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Hydrofluoric acid pre-treatment for improving ^{13}C CPMAS NMR spectral quality of forest soils in south-east Queensland, Australia

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Abstract

Hydrofluoric acid (HF) was used to pre-treat forest soils of south-east Queensland for assessing the effectiveness of iron (Fe) removal, carbon (C) composition using ^{13}C cross-polarisation (CP) with magic-angle-spinning (MAS) nuclear magnetic resonance (NMR) before and after the HF pre-treatment, and the improvement of ^{13}C CPMAS NMR spectra. Soil samples were collected from 4 experimental sites of different soil types, harvest residue management or prescribed burning, and tree species. More than 86% of Fe was in all soil types removed by the HF treatment. The ^{13}C NMR spectral quality was improved with increased resolution, especially in the alkyl C and O-alkyl C regions, and reduced NMR run-time (1–5 h per sample compared with >20 h per sample without the pre-treatment). The C composition appeared to alter slightly after the pre-treatment, but this might be largely due to improved spectrometer conditions and increased resolution leading to more accurate NMR spectral integration. Organic C recovery after HF pre-treatment varied with soil types and forest management, and soluble soil organic matter (SOM) could be lost during the pre-treatment. The Fourier Transform-Infrared (FT-IR) spectra of HF extracts indicated the preferential removal of carboxylic C groups during the pre-treatment, but this could also be due to adsorbed water on the mineral matter. The NMR spectra revealed some changes in C composition and quality due to residue management and decomposition. Overall, the HF treatment was a useful pre-treatment for obtaining semi-quantitative ^{13}C CPMAS NMR spectra of subtropical Australian forest soils.

Additional keywords: NMR spectroscopy, soil organic matter, magnetic materials, iron removal, HF acid.

Introduction

In recent soil organic matter (SOM) studies utilising solid-state nuclear magnetic resonance (NMR) spectroscopy, pre-treatments to remove interfering magnetic materials, such as Fe, have been incorporated (Skjemstad *et al.* 1994; Preston and Newman 1995; Schmidt *et al.* 1997; Dai and Johnson 1999; Knicker and Skjemstad 2000; Mathers *et al.* 2000). Hopkins *et al.* (1997) reported that NMR of soils is restricted by the content of Fe, but also of copper (Cu) and manganese (Mn), because paramagnetic properties interfere with the NMR signals. Paramagnetics severely reduce the proton rotating frame relaxation times, thereby reducing the signal-to-noise (S/N) ratio of a spectrum (Pfeffer *et al.* 1984), and, in severe cases, preventing a spectrum from being obtained at all (Skjemstad *et al.* 1997; Smernik and Oades 1999; Mathers *et al.* 2000). However, techniques to remove Fe have led to

improvements in NMR spectral quality of SOM with different properties (Arshad *et al.* 1988; Skjemstad *et al.* 1994; Schmidt *et al.* 1997).

A number of studies have tested the effectiveness of such techniques on the improvement of solid-state NMR spectroscopy of SOM, including pre-treatments with hydrofluoric acid (HF) at several concentrations (Skjemstad *et al.* 1994; Preston and Newman 1995; Schmidt *et al.* 1997; Dai and Johnson 1999), dithionite over a range of pH (Vassallo *et al.* 1987; Arshad *et al.* 1988; Skjemstad *et al.* 1994; Dai and Johnson 1999), and stannous chloride (Arshad *et al.* 1988; Dai and Johnson 1999), with varying degrees of success. Skjemstad *et al.* (1994) found that 2% HF could improve the visibility of C in a soil sample by as much as 25 times that of the best dithionite treatment and without citrate contamination, whereas Dai and Johnson (1999) reported some losses in organic C and inconsistencies in NMR spectral quality of 3 mineral soils after 2% HF treatment. Both Skjemstad *et al.* (1994) and Schmidt *et al.* (1997) recommended the use of HF (2% and 10%, respectively) as a pre-treatment prior to solid-state NMR analysis of soils because of enhanced S/N ratios, improved resolution, and small losses of organic matter that had little effect on overall C chemistry. However, Dai and Johnson (1999) indicated that it was premature to recommend a routine chemical pre-treatment for improving NMR spectral quality, after not achieving the same degree of success with 2% HF on Spodosols from the north-eastern United States.

Australian soils generally have small quantities of organic matter (<5% C) and high levels of mineral matter, reducing the potential for direct study of SOM structures by solid-state ^{13}C NMR (Skjemstad *et al.* 1994), and resulting in almost unreadable NMR spectra for some soils. The incorporation of a routine pre-treatment for NMR analysis of subtropical Australian forest soils may result in an increased S/N, reduced machine time, and improved NMR spectral quality with the accumulation of fewer scans. From previous studies, the HF pre-treatment represents a promising method to improve NMR spectra of soils originally containing little organic material and large quantities of paramagnetic species (Schmidt *et al.* 1997). However, many previous studies have not compared the C distribution in the soil after pre-treatment with that of the untreated soil (Skjemstad *et al.* 1994), although several studies report a loss in total C after pre-treatment. The nature of soluble C structures preferentially removed by the HF-treatment may be important when evaluating the SOM pool by solid-state ^{13}C NMR spectroscopy.

The objectives of this study were to use the 2% HF pre-treatment procedure recommended by Skjemstad *et al.* (1994) on surface forest soil samples collected from 4 existing experimental sites in south-east Queensland, Australia, to assess: (a) the effectiveness of Fe removal for the soil samples; (b) soil C composition using ^{13}C CPMAS NMR before and after HF pre-treatment; and (c) the improvement of the resultant ^{13}C CPMAS NMR spectra.

Materials and methods

Site description and soil sampling

Soil samples from 4 sites within the Queensland Department of Primary Industries-Forestry (QDPIF) plantation estate in south-east Queensland were selected for this study because of their differences in residue management practice, soil type, and tree species. The soils from Imbil State Forest, Queensland were collected from the 0–10 cm soil depth of a 1-year-old second-rotation (2R) site (26°32' S, 152°37' E) and a 3-year-old 2R site (26°31' S, 152°38' E). These were planted with indigenous hoop pine (*Araucaria cunninghamii* Ait. Ex D. Don) seedlings growing between 2.5-m-wide windrows of harvest residues containing 522 t/ha of dry matter (DM) under the windrows in the 1-year-old site and 348 t DM/ha in the 3-year-old site. The soils of Toolara State Forest (26°00' S, 152°49' E), Queensland, were collected from the 0–10 cm soil depth of an F₁ hybrid between slash pine (*Pinus elliottii*) and Caribbean pine (*P. caribaea*)

Table 1. Location, tree species, residue management, sampling depth, classification, and some properties of the soils in south-east Queensland

Sample code	Location and species	Residue management	Depth (cm)	FAO Soil Classification	Aust. Soil Classification ^A	pH (1:5 H ₂ O)	% Clay
IMB 1.2	Imbil, hoop pine	1-year-old under windrows	0–10	Lithosol	Dermosol	7.05	32
IMB 1.6	Imbil, hoop pine	1-year-old between windrows	0–10	Lithosol	Dermosol	6.51	34
IMB 3.2	Imbil, hoop pine	3-year-old under windrows	0–10	Lithosol	Dermosol	5.28	32
IMB 3.6	Imbil, hoop pine	3-year-old between windrows	0–10	Lithosol	Dermosol	5.17	34
PCH 8	Peachester, blackbutt ^B	Biennially burnt since 1972 (10) ^C	0–5	Ferric Luvisol	Red to Yellow Kandosols	5.26	18
PCH 17	Peachester, blackbutt	Unburnt since 1969	0–5	Ferric Luvisol	Red to Yellow Kandosols	4.90	22
PCH 21	Peachester, blackbutt	Quadrennially burnt since 1972 (7) ^C	0–5	Ferric Luvisol	Red to Yellow Kandosols	5.51	24
TOO 7	Toolara, F ₁ hybrid pine	Double harvest residues	0–10	Gleyic Acrisol	Grey Kandosol	5.57	6
TOO 15	Toolara, F ₁ hybrid pine	No harvest residues	0–10	Gleyic Acrisol	Grey Kandosol	5.55	6

^AIsbell (1996).

^BBlackbutt is dominant in the mixed species of native, eucalypt forest.

^CValues in parentheses are the total number of burns for that treatment.

with either all clearfall harvest residues removed or double residues added. The site at Peachester State Forest (26°50' S, 152°53' E), Queensland, was a natural wet sclerophyll mixed species forest dominated by blackbutt (*Eucalyptus pilularis*). Soil samples from the 0–5 cm soil depth were collected from areas that had no prescribed burns, prescribed burns every 2 years (biennial), and prescribed burns every 4 years (quadrennial), which were described in detail by Guinto *et al.* (2000). Table 1 provides the general chemical and physical characteristics of each site prior to HF-treatment.

Chemical and statistical analyses

A TPS conductivity-salinity-pH-temperature meter (Model WP-81) was used to determine the pH of each sample in a 1:5 soil-to-water extract after filtration. Total soil organic C of the Peachester and Toolara soils was determined by the wet oxidation Walkley-Black method (Walkley and Black 1934; Guinto *et al.* 1999) using a 77% correction factor. Total C of the Imbil soils and total N for all soils were determined by isotope ratio mass spectrometry (IRMS) on a Roboprep CN (7001)/Tracermass System (9001) manufactured by Europa Scientific (Bubb *et al.* 1999; Pu *et al.* 2001). All chemical analyses were conducted in triplicate. Statistical analyses were performed using STATISTICA software (Statsoft 1999).

HCl-extractable Fe

HCl-extractable Fe was determined in duplicate by weighing 500 mg soil into a 50 mL centrifuge tube. After addition of 25 mL of 1 M hydrochloric acid (HCl), samples were shaken end-over-end overnight (approximately 16 h). After shaking, samples were centrifuged at 2000 rpm for 20 min and the supernatant

was filtered through Whatman # 42 filter papers for a clear solution. The filtrates were retained for Fe analysis (in triplicate) by flame atomic absorption spectroscopy (AAS) on a Varian SpectraAA 10/20 atomic absorption spectrometer. The instrumental conditions applied to all samples were an air-acetylene flame, a 0.2 nm slit width at a wavelength of 248 nm, and a lamp current of 5 mA.

Pre-treatment with HF

Following the recommended method (Skjemstad *et al.* 1994), approximately 5 g of each soil was weighed into a 50 mL polyethylene centrifuge tube and shaken end-over-end with 8 (4 × 1 h, 3 × 16 h, and 1 × 64 h) successive 45-mL aliquots of 2% HF acid. Samples were centrifuged after each extraction at 2000 rpm for 20 min and the supernatants were poured through a 5-mm Millipore Durapore membrane filter to recover the light fraction and then discarded. After the final extraction, the residues were combined with any light fraction, washed several times with deionised Millipore water, oven dried at 75°C, and ground to a powder using mortar and pestle for NMR analysis. All HF analyses were conducted on 4 replicates of each of the 9 samples.

Fourier Transform-Infrared spectroscopy

FT-IR spectroscopy was performed on freeze-dried HF extracts. Potassium bromide (KBr) pellets were prepared by pressing a mixture of 1–2 mg dried extract with 100–200 mg KBr (spectrometry grade). The FT-IR spectra were recorded using a Perkin-Elmer 1725 spectrophotometer scanning over the frequency range 4000 cm⁻¹ to 400 cm⁻¹ with a resolution of 4 cm⁻¹.

¹³C CPMAS NMR spectroscopy

The ¹³C CPMAS NMR spectra of all soils were obtained at a frequency of 100.59 MHz on a Varian Unity 400 spectrometer (Varian Inc., CA). Samples were packed in a silicon nitride rotor (OD = 7 mm) and spun at 5 kHz at the magic angle. The untreated Imbil SOM spectra were obtained with single contact times of 1 ms, an acquisition time of 10 ms, and a recycle delay of 1 s. Between 80000 and 100000 transients were collected for each spectrum. The untreated Peachester SOM spectra were collected over 18000 transients with single contact times of 1 ms, an acquisition time of 50 ms, and a recycle delay of 4 s. Untreated SOM spectra from Toolara were obtained with single contact times of 2.5 ms, an acquisition time of 50 ms, and a recycle delay of 4 s. Again 18 000 transients were collected for the Toolara SOM spectra. The HF-treated SOM were collected with single contact times of 1 ms, an acquisition time of 10 ms, and a recycle delay of 1 s. The total transients collected were 3500 for the HF-treated Imbil SOM spectra, and 15000 transients for the HF-treated Peachester and Toolara SOM spectra with the exception of the unburnt Peachester sample (5000 transients). A Lorentzian line broadening function of 50 Hz was applied to all spectra. Carbon-13 CPMAS NMR spectra were referenced externally to hexamethylbenzene at 132.1 ppm, which is equivalent to tetramethylsilane at 0 ppm.

The NMR spectra were divided into 4 regions representing the different chemical environments of a ¹³C nucleus. These were alkyl C (0–45 ppm), O-alkyl C (45–110 ppm), aromatic C (110–160 ppm), and carbonyl C (160–210 ppm). The relative intensities of each C functional group were calculated by integration using the Varian NMR software package (Version 6.1B, Varian Inc., CA) included with the spectrometer. The limiting chemical shift values were not strictly adhered to but used as a guide, especially where peaks overlapped. ‘Valleys’ were then used to represent the chemical shift boundaries. The aromatic and carbonyl C spectral regions were corrected for the carbonyl spinning sideband (SSB) appearing at approximately 223 ppm. Because SSB of equal intensity are produced on either side of the originating centreband (i.e. in this case at 123 and 223 ppm), the visible SSB were integrated and the aromatic and carbonyl C NMR spectral regions were corrected for the presence of SSB.

Results and discussion

Recovery of soil mass, C, and N

Table 2 gives the organic C and total N contents before and after HF treatment, enrichment of C and N, and recoveries of soil mass, C, and N for each of the 4 experimental sites. Dermosols of the hoop pine plantations at Imbil lost 83–92% of their initial soil mass during the HF treatment. In the 1-year old site, SOM from areas under the windrows lost 83% of its initial soil mass, whereas SOM from areas between the windrows lost 92% of its

Table 2. Organic C and total N before and after 2% HF treatment, percentage recoveries of C and N, and percentage loss of soil mass for the four sites

Sample code	% Soil mass lost during HF treatment ^A	Organic C (g/kg) ^A		Enrich. ^B		Total N (g/kg) ^A		Enrich. ^B		% Recovery	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Organic C	Total N
IMB 1.2	83.2±2.1	84.4±3.7 ^C	418±24 ^C	4.95	4.81±0.10	26.5±2.3	5.51	82.9 ^C	92.2		
IMB 1.6	92.1±1.3	43.0±0.5 ^C	382±32 ^C	8.88	3.65±0.04	31.1±1.4	8.52	70.3 ^C	67.5		
IMB 3.2	91.4±1.7	41.1±0.8 ^C	345±25 ^C	8.40	2.77±0.03	19.2±0.8	6.93	72.2 ^C	60.0		
IMB 3.6	84.0±0.8	36.1±1.1 ^C	315±22 ^C	8.71	2.64±0.05	17.7±0.5	6.70	139.0 ^C	106.9		
PCH 8	67.6±3.6	12.9±0.3	59±19	4.60	1.00±0.25	n.d.	n.d.	149.0	n.d.		
PCH 17	70.6±2.5	63.8±0.2	151±22	2.37	1.83±0.21	n.d.	n.d.	69.8	n.d.		
PCH 21	69.2±1.4	12.5±0.3	29±12	2.28	1.60±0.24	n.d.	n.d.	70.3	n.d.		
TOO 7	75.6±2.4	36.7±4.2	46±23	1.26	0.36±0.11	1.0±0.1	2.89	30.7	70.5		
TOO 15	58.9±1.6	19.1±0.6	31±14	1.60	0.28±0.04	0.9±0.2	3.04	65.8	124.8		

n.d. = not determined.

^AMeans ± standard errors.^BEnrich. = (C or N content after HF treatment)/(C or N content before HF treatment), following the calculation of Schmidt *et al.* (1997).^CImbil values are total C not organic C.

initial soil mass. This phenomenon was reversed in SOM from the 3-year-old site (Table 2), with SOM from areas under the windrows losing 91% of its initial soil mass, while SOM from between the windrows lost 84% of its initial soil mass. The Kandosols in the eucalypt-dominated natural forest at Peachester all had soil mass losses around 70%, despite the unburnt plot having a much higher C content (64 g/kg, Table 2) than the burnt plots. The Grey Kandosols from the exotic pine hybrid plantation at Toolara displayed contrasting soil mass losses. SOM from areas under no harvest residues lost 59% of its initial soil mass, while SOM from areas with double harvest residues applied lost 76% of its initial soil mass yet had a higher C content but a low C recovery (Table 2). This was reflected in the 3-year-old Imbil SOM—under the windrows the soil mass loss was greater (91%) and C content higher (41 g/kg) than between the windrows (84% and 36 g/kg) where there were no residues applied (Table 2). Schmidt *et al.* (1997) observed that samples with high organic matter content retained >65% of their soil mass, while those containing high amounts of mineral matter retained only 21% or less of their initial soil mass. Soils from this study retained 30% or less of their initial soil mass (with the exception of TOO 15), consistent with the observation of Schmidt *et al.* (1997). The results of this study also indicate that different harvest residue management or prescribed burning practices in intensively managed plantation forest ecosystems may also affect the mass losses of some soil types during the HF pre-treatment extraction process.

The recoveries of organic C and total N were variable depending on the soil type and residue management practice employed. The Dermosols at Imbil had C recoveries of 70–83% with the exception of IMB 3.6, which had a C recovery of 139%. There was no explanation for this high recovery of C in SOM from areas between the windrows of a 3-year-old hoop pine plantation (Table 2), as C was determined by the combustion method of IRMS. However, it is possible that these older plots containing the first windrows, with previous burnings, different spacings and residue loadings, could have contributed to the large C recovery observed for this sample. The N recoveries of the Imbil SOM were 60–107%.

The Red Kandosols at Peachester were found to have a C recovery of about 70%, except the biennially burnt SOM (PCH 8), which had a C recovery of 149% (Table 2). Guinto *et al.* (1999) reported that the quadrennially burnt site (PCH 21) was more similar to the unburnt site (PCH 17) than the biennially burnt site, which may explain the similarities in C recoveries of these 2 sites. The high C recovery of the biennially burnt site after HF treatment may also be the result of finely divided char C not being detected by the Walkley-Black method before treatment because of protection, such as in microaggregates, metal ion binding, or adsorption to clay surfaces (Knicker and Skjemstad 2000). This is particularly important in the dichromate oxidation method where larger particle sizes may not be fully oxidised, or the optimal temperature not attained during the oxidation process. Although Skjemstad and Taylor (1999) report that the Walkley-Black method does detect char C as efficiently as other methods, they also state that during fire events the formation of char will vary considerably, making it impossible to determine the likely recovery of C by the Walkley-Black method, even if the char content of the soil was known. Therefore, it is possible that char C was released from protection with the removal of Fe and clays, making it more readily oxidisable by the Walkley-Black method. This would increase the C recovery to above 100% in SOM containing large quantities of char or other protected C.

The Grey Kandosols proved to be very different in both C and N recoveries. SOM from areas under double harvest residues had lower recoveries for both C (31%) and N (71%) than the site with no harvest residues (66% C and 125% N, Table 2). This was most likely

Table 3. Simple Pearson linear correlation data for different C components of soils before and after 2% HF treatment

	Alkyl C	O-alkyl C	Aromatic C	Carbonyl C	A/O-A Ratio	Organic C	Total N	HCl-extractable Fe
<i>Untreated (top) v. HF-treated (left side)</i>								
Alkyl C	-0.429	0.155	0.015	0.016	-0.362	-0.383	-0.699*	-0.490
O-alkyl C	0.342	-0.252	-0.087	0.291	0.436	0.626	0.797*	0.582
Aromatic C	-0.433	0.360	-0.049	-0.261	-0.536	-0.717*	-0.637	-0.416
Carbonyl C	-0.674*	0.303	0.032	-0.086	-0.558	-0.550	-0.616	-0.283
A/O-A Ratio	-0.558	0.226	0.010	-0.011	-0.476	-0.550	-0.702*	-0.424
Organic C	0.386	-0.381	-0.011	0.397	0.526	0.680*	0.929***	0.740*
Total N	0.036	-0.502	0.099	0.887*	0.502	0.632	0.949**	0.923**
HCl-extractable Fe	0.406	-0.485	0.125	0.409	0.633	0.499	0.871**	0.723*
<i>Untreated v. untreated</i>								
O-alkyl C	-0.129							
Aromatic C	-0.157	-0.813**						
Carbonyl C	-0.300	-0.714*	0.386					
A/O-A Ratio	0.437	-0.933***	0.702*	0.509				
Organic C	0.025	0.137	-0.295	0.078	-0.040			
Total N	0.182	-0.264	-0.071	0.405	0.346	0.713*		
HCl-extractable Fe	-0.097	-0.197	-0.069	0.480	0.176	0.610	0.928***	
<i>HF-treated v. HF-treated</i>								
O-alkyl C	-0.781*							
Aromatic C	0.506	-0.753*						
Carbonyl C	0.747*	-0.851**	0.827**					
A/O-A Ratio	0.927***	-0.863**	0.770*	0.919***				
Organic C	-0.791*	0.908***	-0.806**	-0.845**	-0.870**			
Total N	-0.861*	0.806	-0.953**	-0.685	-0.910*	0.965**		
HCl-extractable Fe	-0.804**	0.854**	-0.778*	-0.759*	-0.852**	0.940***	0.988***	

* $P < 0.05$; ** $P < 0.01$; and *** $P < 0.001$.

a result of the greater soil mass loss after HF treatment of the soil from the double harvest residues site (TOO 7), which was 76%. However, the SOM from Toolara under double harvest residues had an enrichment factor of 1.26, indicating the organic matter in the remaining 24% soil mass was being concentrated to some extent. The selective removal of minerals during the HF treatment leads to a concentration of organic matter and is expressed by a relative enrichment in organic C and N (Schmidt *et al.* 1997). The C and N enrichment values for our data are shown in Table 2, and the values for organic-rich samples are expected to be less than for mineral rich samples (Schmidt *et al.* 1997).

Skjemstad *et al.* (1994) reported C recoveries between 83 and 92%, and suggested that the C concentrations were increased because of the removal of Fe and other inorganic materials. Dai and Johnson (1999) reported that 2% HF was the most effective treatment in the removal of Fe over other treatments, but that it also removed the largest proportion of the total C (12–67%). Schmidt *et al.* (1997) reported C recoveries of 77–92% and N recoveries of 78–100%, suggesting that the high recoveries were a result of removal of minerals rather than organic material. The relative enrichment of C in the 4 sites was between 1.2 and 8.9, while the enrichment of N ranged between 2.9 and 8.5. Although C recoveries in this study were relatively low compared with the previous studies (Skjemstad *et al.* 1994; Schmidt *et al.* 1997), there was an obvious enrichment of organic matter with the removal of magnetic materials (Fe in particular) and mineral matter, leading to improved NMR spectral quality. The Pearson linear correlation data (Table 3) show that the C and N contents of untreated and HF-treated samples were well correlated ($P < 0.05$ and $P < 0.01$, respectively), suggesting that mostly mineral matter was being removed rather than organic material. However, the low C recovery of some soils in this study suggests that some organic material may be solubilised with the iron oxides in the mineral matter and removed during the HF extraction process, most likely the soluble amino sugars and carboxylic acids. Since these soluble C components could be easily accessible by soil microorganisms and play an important role in soil biological processes, caution would be required when applying the HF pre-treatment to these soils, particularly the sandy soils, with significant C and N losses following the pre-treatment.

To test for any C solubilised during the HF treatment, extracts were analysed for C functional groups using FT-IR spectroscopy. The FT-IR spectra of 2 samples are shown in Fig. 1. All other spectra were similar but for minor differences in intensity levels (spectra not shown). The interpretation of FT-IR spectra was based on previous studies of humic substances by Celi *et al.* (1997), Haberhauer *et al.* (1998), Davis *et al.* (1999), Hsu and Lo (1999), and Haberhauer and Gerzabek (1999). Absorbance in the FT-IR spectra at 1730–1710 and 1620–1600 cm^{-1} arises from the C-O of ketones and COOH groups, and from COO⁻ symmetric stretching, respectively. Changes in the environment of a molecule or compound can either lower or raise the absorption frequency. Schnitzer and Skinner (1963) reported that the addition of increasing amounts of ferric ions diminished the carboxyl band centred at 1725 cm^{-1} and increased that at 1610 cm^{-1} , suggesting a conversion of COOH groups to COO⁻. Confirmation of this work was reported by Byler *et al.* (1987) using FT-IR spectroscopy on a fulvic acid (Celi *et al.* 1997). Parts *a* and *b* of Fig. 1 both show a broad absorption band in this region, albeit of different intensities, indicating the preferential removal of carboxyl C groups during the HF pre-treatment. The broadness of these bands may be indicative of the large amount of soluble Fe contained in the extracts or alternatively it may be indicative of adsorbed water. The broad absorption band between 3000 and 4000 cm^{-1} arises from the OH stretching of COOH or adsorbed water on mineral matter. This region may also show peaks from waxes (aliphatic CH₂ and

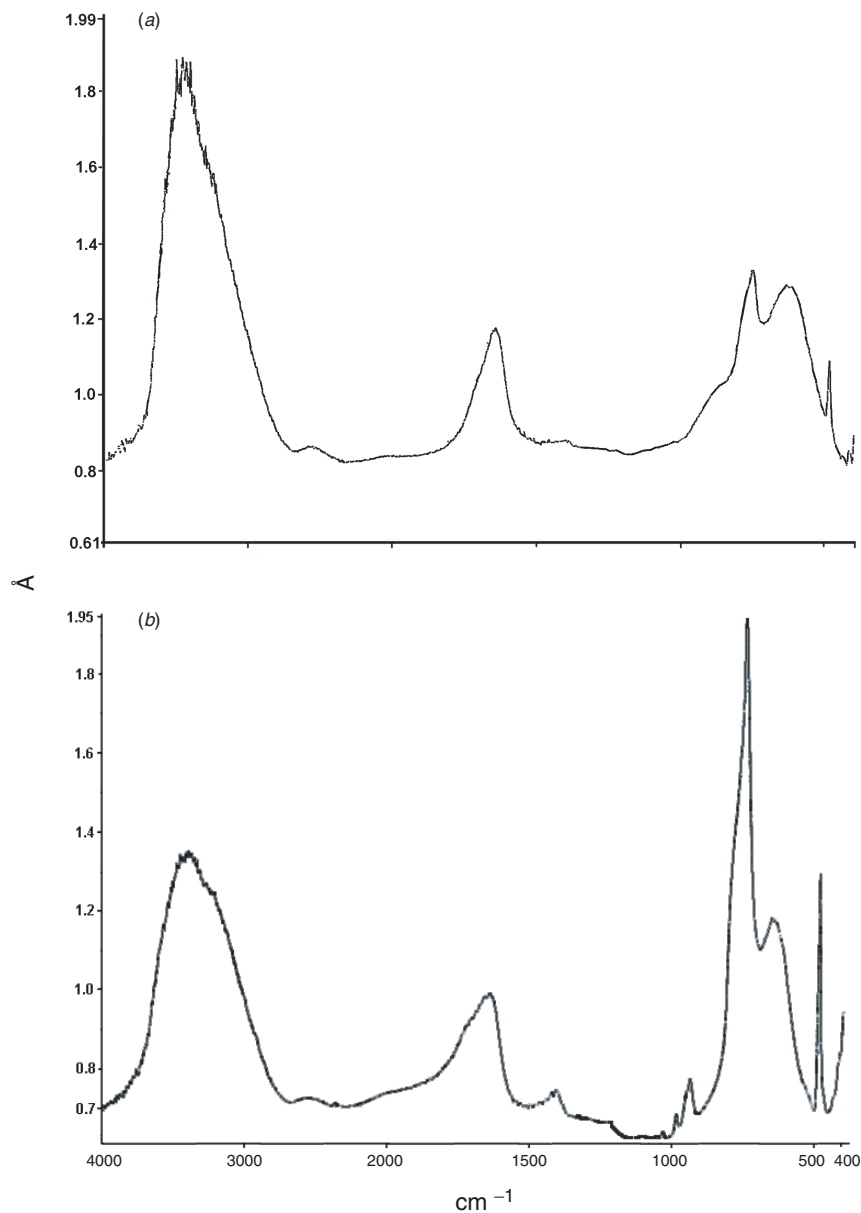


Fig. 1. FT-IR spectra of the 2% HF extracts from: (a) Peachester unburnt; and (b) F₁ hybrid pine containing no harvest residues.

CH_3 at 2800–3000 cm^{-1} , Chapman *et al.* 2001). The absorption peaks between 1000 and 400 cm^{-1} were most likely due to inorganic materials removed with the HF, such as clay and quartz minerals (Orlov 1986; Haberhauer *et al.* 1998). The FT-IR spectra tend to indicate that the main C structures lost during the HF treatment were the soluble carboxylic acids, which appear in the carbonyl C region of the ^{13}C NMR spectrum. However, because the extracts contained large quantities of mineral matter, the absorption bands seen in the FT-IR spectra in Fig. 1 may be partly due to adsorbed water.

Table 4. HCl-extractable Fe and C/Fe ratios before and after 2% HF treatment

Sample code	Fe (g/kg) ^A		% Fe lost	C/Fe ratio (w/w)	
	Before treatment	After treatment		Before treatment	After treatment
IMB 1.2	13.0 ± 0.3	0.90 ± 0.02	93.1	6.49	464
IMB 1.6	10.2 ± 0.2	1.30 ± 0.16	87.2	4.24	294
IMB 3.2	5.34 ± 0.14	0.70 ± 0.02	86.9	7.75	493
IMB 3.6	5.78 ± 0.20	0.75 ± 0.05	87.0	6.25	419
PCH 8	5.20 ± 0.34	0.03 ± 0.01	99.3	2.48	1980
PCH 17	5.80 ± 0.08	0.10 ± 0.002	98.2	11.0	1513
PCH 21	6.80 ± 0.21	0.05 ± 0.003	99.3	1.84	570
TOO 7	1.30 ± 0.06	0.04 ± 0.011	97.0	28.2	1155
TOO 15	1.20 ± 0.10	0.01 ± 0.002	98.2	15.9	3060

^AMeans ± standard errors.

Removal of Fe

To assess the removal of Fe from the soil samples, the Fe contents extractable with HCl before and after HF treatment were determined. This method should remove the organically bound Fe as well as the Fe in Fe (III) oxides and clay minerals (Schmidt *et al.* 1997). Table 4 provides the Fe contents and C/Fe ratios before and after HF treatment for all 4 sites. The HF treatment removed 86–99% of Fe in all soils analysed, reducing the concentration of Fe to <1 g/kg in all samples except one (Table 4). This resulted in an increase of the C/Fe ratios from mostly <10 in the untreated samples to the range between 294 and 3060 after 2% HF pre-treatment. Arshad *et al.* (1988) stated that when C/Fe was much greater than 1, good spectra could be obtained; when C/Fe = 1 then reasonable spectra were obtained; and when C/Fe was <1, poor spectra were expected. However, our results indicate that the C/Fe ratios of all soil samples were >1 before HF treatment, one as high as 28, and still produced poorly resolved NMR spectra. Other studies have also reported instances of high C/Fe ratios producing poor quality spectra (Skjemstad *et al.* 1994; Schmidt *et al.* 1997; Dai and Johnson 1999). Although more than 86% of Fe was removed from all the soil samples, the Kandosols pre-treated with HF removed Fe more efficiently, presumably due to their lower clay contents (Table 1) and lower initial Fe concentrations (Table 4), resulting in much larger C/Fe ratios for these soils.

¹³C CPMAS NMR spectra

Figures 2–5 show the ¹³C CPMAS NMR spectra of the HF-treated and untreated SOM samples obtained from the 4 experimental sites. All 4 figures indicate greater spectral resolution and increased S/N in the HF-treated SOM than the untreated SOM, although the latter accumulated greater numbers of scans. The 1- and 3-year-old Imbil Dermosols (Figs 2 and 3, respectively) showed the greatest improvement in solid-state ¹³C CPMAS NMR spectral quality even though a lower percentage of Fe was effectively removed from these samples (Table 4). This improvement was most probably due to the large portions of clay-metal complexes removed from these soils during the HF extraction. The HF-treated spectra of the Peachester (Fig. 4) and Toolara (Fig. 5) Kandosols also showed improvement in NMR spectral quality, but this was due, in part, to improved spectrometer conditions (see *Materials and methods*), such as a reduction in the recycle delay. The longer delay may have underestimated the aliphatic C regions while overestimating the carbonyl and

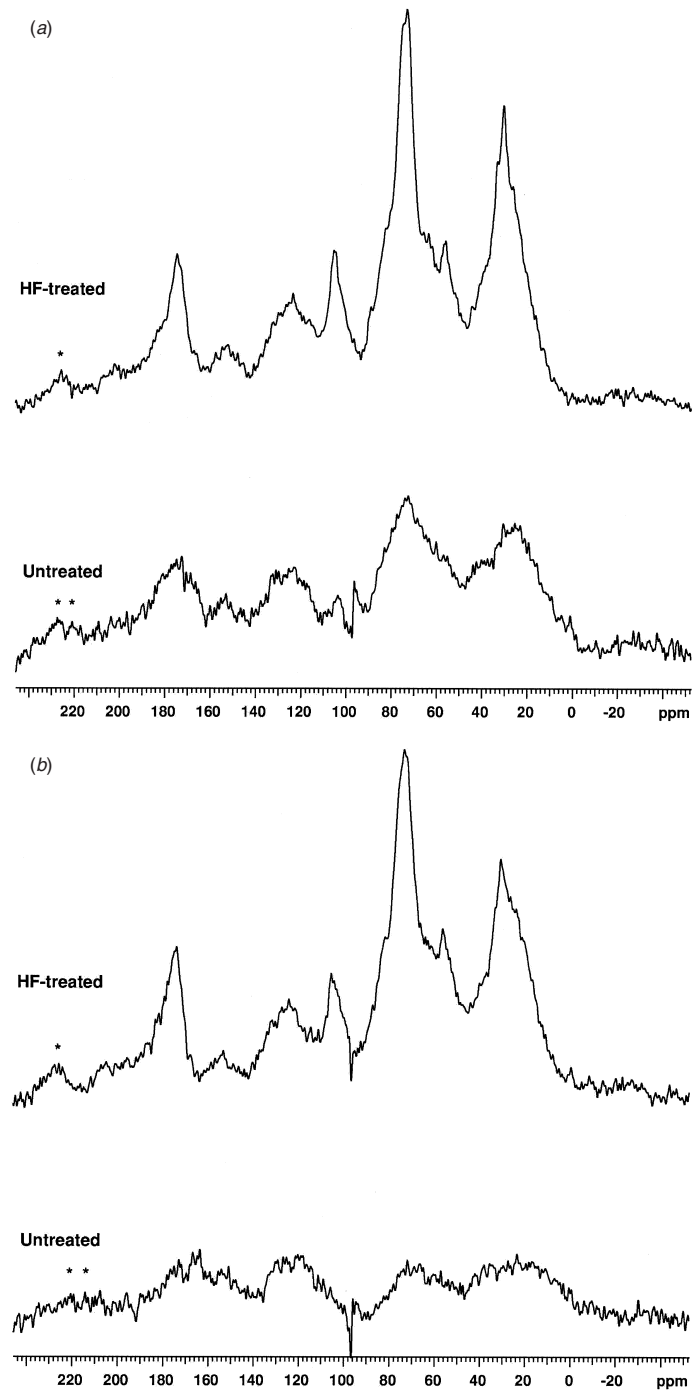


Fig. 2. Carbon-13 CPMAS NMR spectra of 1-year-old Imbil hoop pine SOM from areas: (a) under windrows; and (b) between windrows. NB: Untreated SOM spectra are vertically scaled an order of magnitude greater than the HF-treated SOM.

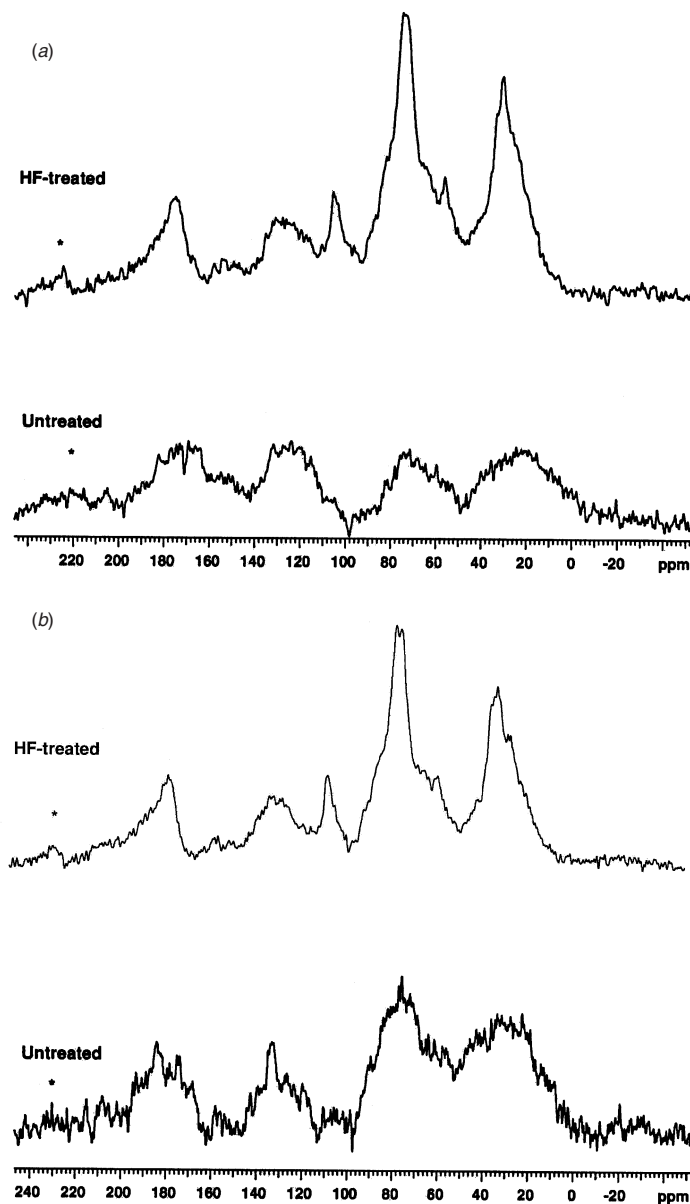


Fig. 3. Carbon-13 CPMAS NMR spectra of 3-year-old Imbil hoop pine SOM from areas: (a) under windrows; and (b) between windrows. NB: Untreated SOM spectra are vertically scaled an order of magnitude greater than the HF-treated SOM.

aromatic C regions. Changes in C composition and quality due to different residue management practices were visible in the HF-treated NMR spectra in Figs 2–5 and also in the graphs displayed in Figs 6–8 of the SSB-corrected relative intensities of the C functional groups. These details will not be discussed here, but can be found in Mathers *et al.* (2002) and Guinto *et al.* (1999) for Imbil and Peachester SOM, respectively.

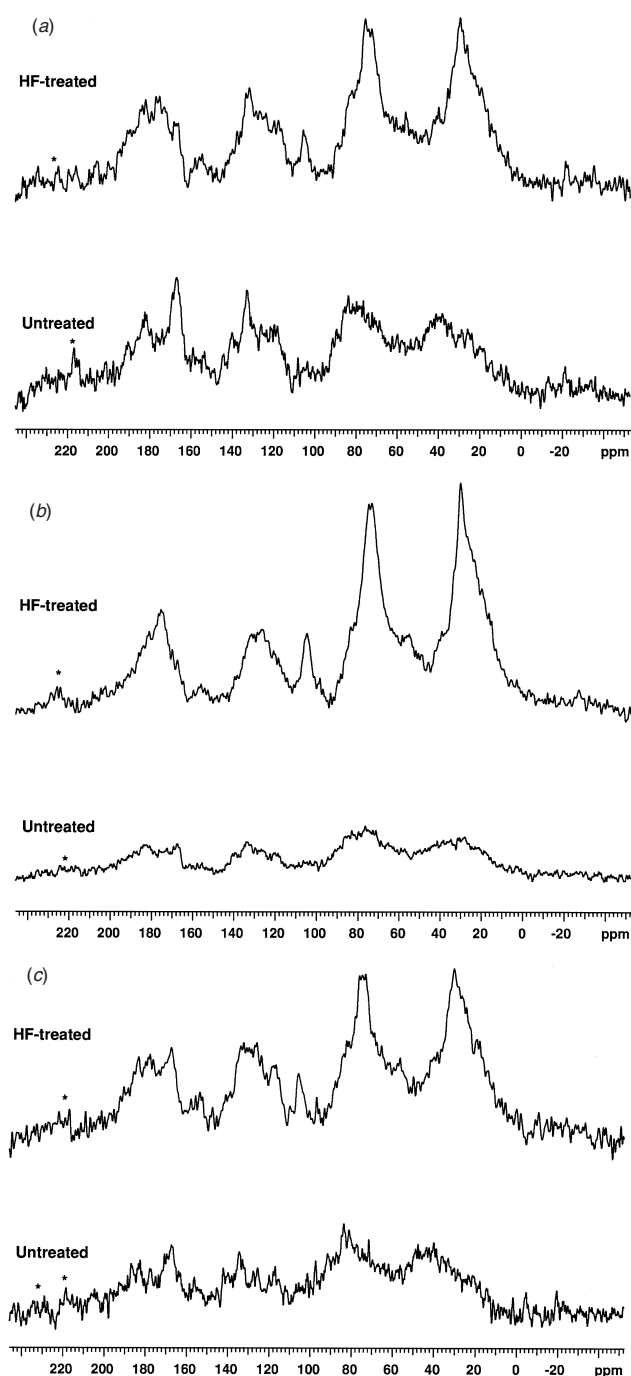


Fig. 4. Carbon-13 CPMAS NMR spectra of native blackbutt SOM from areas that were: (a) burnt every 2 years; (b) unburnt; and (c) burnt every 4 years. NB: The unburnt spectra are vertically scaled at one-third the size of the burnt spectra.

All spectra display major improvements in resolution of the O-alkyl and alkyl C regions after HF treatment, with the O-alkyl C region showing clear separation of the methoxyl/N-alkyl C (45–60 ppm) and di-O-alkyl C (92–110 ppm) signals in most of the HF-treated spectra. This is reflected in Figs 6–8, where the relative intensities of the O-alkyl

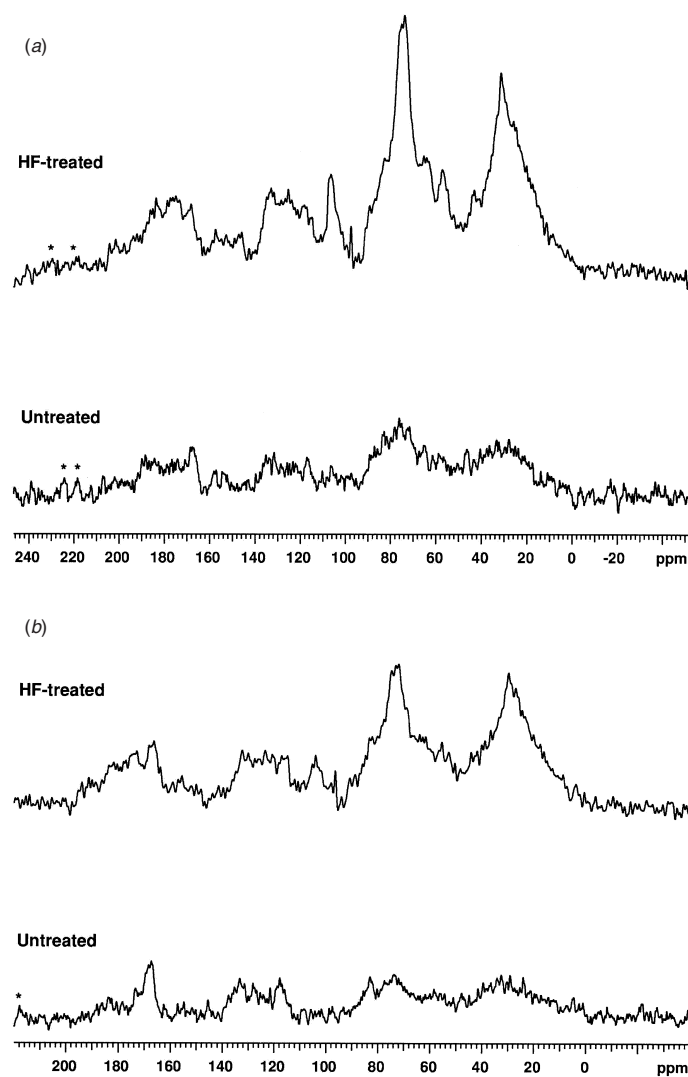


Fig. 5. Carbon-13 CPMAS NMR spectra of exotic F_1 hybrid pine SOM from areas containing: (a) double residues; and (b) no residues.

C region have increased in all but one of the SOM spectra. The intensity of the alkyl C spectral region increased for the Kandosols but decreased in the Dermosols despite the increase in resolution and S/N of the NMR spectra. Figs 6–8 display relative intensity distributions of each C functional group after integration and correction for SSB of Imbil, Peachester, and Toolara SOM, respectively, by comparing the differences between untreated and HF-treated spectra. Integrations and, therefore, relative intensities for untreated spectra were considered estimations because of the huge signal overlap, insensitive spectrometer conditions for SOM, and poor S/N, making quantitation and peak picking difficult. The aromatic C region displays a decrease in intensity after HF treatment for all but 2 samples (IMB 3.6 and TOO 7). This could be due to soil type and/or management practice. The carbonyl C region shows signs of losing intensity in the

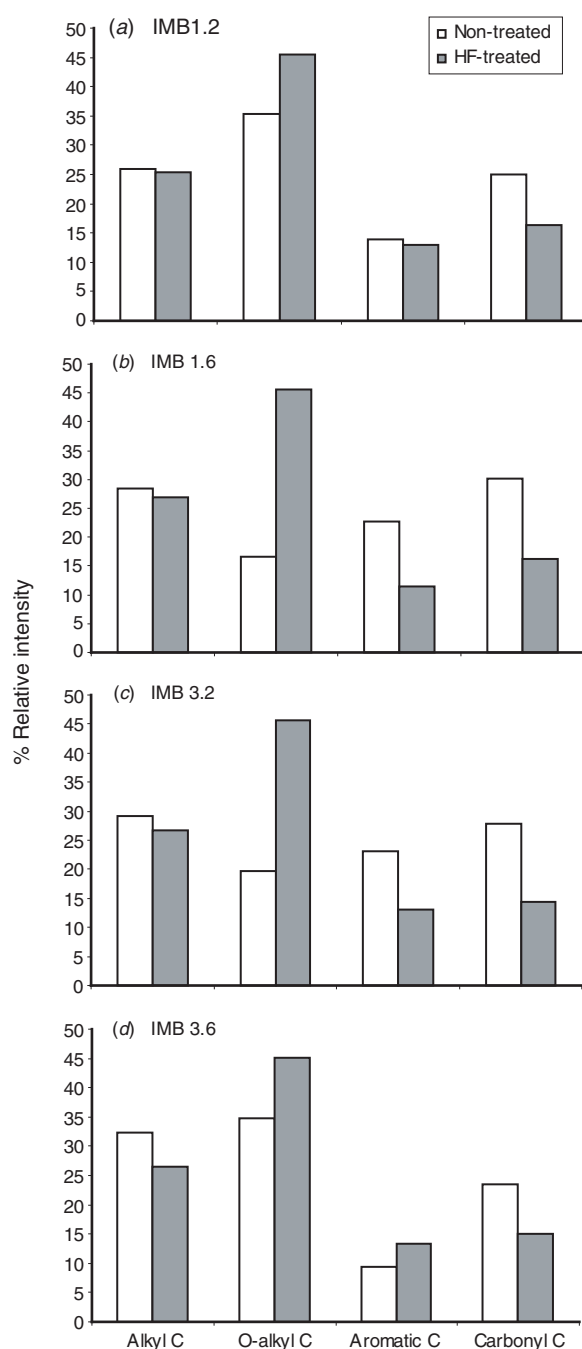


Fig. 6. Relative intensity distribution (%) of the four integrated NMR regions representing C functional groups calculated from the ^{13}C CPMAS NMR spectra from Imbil State Forest of: (a) 1-year-old SOM from under the windrows; (b) 1-year-old SOM from between the windrows; (c) 3-year-old SOM from under the windrows; and (d) 3-year-old SOM from between the windrows.

HF-treated spectra in all but one of the Kandosols (TOO 7), although FT-IR spectra in Fig. 1 indicated that there may be a preferential removal of carboxylic acid groups. The Imbil Dermosols (Figs 2 and 3) also show a variation in chemical shift for the carbonyl C signal between the untreated and HF-treated spectra. The HF-treated spectra show the signal maximum centred at 175 ppm, while untreated spectra display signals at 172 ppm and

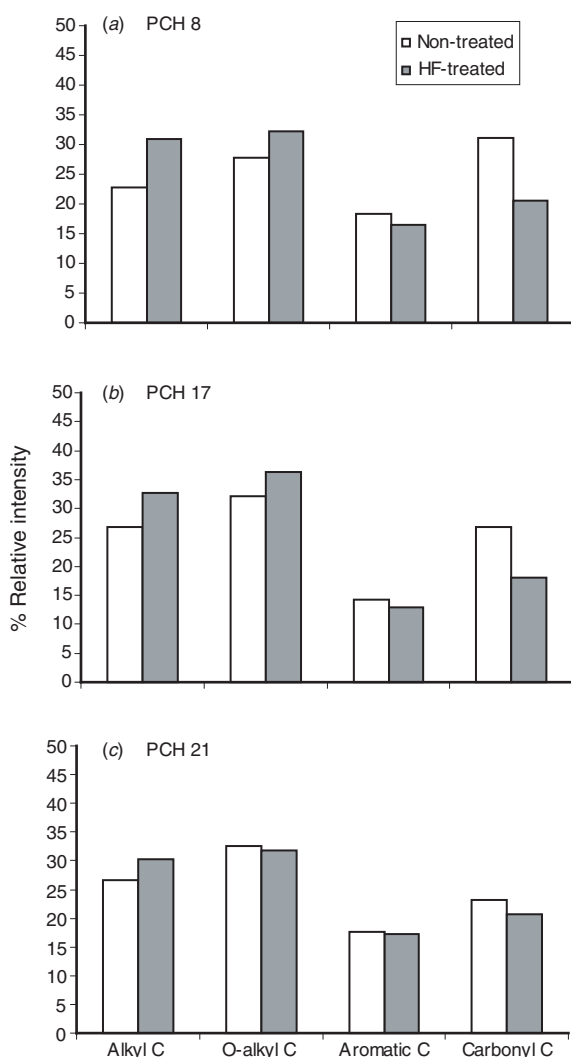


Fig. 7. Relative intensity distribution (%) of the four integrated NMR regions representing C functional groups calculated from the ^{13}C CPMAS NMR spectra from Peachester State Forest of: (a) SOM burnt every 2 years; (b) unburnt SOM; and (c) SOM burnt every four years.

168 ppm. This is most likely the result of signal broadness and a reduction in relaxation time constants (Pfeffer *et al.* 1984) in the untreated spectra induced by the large quantities of Fe (>5 g/kg, Table 4) in these samples.

The FT-IR spectra of the HF extracts indicate that carboxyl C may be preferentially removed during the HF treatment; however, the bands seen in the spectra could also be partly due to adsorbed water. The graphs in Figs 6–8 also show that all but one of the HF-treated NMR spectra lost intensity in the carbonyl C region compared with the untreated spectra. This loss may, in part, be due to the preferential loss of carboxylic C groups observed during the HF treatment, and partly due to the improved resolution of the alkyl and O-alkyl C regions in the NMR spectra. Nevertheless, the relative intensities of the carbonyl C region of the HF-treated NMR spectra were all >10%, with an average of 14%. This is comparable to the ^{13}C CPMAS NMR data of more than 300 whole soils, physical fractions (clay, silt, and sand), and chemical extracts (humic and fulvic acids) collected

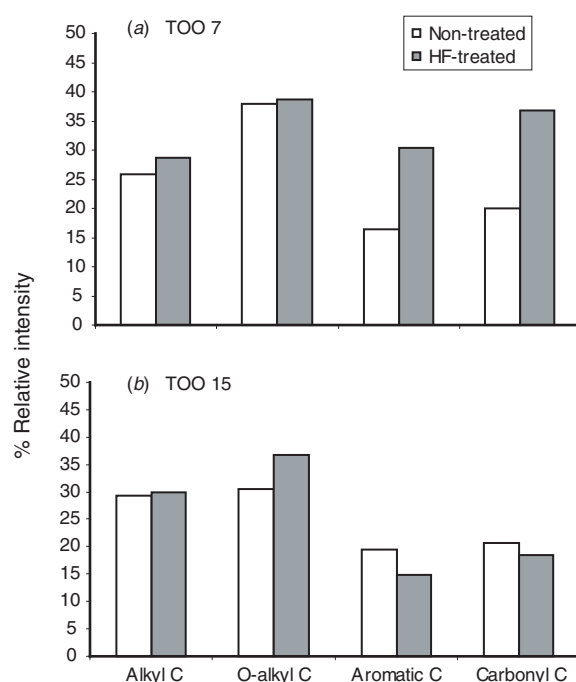


Fig. 8. Relative intensity distribution (%) of the four integrated NMR regions representing C functional groups calculated from the ^{13}C CPMAS NMR spectra from Toolara State Forest of: (a) SOM from areas under double harvest residues; and (b) SOM from areas under no harvest residues.

from different environments under varying land-use practices, pre-treatments, and spectrometer conditions as reported by Mahieu *et al.* (1999). They reported the relative abundance of C functional groups in whole soils to be always in the same order: O-alkyl C (a mean of 45% of the spectrum), followed by alkyl C (mean 25%), aromatic C (mean 20%), and finally carbonyl C (mean 10%). Although some carboxyl/carbonyl C may be solubilised during the HF pre-treatment, the HF-treated ^{13}C CPMAS NMR spectra of subtropical Australian forest SOM in this study display similar characteristics to other published NMR spectra of whole soils and SOM, except for the Imbil Dermosols, which displayed a much lower average for aromatic C (mean 12%) than most SOM (Fig. 1).

Pearson linear correlations were performed on the NMR data obtained from untreated and HF-treated samples together with some of the chemical data to identify any significant relationships between the data (Table 3). Significant correlations that existed in the untreated spectra were total N with HCl-extractable Fe ($P < 0.001$), and O-alkyl C with aromatic C ($P < 0.01$) and carbonyl C ($P < 0.05$). The most significant relationship that appeared in both the untreated and HF-treated soils was a strong positive correlation ($P < 0.001$) between total N and HCl-extractable Fe (Table 3). This may be the result of microbial interactions between organic matter and the iron oxides of clay by nitrate-dependent iron oxidisers (Ratering and Schnell 2000, 2001).

The HF-treated spectra display some significant correlations between NMR functional groups and also between NMR regions and chemical properties (Table 3). The most significant relationships were: a negative correlation between O-alkyl C and aromatic C ($P < 0.05$); strong negative correlations between aromatic C and organic C ($P < 0.01$), and total N and HCl-extractable Fe ($P < 0.001$); positive correlations of O-alkyl C with Fe ($P < 0.01$) and organic C ($P < 0.001$); and a positive correlation between organic C and Fe ($P < 0.001$). The relationship between O-alkyl C and aromatic C may be indicative of decomposition processes. The major changes observed in many published NMR studies on

decomposition of SOM are a decrease in O-alkyl C structures (carbohydrates) that decompose rapidly leading to a selective enrichment of alkyl, aromatic, and carbonyl C structures, which decompose more slowly (Skjemstad *et al.* 1997). This relationship persists in the NMR data even where different harvest residue management practices were in place. Other relationships indicate that organic C, total N, and HCl-extractable Fe were positively correlated to each other and to O-alkyl C, but negatively correlated to aromatic C (Table 3).

These relationships show that organic C, O-alkyl C, and aromatic C may be indicators of decomposition and SOM quality as supported by ^{13}C CPMAS NMR spectroscopy. Hopkins *et al.* (1993) noted that it was not possible to determine which type of C was most influential in controlling decomposition rate because NMR is only semi-quantitative. However, they reported that O-alkyl C content and decomposition rate appeared to be directly related. Our results support this statement. There were also indications that Fe contained in the iron oxides of clay was closely associated with SOM and decomposition processes as observed by ^{13}C CPMAS NMR spectroscopy.

Conclusions

The use of 2% HF as a pre-treatment for improving ^{13}C CPMAS NMR spectral quality has shown positive results in this and previous studies. Apart from displaying increased resolution especially in the alkyl C and O-alkyl C regions and improved S/N in all subtropical Australian forest soils studied, the time on the spectrometer decreased from more than 20 h to between 5 and 1 h, depending on soil type and characteristics. This can allow for greater numbers of samples to be analysed by ^{13}C CPMAS NMR, especially in institutes and research centres where access to NMR spectrometers is limited for soil scientists. These improvements were mostly due to the successful removal of >86% of Fe from all samples, effectively removing paramagnetic interferences with cross-polarisation and NMR signals.

The FT-IR spectra of the HF extracts revealed the preferential removal of carboxyl C groups during the HF pre-treatment process. Despite this, the HF-treated NMR spectra in this study resembled published results of relative abundances for C functional groups in whole soils, and also showed C compositional and quality changes due to different residue management practices. There were also indications that HCl-extractable Fe, total N, and organic C are closely associated with the decomposition processes of SOM as studied by ^{13}C CPMAS NMR spectroscopy, despite the residue management practices in place. Total N and HCl-extractable Fe were also strongly related presumably through microbial interactions between organic matter and the iron oxides of clay. The HF-treated ^{13}C CPMAS NMR spectra indicate that the O-alkyl C and aromatic C regions may be sensitive indicators of SOM quality through their strong relationships with conventional chemical data such as organic C, total N, and HCl-extractable Fe content, which is demonstrated here in a variety of soil types.

These results support previous recommendations for the use of the 2% HF treatment as a pre-treatment for ^{13}C CPMAS NMR spectroscopy of Australian forest soils. However, it is recognised that not all soil organic C may be observed in ^{13}C CPMAS NMR spectroscopy for various reasons, such as preferential removal of certain soluble C types and invisibility or reduction of NMR signals due to interferences. Therefore, NMR data will need to be compared with conventional or other chemical/biological data to be useful. This applies to both HF-treated and untreated samples, indicating that ^{13}C CPMAS NMR spectroscopy should only be used for semi-quantitative analyses.

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