15}\text{N}$  NMR spectroscopy. Because the  $^{15}\text{N}$ -isotope is rare and due to its magnetic properties the sensitivity of an  $^{15}\text{N}$  NMR is

approximately 1/50 of a  $^{13}\text{C}$  NMR. Recent studies, using optimized  $^{15}\text{N}$  NMR spectroscopic parameters, showed that solid-state  $^{15}\text{N}$  NMR spectra of soils with natural  $^{15}\text{N}$  abundance can be obtained with an acceptable signal-to-noise ratio if their organic N-content exceeds 1% (Knicker *et al.*, 1993; Knicker & Lüdemann, 1995). Such concentrations of N are rare in mineral soils and particle size fractions.

An additional problem, which may complicate the interpretation of  $^{13}\text{C}$  and  $^{15}\text{N}$  NMR spectra of soils, derives from the presence of paramagnetic compounds. Such compounds broaden NMR signals and lead to spectra with strongly overlapping resonance lines. The presence of large amounts of paramagnetic compounds can also lead to selective quenching of signal intensity by shortening relaxation times of specific carbon functional groups. Some of the organic carbon, intimately bound to paramagnetic centres, may therefore be invisible for solid-state  $^{13}\text{C}$  NMR spectroscopy. Quantitative interpretation of such spectra becomes difficult and sometimes impossible—see Wilson (1987) and references therein. This is particularly true if the signal observed in the solid-state  $^{13}\text{C}$  NMR spectrum is not representative of the total organic carbon fraction. Such quantification problems may be expected in soil

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samples with a C/Fe ratio of  $< 1$  (Arshad *et al.*, 1988; Preston *et al.*, 1994; Randall *et al.*, 1995). The influence of paramagnetic compounds has also to be considered in NMR spectroscopic investigations of soils contaminated with heavy metals or ash particles from burned coal (Wilson, 1987; Schmidt *et al.*, 1996).

Attempts to circumvent the problems arising from small organic matter content and the effects of paramagnetic compounds have focused on two methodologies, namely the removal of paramagnetic material, and a relative enrichment of organic matter by the removal of mineral matter. Different methods such as the treatment of the samples with citrate, stannous chloride, sodium dithionite, hydrofluoric acid and hydrochloric acid have previously been suggested and evaluated (Calderoni & Schnitzer, 1984; Skjemstad & Dalal, 1987; Vassallo *et al.*, 1987; Arshad *et al.*, 1988; Preston *et al.*, 1989, 1994; Skjemstad *et al.*, 1992, 1994; Preston & Newman, 1995). Briefly, the treatment with stannous chloride and sodium dithionite was found less effective in removing mineral matter and paramagnetic compounds than treatment with a mixture of hydrochloric acid and hydrochloric acid or hydrofluoric acid only (Arshad *et al.*, 1988; Skjemstad *et al.*, 1992, 1994). Skjemstad *et al.* (1994) showed that 2% HF treatment removed iron more efficiently than dithionite-citrate extraction. The solid-state  $^{13}\text{C}$  NMR spectroscopic comparison of bulk soils and their counterparts that had been treated with 1 and 2% HF solution showed no significant change in the distribution of the organic matter significantly (Skjemstad *et al.*, 1994). There was only a small loss of labile carbohydrates. Similar results were obtained by Hatcher *et al.* (1983) in a study of a marine algal sapropel treated with concentrated HCl/HF and by Preston *et al.* (1989) on soil humic fractions treated with an HCl (5%) and HF (5%) aqueous solution. Wet chemical analysis of the latter showed additional minor changes in the molar distribution of amino acids and the distribution of N determined after acid hydrolysis.

Summarizing the results of the above studies, we can conclude that HF treatment represents a promising method to improve the NMR sensitivity of soil samples originally containing little in organic material and with large amounts of paramagnetic compounds. Most of the experiments described above were conducted with HF solutions at concentrations of less than 5%. Skjemstad *et al.* (1994) showed that treatment of bulk soils with a 2% HF solution was more successful in removing minerals and paramagnetic material than with the 1% solution. Based on this result one can assume that treatment of soils with stronger concentrated HF solutions might remove minerals and paramagnetic material even greater efficiently, resulting in greater enrichment of organic matter and solid-state NMR spectra with enhanced signal-to-noise ratios. However, the effect of more concentrated HF on soil organic matter has not yet been examined.

We attempted to evaluate the effects of treating soil organic matter with 10% (v/v) HF solution. We chose this concentra-

tion to increase the efficiency of the removal of mineral matter and without hydrolysing labile organic components. The HF treatment was applied to different bulk soils with less than 35 g organic carbon per 1 kg of soil, associated particle size separates, organic material from a litter layer (Haplic Podzol), and a variety of composts. Some of the samples derived from soils contaminated by airborne coal particles and/or coal combustion emissions from a nearby factory, which produces briquettes from lignite.

## Material and methods

### *Soils and their particle size separates*

Samples were obtained from several sites in Germany, as follows. A Haplic Alisol (sample 1), was sampled under mixed deciduous forest near Siggen in northern Germany. Samples 2 and 3 were collected from a Haplic Phaeozem under agricultural use close to a briquette factory south of Halle/Saale. This site is contaminated with brown coal dust and ash particles (Schmidt *et al.* 1996). Samples from sandy soils were obtained from Podzols in the Ruhr area. Samples 4 and 5 were collected from a Haplic Podzol under coniferous forest close to Flaesheim. Sample 6 derives from another Haplic Podzol under mixed deciduous forest near Bottrop. Sample 7 was obtained from a Haplic Luvisol under agricultural use near Witzhausen. A brief description of the soils is given in Table 1. Horizons were classified according to German Soil Survey Description (AG Boden, 1994).

Roots and visible plant remains were mechanically removed from the samples when possible. Soil aggregates were crushed and the fraction  $> 2$  mm was removed by dry sieving. The remaining fine earth was physically fractionated using a combination of wet sieving and sedimentation after ultrasonic dispersion as described by Schmidt *et al.* (1996). Wet sieving of the suspension resulted in the separation of three sand fractions (630–2000  $\mu\text{m}$ , 200–630  $\mu\text{m}$  and 63–200  $\mu\text{m}$ ). Using sedimentation cylinders, one clay fraction ( $< 2$   $\mu\text{m}$ ) and three silt fractions (20–63  $\mu\text{m}$ , 6–20  $\mu\text{m}$  and 2–6  $\mu\text{m}$ ) were obtained. The fractions were separated from the suspensions by filtration using cellulose nitrate filter with a maximum pore size of 0.45  $\mu\text{m}$ . Bulk soil samples as well as sand and all silt fractions were ground in a ball mill before HF treatment.

### *Plant litter and compost*

The sample of a litter layer was obtained from the Haplic Podzol (sample 6, Flaesheim) described above. The compost material originates from separately collected biological waste material with additional gardening waste from trees and shrubs (Table 1). Three fractions differing in degree of decomposition were investigated. The residue  $> 10$  mm, left after the composting, consists mainly of poorly decomposed material and is called *sieve residue*. Two fractions  $< 10$  mm, obtained after

**Table 1** Horizon designation (AG Boden, 1994) sampling depth, classification according to FAO (1990) of the investigated materials

Sample number	Soil type and horizon	Depth /cm
1	Haplic Alisol (Ah)	0–14
2	Haplic Phaeozem (Ap)	0–20
3	Haplic Phaeozem (BvCv)	50–90
4	Haplic Podzol (Bh)	25–31
5	Haplic Podzol (Bs)	31–35
6	Haplic Podzol (Bhs)	17–22
7	Haplic Luvisol (Ap)	0–20
	<i>Particle size fractions</i>	<i>Particle size /<math>\mu\text{m}</math></i>
8	Haplic Alisol (Ah) clay	<2
9	Haplic Phaeozem (Ap) clay	<2
10	Haplic Phaeozem (Ap) fine silt	2–6
11	Haplic Phaeozem (Ap) medium silt	6–20
12	Haplic Phaeozem (Ap) coarse silt	20–63
13	Haplic Luvisol (Ap) fine silt	2–6
14	Haplic Luvisol (Ap) fine sand	63–200
	<i>Organic materials</i>	
15	Haplic Podzol (L)	
16	Compost sieve residue	
17	Compost immature	
18	Compost mature	

different duration of composting, were classified according to a self-heating test (BGK, 1993) and called *immature* and *mature compost*. Samples were homogenized in a plant mill before HF treatment.

#### Hydrofluoric acid method

Each sample, weighing approximately 5 g was weighed into a 50 ml polyethylene beaker. After addition of approximately 40 ml of 10% (v/v) HF the closed beaker was shaken for approximately 30 s and the suspension was allowed to settle for at least 12 h. The rising temperatures caused by the exothermic reaction of HF with mineral matter did not exceed 40°C. The supernatant was removed with a tube attached to a plastic syringe to prevent the loss of fine material by decanting. The HF treatment as described above was repeated twice. The remaining sediment was washed with distilled water and vacuum-filtered on 0.45- $\mu\text{m}$  pore-size cellulose nitrate to remove HF. Finally, the isolated pellet was freeze-dried.

#### Elemental analysis

For chemical analysis, a subsample was milled in a ball mill for 10 min. Carbon and nitrogen contents were determined in duplicate with an Elementar Vario EL. The minimum detection concentrations were  $0.1 \pm 0.3 \text{ g kg}^{-1}$  for C and N.

To check if most reactive Fe (such as organically bound Fe and Fe in more reactive Fe(III) oxides and clay minerals), which would interfere with the NMR spectra, had been

removed, subsamples of the bulk soils were extracted with 1 M HCl at 60°C for 1 h. After filtering and decanting of the solution, the amount of Fe in the extracts was determined by atomic absorption spectrometry.

#### Magnetic susceptibility

The magnetic susceptibility of the untreated bulk soils was measured at room temperature (22°C) with a FMA 5000 instrument (Forgenta GmbH, Berlin) using 5 ml of sample. The mass of the sample was determined, and results are given as mass specific magnetic susceptibility.

#### CPMAS NMR analysis

The solid-state  $^{13}\text{C}$  NMR spectra were run on a Bruker MSL 100 (25.178 MHz) and a Chemagnetics M-100 (25.035 MHz), applying the cross polarization magic angle spinning technique (CPMAS) (Schaefer & Stejskal, 1976) with spinning speeds of 4 kHz and 3.5 kHz, respectively. Solid-state  $^{13}\text{C}$  NMR experiments were done with a contact time of 1.0 ms, a  $90^\circ$   $^1\text{H}$ -pulse width of 6.6 ms and a pulse delay from 100 to 600 ms. For each spectrum between  $20 \times 10^3$  and  $800 \times 10^3$  scans were obtained. The chemical shifts of  $^{13}\text{C}$  are reported relative to tetramethylsilane (= 0 ppm). The solid-state CPMAS  $^{15}\text{N}$  NMR spectra were obtained on a Bruker MSL-300 spectrometer at a frequency of 30.398 MHz, with a  $90^\circ$   $^1\text{H}$ -pulse width of 5.5 ms and a contact time of 0.7 ms, spinning at the magic angle at 4.5 kHz frequency. The chemical shift of  $^{15}\text{N}$  are reported relative to nitromethane (= 0 ppm). Using this scale the chemical shift of liquid ammonia is reported at  $-381.9$  ppm (Martin *et al.*, 1981). The spectra were obtained after accumulation of  $500 \times 10^3$  to  $770 \times 10^3$  scans. Knicker & Lüdemann (1995) describe the experimental conditions in more detail.

## Results and discussion

#### Removal of iron

To assess the removal of iron which influences the resolution of NMR spectra we measured the Fe content of bulk soils extractable with HCl before and after the HF treatment. With this method organically bound Fe and iron in Fe(III) oxides and clay minerals, influencing the NMR most strongly, should be removed. As seen from Table 2, the HCl extractable Fe was indeed minimized by the HF treatment to less than  $0.3 \text{ g kg}^{-1}$ . This resulted in an increase of the C/Fe (w/w) ratios from < 10 in the untreated samples to values between 136 and 654 after their treatment with HF.

#### Quantitative alterations

Alteration of the bulk chemical composition of organic matter was assessed by the recoveries of dry matter from the particle size fractions, the litter layer, and the composts after

**Table 2** Magnetic susceptibility of the untreated bulk soils, the amount of Fe extractable with HCl before and after treatment with HF and the C/Fe (w w<sup>-1</sup>) ratio (Fe extractable with HCl) before and after treatment with HF

Number	Soil and horizon	Magnetic susceptibility /10 <sup>-8</sup> m <sup>3</sup> kg <sup>-1</sup>	Fe g <sup>-1</sup> kg <sup>-1</sup>		C/Fe-ratio by weight	
			Before	After	Before	After
1	Haplic Alisol (Ah)	3	4.0	0.2	5	240
2	Haplic Phaeozem (Ap)	21	3.3	0.1	7	654
3	Haplic Phaeozem (BvCv)	11	3.2	0.3	4	309
4	Haplic Podzol (Bh)	1	3.1	0.1	11	404
5	Haplic Podzol (Bs)	1	8.0	0.1	1	136
6	Haplic Podzol (Bhs)	1	2.5	0.1	7	192

Number	Soil type, horizon and particle size	Dry matter	C <sub>org</sub>	N <sub>t</sub>
		recovery <sup>a</sup> /% of initial mass		
8	Haplic Alisol (Ah) clay	ND	ND	ND
9	Haplic Phaeozem (Ap) clay	18.4	77.2	79.0
10	Haplic Phaeozem (Ap) fine silt	17.7	88.9	98.5
11	Haplic Phaeozem (Ap) medium silt	12.3	86.8	103.2
12	Haplic Phaeozem (Ap) coarse silt	21.0	79.1	93.7
13	Haplic Luvisol (Ap) fine silt	12.2	81.5	78.2
14	Haplic Luvisol (Ap) fine sand	4.4	91.3	84.8
15	Haplic Podzol (L)	90.9	91.7	85.6
16	Compost sieve residue	75.2	88.2	83.3
17	Compost immature	66.9	79.5	79.0
18	Compost mature	64.9	84.7	81.8

**Table 3** Calculated recoveries of dry matter, C and N after treatment with 10% (v/v) HF

ND not determined. <sup>a</sup>The amount of dry matter before and after the treatment (data not shown) and the measured C and N contents (Table 2) are used to calculate their recovery. <sup>b</sup>Six samples were treated according to the procedure described in the text except that HF was replaced by distilled water.

HF treatment (Table 3). These samples and the bulk soils were also analysed for C and N content before and after HF treatment. From these data the enrichment of C and N and changes in C N<sup>-1</sup> ratios before and after HF treatment were calculated and are listed in Table 4.

The samples containing much organic matter lost less than 35% of their dry matter, whereas those containing much mineral matter lost more than 79%. In these samples 77 to 92% of the carbon and 78 to 100% of the nitrogen was recovered. Skjemstad *et al.* (1994) reported a similar recovery for C (83 to 92%) after treatment with 2% (v/v) HF. The large recovery of carbon and nitrogen for this set of samples following HF treatment indicates that most of the dry matter is lost in the removal of minerals rather than the loss of organic material. The selective removal of minerals, however, leads to a concentration of organic matter in the samples, which is expressed by a relative enrichment in organic C and N (Table 4). This relative enrichment of organic matter is, as expected, less effective for organic-rich samples than for mineral rich samples. The relative enrichment of organic C and N in the bulk samples lay between 1.2 and 3.2, whereas they were between 3.2 and 20.8 in the particle size fractions. The larger

enrichment in organic material in the particle size fractions may be because particle size fractionation makes the mineral matrix more accessible of HF. Some of the organic matter was removed by the HF treatment, as shown by the recovery of organic C (Table 3). The loss of organic matter may be the result of handling or the removal of labile material soluble in aqueous solutions or both. To test this effect, the Ap horizon of the Haplic Phaeozem (sample 2) was treated using the standard procedure except that HF was replaced by distilled water. It lost 5% of its dry matter. This result supports the above conclusion, namely that incomplete recovery of organic material after HF treatment is due mainly to the handling procedure or to the removal of labile compounds soluble in water, rather than to the hydrolysis of organic compounds with HF.

For further evaluation of possible alterations of the organic material caused by HF treatment a parameter *R* is introduced to describe the changes in the C/N ratio after treatment with HF. The factor *R* was calculated from the C/N ratios of the samples before and after HF treatment (Table 4) and is defined as follows:

$$R = [\text{C/N value before treatment}] / [\text{C/N value after HF treatment}]$$

**Table 4** Enrichment of C and N and alterations of bulk chemical composition due to treatment with 10% (v/v) HF

Number	Sample	$\text{C}_{\text{org}} / \text{g kg}^{-1}$			$\text{N}_t / \text{g kg}^{-1}$			$R^b$
		Before treatment	After treatment	Enrichment <sup>a</sup>	Before treatment	After treatment	Enrichment <sup>a</sup>	
<i>Bulk soils</i>								
1	Haplic Alisol (Ah)	19.4	48.0	2.5	1.6	3.2	2.0	0.8
2	Haplic Phaeozem (Ap)	22.6	65.4	2.9	2.0	5.3	2.7	0.9
3	Haplic Phaeozem (BvCv)	12.4	92.8	7.5	1.1	7.8	7.1	1.0
4	Haplic Podzol (Bh)	34.5	40.4	1.2	1.0	1.3	1.3	1.1
5	Haplic Podzol (Bs)	8.1	13.6	1.7	0.3	0.5	1.7	1.0
6	Haplic Podzol (Bhs)	17.0	19.2	1.1	0.6	0.7	1.2	1.1
7	Haplic Luvisol(Ap)	13.8	37.0	3.2	1.5	3.4	2.3	0.8
<i>Particle size fractions</i>								
8	Haplic Alisol (Ah) clay	54.0	172.9	3.2	5.8	17.6	3.0	1.0
9	Haplic Phaeozem (Ap) clay	62.9	253.6	4.0	5.7	24.4	4.3	1.0
10	Haplic Phaeozem (Ap) fine silt	62.9	316.4	5.0	4.1	22.8	5.6	1.1
11	Haplic Phaeozem (Ap) medium silt	19.2	136.0	7.1	1.0	8.4	8.4	1.2
12	Haplic Phaeozem (Ap) coarse silt	3.2	12.1	3.8	0.1	0.5	5.0	1.2
13	Haplic Luvisol (Ap) fine silt	39.8	266.3	6.7	3.8	24.2	6.4	1.0
14	Haplic Luvisol (Ap) fine sand	17.4	362.4	20.8	1.2	24.0	20.0	0.9
<i>Organic materials</i>								
15	Haplic Podzol (L)	496.3	501.1	1.0	13.3	12.5	0.9	0.9
16	Compost sieve residue	347.0	406.9	1.2	14.9	16.5	1.1	0.9
17	Compost immature	258.9	307.8	1.2	19.3	22.8	1.2	1.0
18	Compost mature	198.1	258.7	1.3	16.7	21.0	1.3	0.9

<sup>a</sup>Enrichment = [content of C or N before HF treatment]/[content of C or N after HF treatment]. <sup>b</sup> $R = [\text{C/N untreated}]/[\text{C/N treated}]$ .

If  $R = 1.0$ , then we assumed that there was no change in C/N ratio due to the treatment. Values of  $R < 1.0$ , however, indicate an increase of C relative to N, whereas  $R > 1.0$  reveals that there is a decrease in C content compared to N. All samples, examined, had  $R$  values of  $1.0 \pm 0.2$ , which indicates that the applied HF treatment did not result in major changes of bulk organic chemical composition. Samples containing much C ( $>54.0 \text{ g kg}^{-1}$ ) and N ( $>5.7 \text{ g kg}^{-1}$ ) before the treatment varied less, probably because error of elemental analysis was less.

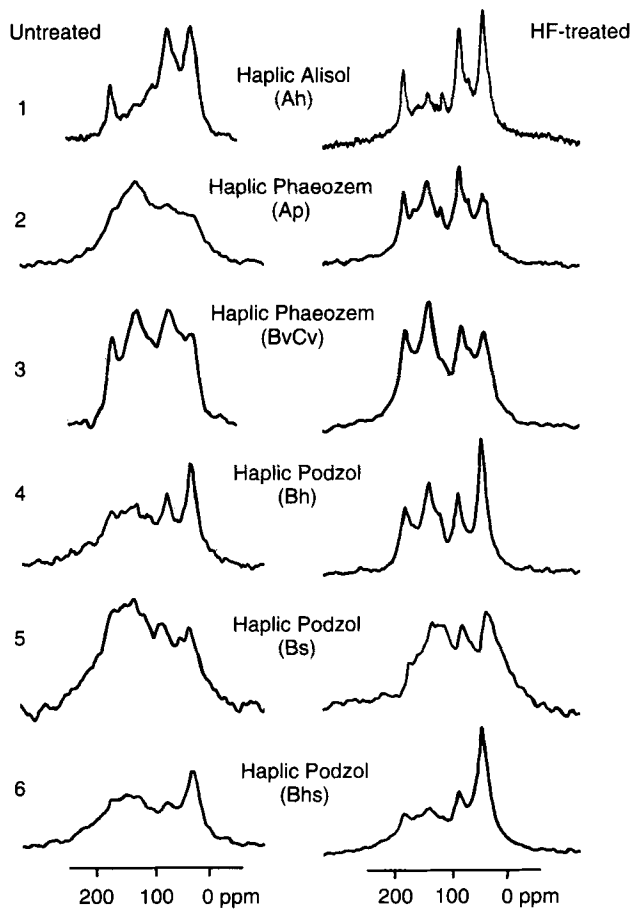
#### NMR spectra

Figure 1 shows the solid-state  $^{13}\text{C}$  NMR spectra obtained from untreated and HF treated soil samples. The solid-state  $^{13}\text{C}$  NMR spectrum of the Ah horizon of the Haplic Alisol (sample 1), was obtained after accumulation of 800 000 scans (Table 5). It reveals the typical pattern of organic material at an early stage of humus maturation with high intensities in the regions tentatively assigned to carbohydrates (100–60 ppm), alkyl C (60–0 ppm) and in the chemical shift region of carboxylic C, carbonylic C and amide C (220–160 ppm). The presence of aromatic compounds is indicated by broad overlapping signals in the chemical shift region between 160 and 100 ppm. Between 60 and 45 ppm a shoulder is apparent, originating from methoxylic C or from

NH-substituted C, or both. The solid-state  $^{13}\text{C}$  NMR spectrum of the HF treated sample shows a clear improvement in resolution despite fewer scans (117 000) (Table 5). The shoulder between 60 and 45 ppm becomes more distinguishable, and there is less overlapping of signals in the aromatic region. This can be explained by an efficient removal of interfering paramagnetic material, indicated by a decrease of HCl-extractable Fe from  $4 \text{ g kg}^{-1}$  in the untreated sample to  $0.2 \text{ g kg}^{-1}$  in the HF-treated sample (Table 2). Together with the C enrichment of factor 2.5 (Table 4) this results in an increasing C Fe-1 ratio from 5 to 240.

The solid-state  $^{13}\text{C}$  NMR spectrum of the Ap horizon of a Haplic Phaeozem can be compared to its HF treated counterpart (sample 2), obtained after accumulation of 315 000 and 139 000 scans, respectively (Table 5). This sample originates from a site contaminated with coal dust and ash, resulting in a magnetic susceptibility of  $21 \times 10^{-8} \text{ m}^3 \text{ kg}^{-1}$ . High magnetic susceptibility leads to a decrease in sensitivity of the samples for NMR spectroscopy. This might explain why the solid-state  $^{13}\text{C}$  NMR spectra of the Ap horizon of the Haplic Phaeozem (sample 2) shows less resolution than that of the Ah horizon of the Haplic Alisol (sample 1) which contains slightly less C (Table 4) and more HCl-extractable Fe (Table 2).

The signals overlap to a large extent, which makes a quantification of the solid-state  $^{13}\text{C}$  NMR spectrum of the untreated sample impossible. Even a qualitative interpretation



**Fig. 1** Solid-state  $^{13}\text{C}$  NMR spectra of untreated (left) and HF treated (right) bulk soils (samples 1–6). Numbers also refer to the numbers given in other Tables and Figures.

of this spectrum is questionable since, with the exception of a broad signal in the aromatic region, no signals can be resolved. The increase in spectral resolution after HF treatment of the Ap horizon of the Haplic Phaeozem indicates that the interference of coal dust and ash can be efficiently removed by HF. Additionally the C/Fe ratio increased from 7 before HF-treatment to 654 after HF treatment (Table 2).

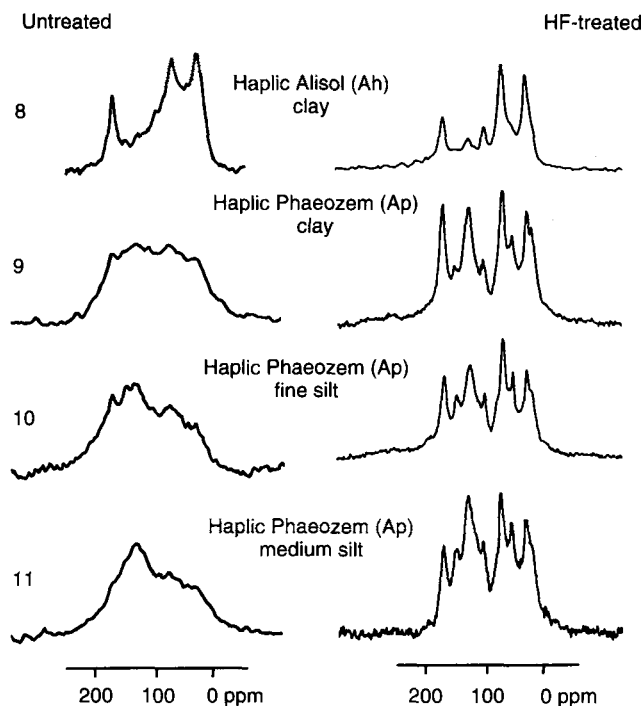
The solid-state  $^{13}\text{C}$  NMR spectrum of the BvCv horizon of a Haplic Phaeozem is contaminated with brown coal residues (sample 3) (Schmidt *et al.*, 1997). Although this sample has a high magnetic susceptibility of  $11 \times 10^{-8} \text{ m}^3 \text{ kg}^{-1}$ , a solid-state  $^{13}\text{C}$  NMR spectrum with distinguishable peaks was obtained after accumulation of 696 000 scans. For the solid-state  $^{13}\text{C}$  NMR spectrum of the HF-treated counterpart only 123 000 scans were accumulated (Table 4). The solid-state  $^{13}\text{C}$  NMR spectrum of the HF treated sample has a slightly better signal-to-noise ratio and a small increase in resolution than that of the untreated sample. This is best explained by the decrease of HCl-extractable Fe from  $3.2 \text{ g kg}^{-1}$  to  $0.3 \text{ g kg}^{-1}$  (Table 2) and by the enrichment of carbon by a factor of 7.5 (Table 4) due to the HF extraction. The HF treatment obviously allowed a reduction in the acquisition time for the  $^{13}\text{C}$  NMR spectroscopic analysis by a factor of 6.

The solid-state  $^{13}\text{C}$  NMR spectra of the Bh and Bs horizons of a Haplic Podzol from Bottrop (samples 4 and 5) before and after HF treatment were obtained after accumulation of approximately 100 000 scans (Table 5). The broad lines in the solid-state  $^{13}\text{C}$  NMR spectra of the untreated samples are most likely due to interfering paramagnetic material, particularly in the Bs horizon, which contained much HCl extractable Fe of  $8 \text{ g kg}^{-1}$ . The HF treatment of the Bh horizon (sample 4) resulted in a relative enrichment in organic carbon by a factor of 1.7 (Table 4). In spite of the minimal carbon enrichment a major improvement in signal-to-noise ratio and resolution of their solid-state  $^{13}\text{C}$  NMR spectra was observed. We concluded from this that the increase in the quality of the spectra is mainly due to the removal of paramagnetic Fe, indicated by an increase of the C/Fe extractable with HCl from 11 before to 404 after HF-treatment, respectively.

Although HF-treatment of the Bs horizon of the Haplic Podzol (sample 5) resulted in a decrease of Fe concentration from  $8 \text{ g kg}^{-1}$  to  $0.1 \text{ g kg}^{-1}$ , there was only a slight improvement in the spectrum. However, one has to bear in mind that HF treatment of this sample resulted in a small organic C enrichment factor of only 1.7. The small amount of organic C of  $13.6 \text{ g kg}^{-1}$  (Table 3) in the HF treated sample is still at a concentration at which well-resolved solid-state  $^{13}\text{C}$  NMR

Number	Soil type, horizon, particle size	Number of scans $\times 10^3$	
		Without treatment	With treatment
1	Haplic Alisol (Ah)	793	117
2	Haplic Phaeozem (Ap)	315	139
3	Haplic Phaeozem (BvCv)	696	123
4	Haplic Podzol (Bh)	106	101
5	Haplic Podzol (Bs)	106	101
6	Haplic Podzol (Bhs)	106	96
8	Haplic Alisol (Ah) clay	341	25
9	Haplic Phaeozem (Ap) clay	170	49
10	Haplic Phaeozem (Ap) fine silt	156	35
11	Haplic Phaeozem (Ap) medium silt	166	104

**Table 5** Comparison of the number of scans obtained for the solid-state  $^{13}\text{C}$  NMR spectra shown in Fig. 1 before and after treatment with 10% (v/v) HF

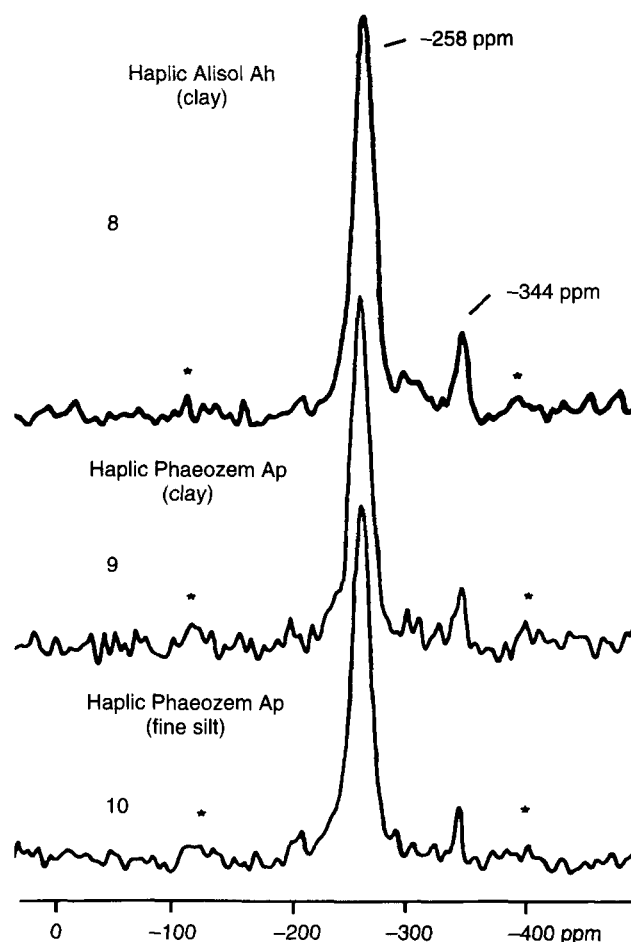


**Fig. 2** Solid-state  $^{13}\text{C}$  NMR spectra of untreated (left) and HF treated (right) particle size fractions (samples 8–11). Numbers refer to the numbers given in other Tables and Figures.

spectra are difficult to obtain. A similar explanation may be valuable for the small improvement of spectrum quality observed after comparison of the solid-state  $^{13}\text{C}$  NMR spectra of the Bhs horizon of the Haplic Podzol from Flaesheim (sample 6) before and after HF treatment.

Figure 2 shows the solid-state  $^{13}\text{C}$  NMR spectra of different particle size fractions obtained before and after HF treatment. The solid-state  $^{13}\text{C}$  NMR spectrum of the clay fraction of the untreated noncontaminated Ah horizon of the Haplic Alisol (sample 8) was obtained after accumulation of 340 000 scans (Table 5). Although the number of accumulated scans for the solid-state  $^{13}\text{C}$  NMR spectrum of the HF treated sample was reduced to 25 000, this spectrum shows much better resolution, with signals in the aromatic region clearly separated. The improvement of the spectrum results from the relatively enrichment factor of organic carbon (3.2) after HF treatment and by the efficient removal of paramagnetic iron, expected to be larger in clay fractions (Randall *et al.*, 1995). This increase in spectrum quality becomes even more obvious in the clay fraction of the Ap horizon of the Haplic Phaeozem contaminated with coal ash particles (sample 9). The removal of paramagnetic compounds associated with the contaminant may be the main reason for the improvement. Similar results were obtained from the fine silt and medium silt fraction of the same soil (samples 10 and 11).

The enrichment of organic matter in the particle size fractions after HF treatment is accompanied by a relative



**Fig. 3** Solid-state  $^{15}\text{N}$  NMR spectra of the HF treated clay fraction of the Haplic Alisol (sample 8) and the clay and fine silt fractions of the Haplic Phaeozem (samples 9 and 10). The number refers to the number given in other Tables and Figures.

enhancement of the organic nitrogen content. The enhancement is sufficient to provide solid-state  $^{15}\text{N}$  NMR spectra with greatly improved signal-to-noise ratios. Inorganic nitrogen, however, is removed by the aqueous HF solution. Figure 3 shows the solid-state  $^{15}\text{N}$  NMR spectra of the HF treated clay fraction of the Ah horizon of the Haplic Alisol (sample 8) after 517 000 scans and the HF treated clay and fine silt fractions of the Ap horizon of the Haplic Phaeozem (samples 9 and 10) after accumulation of 509 000 and 770 000, respectively. Without HF treatment no  $^{15}\text{N}$  NMR spectrum could be obtained from any of these samples even after accumulation of more than 1 000 000 scans. The solid-state  $^{15}\text{N}$  NMR spectra of these HF treated fractions show the pattern typically observed for other agricultural soils (Knicker *et al.*, 1993). They are dominated by a signal in the chemical shift region of amide functional groups between  $-220$  and  $-285$  ppm. Another pronounced resonance line is observed at  $-344$  ppm and is tentatively assigned to the free amino group in amino acids and

**Table 6** Comparison of the number of scans and the relative intensity distribution in the solid-state  $^{13}\text{C}$  NMR spectra of some organic materials before and after treatment with 10% (v/v) HF

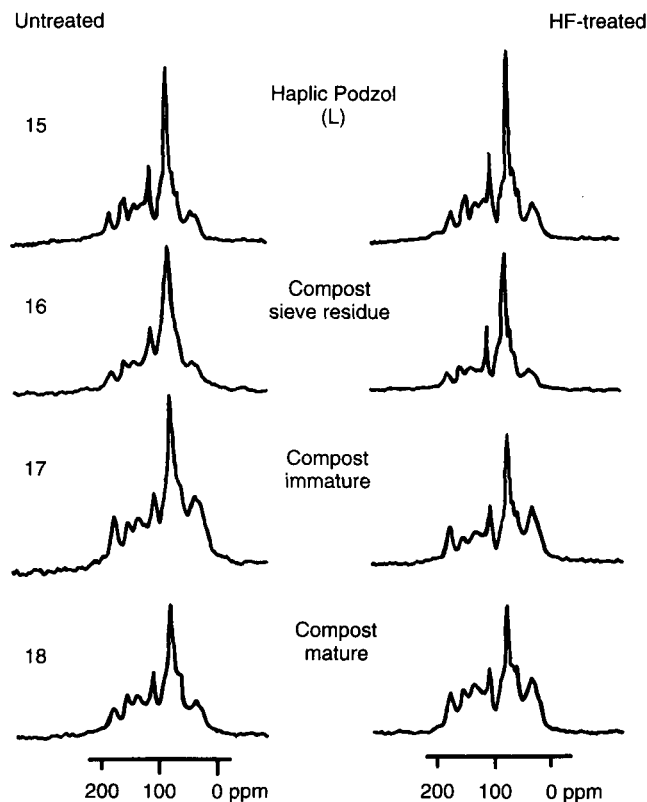
Number	Sample	Number of scans $\times 10^{3b}$	% of total signal intensity <sup>a</sup> (in parenthesis: alteration after HF treatment)			
			Ccarboxyl C	Aromatic C	O-alkyl C	Alkyl C
15	Haplic Podzol (L)	30 (34)	11 (-1)	24 ( $\pm 0$ )	53 ( $\pm 0$ )	12 (+1)
16	Compost sieve residue	22 (18)	5 (+2)	18 (+1)	62 ( $\pm 0$ )	15 (-2)
17	Compost immature	36 (15)	11 ( $\pm 0$ )	19 (0)	49 (-2)	22 (+1)
18	Compost mature	21 (15)	9 ( $\pm 0$ )	22 (+3)	54 (-7)	15 (+4)

<sup>a</sup>Data in percentage of total signal intensity (alkyl C, -10–45 ppm; O-alkyl C, 45–110 ppm; aromatic C, 110–160 ppm; carboxyl C, 160–220 ppm). <sup>b</sup>Value in parenthesis is for HF treated samples.

sugars. This feature of the spectra suggests that amide functional groups, probably from peptide-like materials, are the major forms of nitrogen in these samples.

Although an improvement of the solid-state  $^{13}\text{C}$  NMR spectra after HF treatment was observed, structural changes in the organic matter might have occurred. The small signal-to-noise ratio and low resolution of most  $^{13}\text{C}$  NMR spectra of the untreated soil samples (Figs 1 and 2) does not allow a quantitative interpretation of the spectra. Therefore, to investigate the effect of HF treatment we have examined materials rich in organic matter. The samples were from a litter layer of a Haplic Podzol (sample 15) and compost. According to a self-heating test (BGK, 1993) the degree of decomposition of the compost increases from sieve residue (sample 16) to immature compost (sample 17) and mature compost (sample 18). Their solid-state  $^{13}\text{C}$  NMR spectra before and after HF treatment are shown in Fig. 4. The intensity distributions of different functional groups were obtained by integration of the representative chemical shift regions in the solid-state  $^{13}\text{C}$  NMR spectra and are presented in Table 6. For solid-state  $^{13}\text{C}$  NMR studies of bulk soils and associated fractions Preston *et al.* (1994) found a standard deviation of the integrated spectra of 5% and 10%, respectively. Since we examined the same type of samples under similar conditions we might expect a similar standard deviation in the integration values from these experiments.

The solid-state  $^{13}\text{C}$  NMR spectra of the slightly decomposed organic material (samples 15–17) before and after HF treatment have a very similar pattern (Fig. 4) and show a similar relative intensity distribution (Table 6). Differences of the latter vary in the range of experimental error. It seems that no major alteration in organic matter composition was induced by the HF treatment. In contrast, the spectrum of the mature compost (sample 18) shows a relative decrease in the O-alkyl C region (-7% of the total signal intensity) and therefore a relative increase in the other regions, indicating a possible loss of carbohydrates. Hatcher *et al.* (1983) and Preston *et al.* (1989) found that some carbohydrates are found to be labile components easily solubilized and removed from soil and sediment by circulating water. Therefore we assume that the



**Fig. 4** Solid-state  $^{13}\text{C}$  NMR spectra of untreated (left) and HF treated (right) different organic materials (samples 15–18). Numbers refer to the numbers given in other Tables and Figures.

removal is the effect of solubilization by water rather than by extraction with HF. Considering this fact and the small decrease of O-alkyl C we can expect the loss of carbohydrates caused by HF extraction to be negligible for bulk soils and size fractions.

Further alterations of C species could not be detected by solid-state  $^{13}\text{C}$  NMR. If qualitative alterations of the organic matter due to HF treatment occur they obviously affect all C-species to the same extent and cannot be detected by NMR spectroscopy.

**Table 7** Comparison of the relative intensity distribution in the solid-state  $^{13}\text{C}$  NMR spectra of the Ah horizon of an Haplic Alisol before and after treatment with 10% (v/v) HF

Number	Sample	Carboxyl C Aromatic C O-alkyl C Alkyl C % of total signal intensity <sup>a</sup> (in parenthesis: alteration after HF treatment)			
		1	Haplic Alisol (Ah)	10 (+5)	14 (+4)
8	Haplic Alisol (Ah) clay	12 (+4)	14 (+1)	45 (-2)	29 (-2)

<sup>a</sup>Data in percentage of total signal intensity (alkyl C, -10-45 ppm; O-alkyl C, 45-110 ppm; aromatic C, 110-160 ppm; carboxyl C, 160-220 ppm).

Paramagnetic compounds can lead to selective quenching of the signal's intensity by shortening relaxation times of specific functional groups. Some of the organic carbon, intimately bound to paramagnetic centres, may, therefore, be invisible for solid-state  $^{13}\text{C}$  NMR spectroscopy. This is of particular concern when examining clay fractions, which can contain much paramagnetic iron. Preston *et al.* (1994) reported that only 16-30% of the organic carbon was detected in solid-state  $^{13}\text{C}$  CPMAS NMR spectra of some soils and soil size fractions having C/Fe ratios between 0.9 and 10.2. Randall *et al.* (1995) compared the total signal intensity of solid-state  $^{13}\text{C}$  CPMAS NMR spectra of different soil samples obtained under the same spectroscopic conditions in relation to the total carbon determined gravimetrically. They found that the carbon in the clay fraction in a Rothamsted soil observed by solid-state  $^{13}\text{C}$  NMR ranges from 24% to 39% relative to the silt fraction. Some of the carbon was not detected with this technique, possibly due to the influence of neighbouring paramagnetic centres. The question resulting from these observations is whether the carbon observed in solid-state  $^{13}\text{C}$  NMR spectra of soil samples containing much of paramagnetic material is representative for the total carbon of the sample (Preston *et al.*, 1994). The HF treatment should reduce the influence of paramagnetic compounds by removing them, and a greater proportion of the hidden carbon should be visible in the NMR spectrum. If this hidden carbon was chemically different from the C detected by NMR, a comparison of the solid-state  $^{13}\text{C}$  NMR of the untreated and the HF treated sample should reveal differences in their relative intensity distribution. However we cannot interpret most of the solid-state  $^{13}\text{C}$  NMR spectra of the untreated soil samples we show here because of their poor signal-to-noise ratios. Therefore we limited our comparison of the relative intensity distribution before and after HF treatment to the bulk soil and the clay fraction of the Ah horizon of the Haplic Alisol (samples 1 and 8). These data are listed in Table 7. The solid-state  $^{13}\text{C}$  NMR spectrum of the bulk soil shows a relative decrease in signal intensity in the chemical shift region of O-alkyl C (carbohydrates) with a simultaneous relative increase in signal intensity in the regions assigned to carboxylic C and aromatic C. This may be due to the loss of water-soluble carbohydrates during the HF treatment, discussed above. Another explanation for the difference in the relative intensity distribution in the solid-state  $^{13}\text{C}$  NMR spectra of both these counterparts may be the increase of resolution in the spectrum of the HF treated sample. The

removal of paramagnetic compounds with HF results in less overlapping resonance lines, which allows a more realistic assignment of signal intensity to any specific integration region. However, the results may underestimate carboxylic C and aromatic C in the solid-state  $^{13}\text{C}$  NMR spectrum of the untreated soil sample. In contrast to the bulk soil of the Ah horizon of the Haplic Alisol, the solid-state  $^{13}\text{C}$  NMR spectra of the untreated and HF treated clay fraction of the same horizon reveals only minor differences in their signal intensity distribution. The variations are in the range of experimental error. This strongly indicates that here the organic carbon distribution observed in the solid-state  $^{13}\text{C}$  NMR spectrum of the untreated sample represents that of the total carbon.

#### Summary and conclusions

Samples of several soils, some contaminated with brown coal and ash were extracted with hydrofluoric acid and their chemical composition examined by elemental analysis and solid-state  $^{13}\text{C}$  NMR. Some organic matter was lost due to the HF treatment (C: 8-23%, N: 0-23%), but there were no evident changes in bulk chemical composition. Except for the loss of some carbohydrates, no qualitative changes of C species in organic matter could be detected by solid-state  $^{13}\text{C}$  NMR.

Extraction of bulk soils and particle size fractions with 10% HF effectively removed mineral matter and enriched the sample in organic matter. Comparing untreated and HF-treated samples of bulk soils (samples 1-3) and particle size fractions (samples 8-11) showed that the HF treatment results in solid-state  $^{13}\text{C}$  NMR spectra with enhanced signal-to-noise ratios and improved resolution, although fewer scans were accumulated. Solid-state  $^{13}\text{C}$  NMR spectra of soil contaminated with coal dust and ash showed major improvements in resolution and signal-to-noise ratio after HF treatment. Solid-state  $^{13}\text{C}$  NMR spectra of samples from podzol horizons containing sesquioxides (samples 4-6) still suffered from poor resolution, probably because of incomplete removal of mineral matter by the HF-treatment and less efficient concentration of C. However, the improvement of most solid-state  $^{13}\text{C}$  NMR spectra after HF treatment allowed their qualitative analysis. The efficient removal of minerals with HF decreases the amount of hidden carbons, influenced by paramagnetic compounds. Despite the loss of some C, HF treatment of soil samples might increase the reliability of organic carbon distributions, determined by means of solid-state  $^{13}\text{C}$  NMR spectroscopy.

The relative enrichment in organic material in mineral rich soil samples after HF treatment allows the elucidation of the chemical structure of organic N in soils with natural  $^{15}\text{N}$  abundance by solid-state  $^{15}\text{N}$  NMR spectroscopy.

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