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Aspects of the chemical structure of soil organic materials as revealed by solid-state ^{13}C NMR spectroscopy

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Abstract. Solid-state cross-polarisation/magic-angle-spinning ^{13}C nuclear magnetic resonance (CP/MAS ^{13}C NMR) spectroscopy was used to characterise semi-quantitatively the organic materials contained in particle size and density fractions isolated from five different mineral soils: two Mollisols, two Oxisols and an Andosol. The acquired spectra were analysed to determine the relative proportion of carboxyl, aromatic, O-alkyl and alkyl carbon contained in each fraction. Although similar types of carbon were present in all of the fractions analysed, an influence of both soil type and particle size was evident.

The chemical structure of the organic materials contained in the particle size fractions isolated from the Andosol was similar; however, for the Mollisols and Oxisols, the content of O-alkyl, aromatic and alkyl carbon was greatest in the coarse, intermediate and fine fractions, respectively. The compositional differences noted in progressing from the coarser to finer particle size fractions in the Mollisols and Oxisols were consistent with the changes noted in other studies where CP/MAS ^{13}C NMR was used to monitor the decomposition of natural organic materials. Changes in the C:N ratio of the particle size fractions supported the proposal that the extent of decomposition of the organic materials contained in the fine fractions was greater than that contained in the coarse fractions. The increased content of aromatic and alkyl carbon in the intermediate size fractions could be explained completely by a selective preservation mechanism; however, the further accumulation of alkyl carbon in the clay fractions appeared to result from both a selective preservation and an *in situ* synthesis.

The largest compositional differences noted for the entire organic fraction of the five soils were observed between soil orders. The differences within orders were smaller. The Mollisols and the Andosol were both dominated by O-alkyl carbon but the Andosol had a lower alkyl carbon content. The Oxisols were dominated by both O-alkyl and alkyl carbon.

A model describing the oxidative decomposition of plant materials in mineral soils is proposed and used to explain the influence of soil order and particle size on the chemical composition of soil organic matter in terms of its extent of decomposition and bioavailability.

Introduction

The importance of soil organic matter to such soil properties as fertility, structural stability, cation exchange capacity and water holding capacity, to name a few, has been well documented. However, despite its important role in soils and approximately 200 years of research, the chemical structure of soil organic matter remains controversial.

As a result of the strong association between soil organic and mineral fractions, a prerequisite to the selective characterisation of soil organic materials has been its extraction from soil using various chemical reagents. Classical extraction schemes involve the use of alkaline solutions (e.g. sodium hydroxide or sodium pyrophosphate) to extract the organic materials from soil followed by acidification with hydrochloric acid to separate humic and fulvic acids. The use of such an extraction scheme has been criticised because only a portion (20—60%) of the total soil organic matter is extracted and the extreme pH values used may create artifacts (Poppoff and Theander 1976a, b, Forsskahl et al. 1976, Worobey and Webster 1981).

Solid-state ^{13}C NMR (nuclear magnetic resonance) spectroscopy offers the possibility of direct chemical characterisation of organic materials in soils. The initial application of solid-state ^{13}C NMR spectroscopy to soil organic matter studies produced spectra containing only broad featureless resonances due to strong ^{1}H-^{13}C dipolar interactions, low sensitivity and chemical shift anisotropy. However, the use of high-power dipolar-decoupling and the development of cross-polarisation, CP, and magic-angle-spinning, MAS, techniques has allowed high resolution solid-state ^{13}C NMR spectra of soil organic matter to be acquired. In the cross-polarisation pulse sequence sensitivity is enhanced by the transfer of energy from the more abundant ^{1}H nuclei to ^{13}C nuclei. Under optimum conditions this results in an enhancement of ^{13}C signals by a factor of four. The magic-angle-spinning (rapid spinning of a sample at the magic angle of 54.74° to the applied magnetic field) and dipolar-decoupling techniques are used to eliminate signal broadening arising from chemical shift anisotropy and ^{1}H-^{13}C dipolar interactions. Using CP/MAS ^{13}C NMR techniques, Barron and Wilson (1981) and Wilson et al. (1981) acquired well resolved spectra which provided detailed information on the chemical structure of soil organic matter and established the potential usefulness of the technique as a tool for characterising soil organic materials.

Two limitations to the use of CP/MAS ^{13}C NMR in its application to the study of organic materials in mineral soils exist. Firstly, by definition, mineral soils contain $< 17\%$ organic carbon by mass of which only 1.1%

exists as ^{13}C. The low carbon content coupled with the low natural abundance of ^{13}C nuclei makes the acquisition of spectra with an adequate signal resolution for mineral soils difficult unless exceedingly long scan periods are used. The detection problems are compounded by the wide range of carbon structures present in soils, which lowers the concentration of each type of ^{13}C nucleus. For soils containing $< 5\%$ organic carbon it may be necessary to use various physical methods to concentrate the carbon. Particle size and density fractionation schemes have been used successfully by Barron et al. (1980), Skjemstad et al. (1986), Oades et al. (1987, 1988), Arshad et al. (1988) and Baldock et al. (1990a) to acquire well resolved spectra of the organic materials associated with mineral soils.

The second limitation of applying CP/MAS ^{13}C NMR to mineral soils is caused by paramagnetic species with contain unpaired electrons and reduce the efficiency of the cross polarisation process such that ^{13}C nuclei in proximity to paramagnetics may be rendered NMR 'invisible'. Preston et al. (1984) demonstrated a selective decrease in the amount of carbohydrate carbon observed as the Cu^{2+} content of organic soils increased and a similar effect of Fe^{3+} was observed by Pfeffer et al. (1984) for sewage sludge and the humic and fulvic acids derived from a composted sludge. These results indicate that the relative signal intensities observed in CP/MAS ^{13}C NMR spectra acquired for samples containing paramagnetics may not reflect the actual distribution of carbon within the various chemical structures because of a selective interaction of the paramagnetics with certain structural groups. The major paramagnetic component in soils is Fe^{3+} but other transition metal cations containing unpaired electrons and organic free radicals may also cause problems if present in high enough quantities. Oades et al. (1987) showed how, in a < 0.2 μm diameter fraction of soil, the chemical reduction of paramagnetic Fe^{3+} to nonparamagnetic Fe^{2+} with dithionite improved signal resolution. Resonances arising from carbohydrate (62 ppm), aromatic (127 ppm) and carboxyl (171 ppm) carbon, which were not observed for the untreated sample, became evident despite the fact that 18% of the carbon contained in the fraction was removed by the dithionite treatment. Subsequent analysis of the dithionite extract by solution-state ^{13}C NMR revealed that the extracted carbon was dominated by carbohydrate structures. Similar results were obtained by Arshad et al. (1988) using sodium dithionite or stannous chloride to reduce Fe^{3+} to Fe^{2+}. Although there is evidence that dithionite extraction may alter the chemical composition of the carbon in soil fractions by removing certain chemical structures selectively, the improvements in signal quality and resolution often warrant its use. Arshad et al. (1988) indicated that the ratio of organic carbon content to iron content

was a critical factor, and that, unless this ratio was much greater than 1, the use of reducing agents was warranted to obtain acceptable solid-state ^{13}C NMR spectra of mineral soils and their particle size and density fractions.

In this study, solid-state CP/MAS ^{13}C NMR spectra have been acquired for particle size and density fractions isolated from five different mineral soils to illustrate the following:

(1) differences in the chemical structure of the soil organic materials in particle size and density fractions isolated from mineral soils,
(2) variations in the chemical structure of the entire organic fraction of mineral soils of different orders, and
(3) proposed relations between chemical structure and extent of decomposition and/or bioavailability of the organic materials in different soils and particle size fractions.

Solid-state CP/MAS ^{13}C NMR results from Oades et al. (1987, 1988), Baldock (unpublished) and Preston et al. (1987, 1989) have been included with those collected in this study to extend the range of soil orders.

Materials and methods

Soils

A description of the soils used in this study and those included from previous work is given in Table 1. For the soils used in this study a composite sample of the A horizon (0—10 cm) was collected, air dried and sieved $\leqslant 2$ mm. Approximately 2 kg of air-dried soil was disrupted using a Branson Sonifier (Model 250) by adding 20 g soil to 50 cm^3 deionised water in a 150 cm^3 beaker and sonifying for 5 minutes at 50 percent of the maximum 200 watt output. Ice was packed around the beaker to reduce sample heating during sonification. The disrupted soil was fractionated quantitatively on the basis of particle size and density according to Oades et al. (1987) with the exception that a sodium polytungstate, $Na_3WO_4 \cdot 9WO_3 \cdot H_2O$ (Sometu, Falkenried 4, D-1000 Berlin, FRG), solution of density 2.0 Mg m^{-3} was used for the Henongjiang Mollisol and the Guangdong Oxisol and deionised water was used for the Mount Schank Andosol in place of the $ZnBr_2$ solutions used by Oades et al. (1987). The total carbon content of each fraction was measured using a LECO CR12 combustion furnace. Inorganic carbon contents were determined using a volumetric calcimeter (Allison and Moodie 1965) and organic carbon contents were calculated as the difference between the total and inorganic carbon contents. Total nitrogen

Table 1. Description of the soils used in this study and those included from other studies.

Soil	Location[1]	Climate	Vegetation[2]	pH[3]	Organic carbon[4] (%)
Millicent Mollisol	Millicent, S.A.	temperate	cultivated (wheat/pasture)	8.5	3.7
Henongjiang Mollisol	Henongjiang, China	temperate	cultivated (wheat/soybeans)	5.9	3.2
Mount Schank Andosol	Mount Schank, S.A.	temperate	native and introduced grasses with bracken	6.1	4.8
Malanda Oxisol	Malanda, Qld.	tropical	rainforest	5.4	6.1
Guangdong Oxisol	Guangdong, China	tropical	eucalyptus forest (> 30 years)	4.6	2.2
Urrbrae Alfisol[5]	Glen Osmond, S.A.	mediterranean	old pasture (> 40 years)	5.6	2.3
Meadows Alfisol[6]	Meadows, S.A.	mediterranean	mixed forest with grass ground cover	6.4	2.7
Gatineau Histosol[7]	Gatineau, Quebec Canada	temperate	sphagnum peat dominated by mosses	3.3	44.1
Farnham Histosol[7]	Farnham, Quebec Canada	temperate	derived from woody plants (50% wood, 40% sedge and 10% moss)	3.3	54.9
Ormstown Histosol[8]	Ormstown, Quebec Canada	temperate	derived from sedge fen (15% wood, 77% sedge and 8% moss)	3.1	45.2

[1] unless otherwise stated the soils were located within Australia.
[2] for mineral soils the vegetation refers to that above ground, and for the Histosols it refers to the botanical composition of the peat material.
[3] pH was determined in the clear supernatant of a 1:5 soil:water extract after removal of soil particles by centrifugation for the mineral soils and according to Lévesque et al. (1980) for the Histosols.
[4] determined using either a LECO CR12 combustion furnace or a Walkley-Black procedure. The LECO CR12 data were corrected for inorganic carbonate contents as determined using the volumetric calcimeter method of Allison and Moodie (1965).
[5] from Oades et al. (1987).
[6] from Baldock (unpublished). Soil properties, sampling and sampling procedure have been described by Baldock et al. (1989, 1990a).
[7] from Preston et al. (1989).
[8] from Preston et al. (1987). Sampling site and samples have been described by Lévesque et al. (1982) and Mathur et al. (1982).

contents were determined using a Kjeldahl procedure (Bremner and Mulvaney 1982).

The total iron content of the fractions was determined by X-ray fluorescence (Norrish and Hutton 1969). Fractions containing > 3 per cent Fe_2O_3 by weight were treated with a dithionite solution (Mitchell and McKenzie 1954) to reduce Fe^{3+} to Fe^{2+} and decrease the influence of paramagnetic species in the CP/MAS ^{13}C NMR analyses.

Solid-state CP/MAS ^{13}C NMR spectroscopy

Solid-state CP/MAS ^{13}C NMR spectra were obtained using a Bruker CXP 100 instrument as described by Oades et al. (1987). A CP/MAS ^{13}C NMR spectrum was acquired for each of the particle size and density fractions indicated in Table 2. The remaining fractions (those included in Appendix 1 but omitted from Table 2) were not analysed because their contribution to the total organic carbon content of the soils was < 1% and/or their low organic carbon contents precluded the acquistion of spectra with adequate signal resolution in reasonable scan times (see Appendix 1 for organic carbon contents and contributions).

The total signal intensity and the proportion contributed by each carbon grouping were determined by integration using the four spectral areas delineated in Table 3. Although the signal resolution in some of the acquired spectra was sufficient to allow the identification of additional types of carbon to those listed in Table 3, in several spectra signal resolution was poor making it impractical to identify more than four general types of carbon based on spectral areas. The chemical shift limits of each region are approximate as there would undoubtedly be some overlap of signals between adjacent regions. As indicated by the dominant forms of carbon associated with each spectral region (Table 3), it is important to note that the label given to each region is only general and thought to be indicative of the major type of carbon present.

Resonances in the vicinity of 30—32 ppm may be ascribed to alkyl carbon in long chain polymethylene type structures (e.g. fatty acids, waxes and resins). A decrease in the chemical shift of this resonance towards 25 ppm indicates a reduction in the amount of long chain polymethylene material and an increase in short chain material. Resonances in the vicinity of 17 ppm arise from methyl carbon. The methoxyl resonance occurs at 57 ppm. The resonance near 72 ppm is attributed to the ring carbons of carbohydrates and is accompanied by resonances near 105 ppm and at 62 ppm for the anomeric carbon and C-6 carbon (CH_2) of carbohydrate structures, respectively. The 62 ppm resonance is often poorly resolved and seen as a shoulder on the 72 ppm resonance. The amine carbon of

Table 2. Particle size and density fractions for which solid-state CP/MAS ^{13}C NMR spectra were acquired.

Soil	Particle size (μm)	Particle density (Mg m^{-3})	Dithionite treated
Millicent Mollisol	250—2000	⩽2.0	no
	53—250	⩽2.0	no
	20—53	⩽2.0	no
	2—20	⩽2.0	no
	0.2—2	whole fraction	no
	<0.2	whole fraction	no
Henongjiang Mollisol	53—2000	⩽2.0	no
	20—53	⩽2.0	no
	2—20	⩽2.0	no
	0.2—2	whole fraction	no
	<0.2	whole fraction	no
Mount Schank Andosol[1]	250—2000	⩽1.0	yes
	53—250	⩽1.0	yes
	2—20	whole fraction	yes
	<2	whole fraction	yes
Malanda Oxisol	53—2000	⩽2.0	yes
	20—53	⩽2.0	yes
	2—20	⩽2.0	yes
	0.2—2	whole fraction	yes
	<0.2	whole fraction	yes
Guangdong Oxisol	53—2000	⩽2.0	yes
	20—53	⩽2.0	yes
	2—20	⩽2.0	yes
	0.2—2	whole fraction	yes
	<0.2	whole fraction	yes

[1] No CP/MAS ^{13}C NMR spectrum could be acquired for the 20—53 μm fraction of the Andosol regardless of how many transients were collected. The inability to acquire a spectrum was suspected to be due to the high iron content of this fraction even after dithionite treatment (13.5% Fe_2O_3 by mass).

protein structures resonates in the region of 45—65 ppm (Duncan 1987) but is often not resolved completely from the more intense carbohydrate resonances. Signals for C- or H-substituted aromatic carbon occur in the vicinity of 130 ppm while those arising from O-substituted aromatic carbon such as that in phenols resonate near 150 ppm. The resonance near 175 ppm can originate from carboxylic, amide or ester carbon; however, it is thought to be dominated by carboxyl carbon. Ketone and aldehyde carbon resonates at approximately 200 ppm but for the spectra

Table 3. Chemical shift limits and assignments of the four spectral regions into which the CP/MAS ^{13}C NMR spectra were divided.

Label	Chemical shift range (ppm)	Dominant forms of carbon
Alkyl	10—45	methyl, methylene, methine and quaternary carbon
O-Alkyl	45—110	Oxygenated alkyl, alkyl-amino, methoxyl, acetal and ketal carbon
Aromatic	110—160	Protonated and carbon substituted aromatics and unsaturated carbon and oxygenated aromatics and unsaturated carbon
Carboxyl	160—200	Carboxylic carbon, esters and amides

acquired in this study there is little evidence of any well resolved resonances in this region.

The information obtained by integration of CP/MAS ^{13}C NMR spectra is only quantitative provided that: (1) a long enough contact time to excite all carbons equally is used, (2) the relaxation of protons in the rotating frame is homogeneous, (3) the recycle delay period is long enough to allow all nuclei to relax fully and (4) the rate of sample spinning at the magic angle is sufficient to remove chemical shift anisotropy. In this study a contact time of 1 ms and a recycle delay of 1 s, typical of the analysis of natural organic materials (Wilson 1987), and an adequate sample spinning speed to remove chemical shift anisotropy were used to acquire all spectra. The large number of samples analysed and the low carbon content of some samples collected in this study made the amount of spectrometer time required to complete NMR experiments designed to determine optimum conditions prohibitive. As a result, the distribution of the total acquired signal intensity within the delineated chemical shift regions should be taken as semi-quantitative.

Results

Particle size and density fractions

The distribution of soil and organic carbon in the fractions isolated from each soil and the mass balance and carbon balance data obtained are presented in Appendix 1. Except for the Millicent Mollisol and the Malanda Oxisol, in which a portion of the 0.2—2 and <0.2 μm fractions

was lost due to the breakage of freeze drying flasks, the recovery of soil and organic carbon was complete (Table 4).

Table 4. Recovery of soil and organic carbon after fractionation of the soils used in this study.

Soil	Recovery of soil (% of initial soil mass)	Recovery of organic carbon (% of initial)
Millicent Mollisol	83.2	94.7
Henongjiang Mollisol	99.2	96.4
Mount Schank Andosol	97.8	99.4
Malanda Oxisol	89.5	88.5
Guangdong Oxisol	100.1	102.0

The extent to which the ultrasonic vibration treatment used in the fractionation procedure disrupted soil aggregates was assessed by comparing the proportion, on a total soil mass basis, of clay size particles released as a result of the fractionation procedure with that obtained from a soil textural analysis (Table 5). In addition to the same ultrasonic treatment as was used in the fractionation procedure, the textural analysis included a chemical pretreatment in which 20 g of soil was soaked for 16 hours in a solution consisting of 25 cm^3 of a 50 g sodium hexametaphosphate L^{-1} solution, 5 cm^3 of 2.0 M NaOH and 20 cm^3 of deionised water. Since experimental problems (a lack of soil and the breakage of freeze drying flasks) allowed only one direct comparison for the soils used in this study, the results obtained from several other studies in which similar fractionation and textural analysis procedures were used have also been included in Table 5. Except for the Millicent Mollisol and the Mount Schank Andosol, chemical pretreatment prior to ultrasonic disruption caused only small increases in the proportion of soil collected in the clay fraction, indicating that the extent of disruption induced by the ultrasonic treatment used in the fractionation procedure was high. In the absence of chemical pretreatments, it was presumed that complete disruption of the Millicent Mollisol was limited by the presence of calcium dominated smectite clays and significant amounts of calcium carbonate and organic material while that of the Mount Schank Andosol was limited by the presence of amorphous allophanic materials which would have resisted dispersion under the near neutral pH conditions used during the disruption procedure.

Most of the soil organic carbon (79—91%) was contained in the silt

Table 5. Percentage of soil collected in the clay fraction after the fractionation procedure used in this study and a soil textural analysis.

Soil		Clay content (% of soil on a mass basis)	
		Fractionation procedure	Soil textural analysis
Millicent Mollisol	(this study)	> 45.0	62.3
	(Turchenek 1975)	46.0	60.8
Henongjiang Mollisol	(this study)	29.0	not determined
Mount Schank Andosol	(this study)	45.9	19.1
Malanda Oxisol	(this study)	> 55.6	69.9
	(Oades and Waters 1992)	62.5	69.9
Guangdong Oxisol	(this study)	70.5	not determined
Meadows Alfisol	(Baldock et al. 1990a)	9.4	9.3
Urrbrae Alfisol (permanent pasture)	(Oades et al. 1988)	15.4	17.3
Urrbrae Alfisol (wheat/fallow)	(Oades et al. 1988)	10.7	13.8

(2—20 μm) and clay (< 2 μm) fractions of each soil. With the exception of the Mount Schank Andosol, the clay fractions contained the largest proportion of the soil organic carbon (46—70%). In the Mount Schank Andosol, the largest proportion of the soil organic carbon was observed in the silt fraction (53%). Accumulations of soil organic carbon in clay fractions have been noted previously by Turchenek and Oades (1979), Christensen (1985), Dalal and Mayer (1986) and Oades et al. (1987, 1988); however, McKeague (1971) indicated that soils high in silt content may contain a high proportion of the soil organic carbon in that fraction, as was observed for the Mount Schank Andosol in this study.

The densimetric fractionation procedure used to separate the light (density < 2.0 Mg m^{-3}) and heavy (> 2.0 Mg m^{-3}) soil materials in the > 2 μm particle size fractions concentrated organic carbon in the light fractions, with 3.2—18.9 fold increases in organic carbon content being obtained when compared with that of the whole soil. For all soils except the Mount Schank Andosol, where the density separations were performed using water, the majority of the organic carbon contained in particle size fractions > 53 μm was in the light fractions (74—92%). For 20—53 μm and 2—20 μm particle size fractions, significant amounts of

carbon were observed in the heavy fraction of some soils (e.g. the 2—20 μm fraction of the Henongjiang Mollisol). The carbon contained in the heavy fractions must be strongly associated with the soil mineral fraction, presumably through some form of adsorption, since it was not removed during the ultrasonic vibration procedure used to disperse the soil. For the Mount Schank Andosol, the majority of the organic carbon contained in the 53—250 μm and 250—2000 μm particle size fractions was in the heavy (density > 1.0 Mg m^{-3}) fraction; however, the low organic carbon content (Appendix 1) and high iron content (15.3 and 15.5% Fe_2O_3, respectively) of these fractions precluded the acquisition of any solid-state CP/MAS ^{13}C NMR spectra.

A decrease in the C:N ratio of both the heavy and light fractions, away from that of plant materials (30—80) and towards that of the soil microbial biomass (8—12), was observed in progressing from the coarser to finer particle size fractions (Appendix 1). The C:N ratios of the light fractions were greater than that of the heavy fractions and the difference in C:N ratio of the light fractions between the fine and coarse particles was much more pronounced than that observed in the heavy fractions. A similar difference in C:N ratio between fine and coarse particles was noted by Oades et al. (1987) for an Alfisol (Rhodoxeralf) and by Catroux and Schnitzer (1987) for a Mollisol (Aquoll). Almendros et al. (1987) showed that, as the decomposition of leaves from the plants *Eucalyptus amygdalina* and *Salix alba* progressed, the C:N ratio of the remaining leaves and their residues decreased from initial values of 47.4 and 37.7 to 18.7 and 14.8, respectively, after 22 weeks of incubation. Decreases in C:N ratio as the extent of decomposition of organic materials increased were also noted by Hempfling et al. (1987) for spruce (*Picea abies*) and beech (*Fagus sylvatica*) litter, Zech et al. (1987) for spruce (*Picea abies*) and pine (*Pinus sylvestris*, L.) litter and Preston et al. (1990) for fallen douglas-fir (*Peudotsuga menziesii*), western hemlock (*Tsuga heterophylla*) and western red cedar (*Thuja plicata*) logs. The changes observed in the C:N ratio of the particle size fractions collected in this study may indicate that the organic materials contained in the finer particle size fractions were decomposed to a greater extent than those in the larger fractions. The greater C:N ratios measured for the light fractions compared to the heavy fractions of a given particle size lends support to this suggestion. The organic materials contained in the light fractions would be dominated by plant fragments, while those contained in the heavy fractions would have to exist as molecules capable of being adsorbed onto mineral surfaces. The organic materials associated with the heavy fractions are therefore likely to be decomposed to a greater extent, as suggested by the differences observed in C:N ratios.

Solid-state CP/MAS ^{13}C NMR analyses

Particle size and density fractions

Figs 1—5 show the solid-state CP/MAS ^{13}C NMR spectra acquired for the particle size and density fractions isolated from the five soils included in this study. Although similar types of carbon are present in all spectra, as indicated by chemical shift values, an influence of both soil type and particle size on the spectra is evident.

The distribution of each type of carbon in the particle size and density fractions of the soils used in this study, as determined by integration of the spectral areas indicated in Table 3, are shown in Fig. 6 (actual values are listed in Appendix 2), while those taken from other studies (Oades et al. 1987, Baldock unpublished and Preston et al. 1989) are presented in Fig. 7. The total signal intensities have been normalised to 100 so that comparisons of the relative contents of each type of carbon in the fractions isolated from the soils could be made.

Mollisols and Oxisols. Differences in the composition of the organic materials contained in the particle size and density fractions isolated from the two Mollisols and the two Oxisols were similar and several trends were evident. O-alkyl carbon (72 and 105 ppm) was concentrated in coarser particles while alkyl carbon (30 ppm) was concentrated in the finer particles. The proportion of aromatic carbon was greatest in the fine sand (20—53 μm diameter particles) and silt (2—20 μm diameter particles) fractions and lowest in the clay fractions. Differences in the proportion of carboxyl carbon were small and variable, but there did appear to be a trend of more carboxyl carbon in the finer particles of the Millicent Mollisol and to some extent in the Henongjiang Mollisol.

The most pronounced differences in the chemical structure of the organic materials contained in the particle size fractions were noted for the Millicent Mollisol, where alkyl and O-alkyl carbon accounted for 21% and 57% of the carbon in the 250—2000 μm fraction and 36% and 37% in the <0.2 μm fraction, respectively. For the Henongjiang Mollisol and the two Oxisols, the decrease in the proportion of O-alkyl carbon in progressing from the larger to finer particle size fractions was interrupted in the clay fractions where an increase in the proportion of O-alkyl carbon was observed. The increased signal intensity in the O-alkyl spectral region of the clay fractions resulted from an increase in the relative contribution of resonances in the vicinity of 45—60 ppm to the total O-alkyl spectral region and not from an increase in the 72 and 105 ppm resonances which dominated the O-alkyl region of the larger particle size fractions (Figs 2, 4 and 5). Resonances in the 45—60 ppm region may be derived from

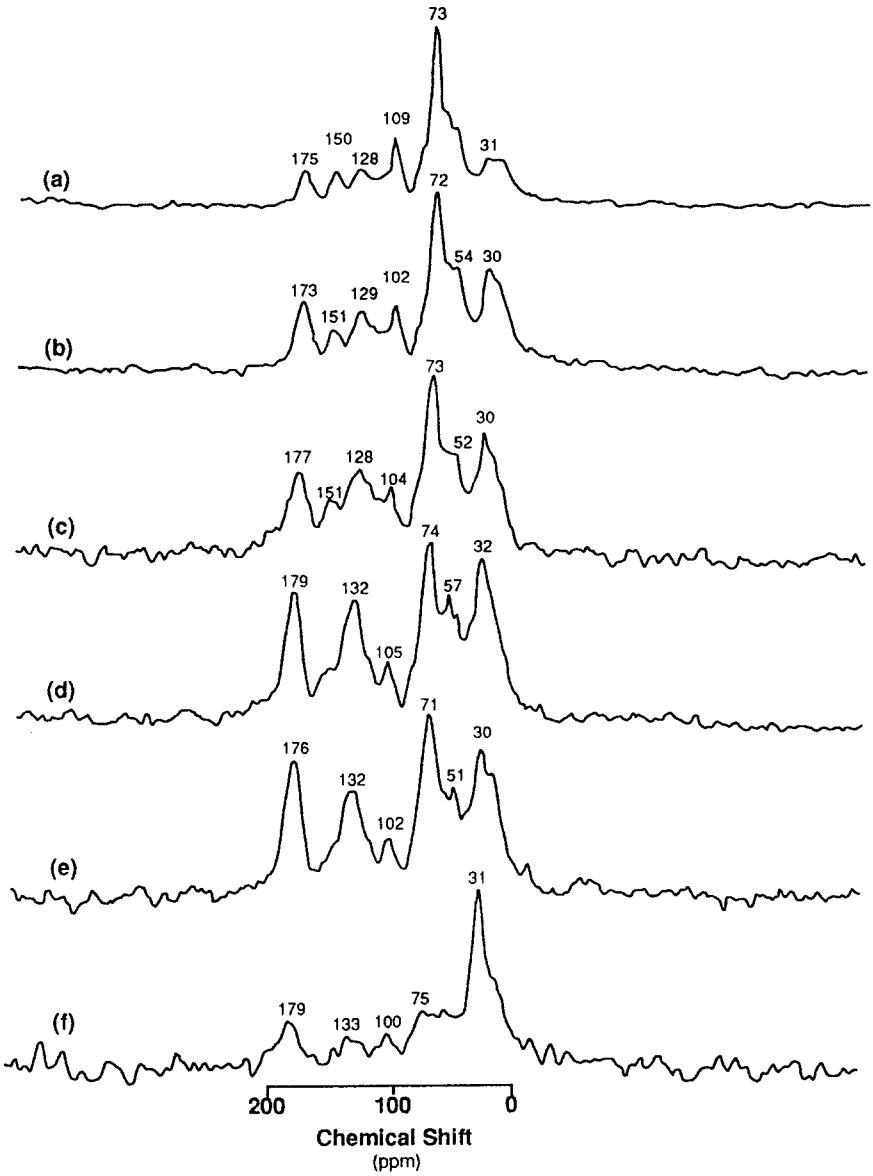

Fig. 1. Solid-state CP/MAS ^{13}C NMR spectra acquired for the Millicent Mollisol. (a) 250–2000 μm diameter, $\leq$2.0 Mg m^{-3}, (b) 53–250 μm diameter, $\leq$2.0 Mg m^{-3}, (c) 20–53 μm diameter, $\leq$2.0 Mg m^{-3}, (d) 2–20 μm diameter, $\leq$2.0 Mg m^{-3}, (e) 0.2–2 μm diameter, whole fraction, and (f) $<$0.2 μm diameter, whole fraction.

either methoxyl carbon or the amine carbon found in proteins (Duncan 1987). Since removal of methoxyl groups is known to be one of the early stages of lignin decomposition (Oades et al. 1987) and nitrogen tends to

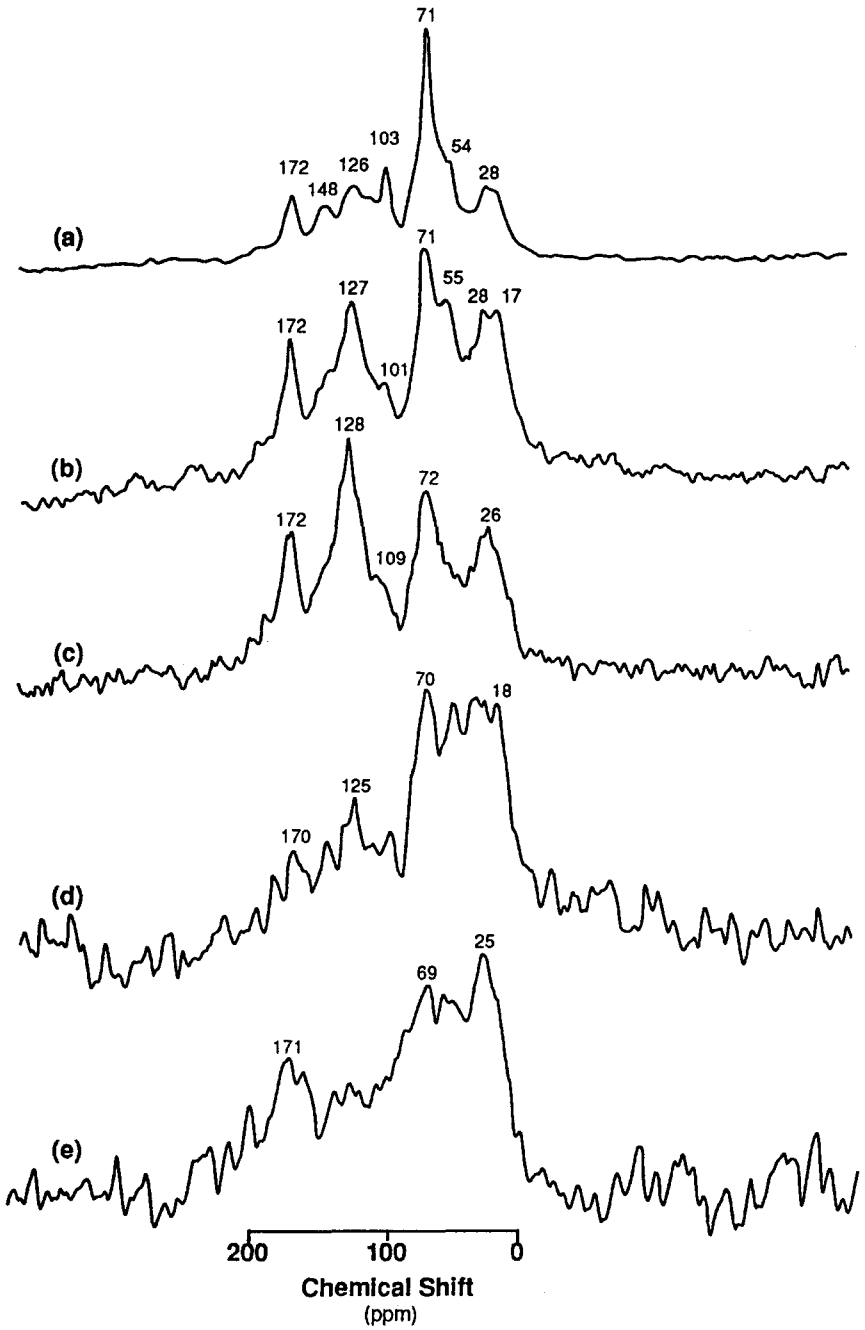

Fig. 2. Solid-state CP/MAS ^{13}C NMR spectra acquired for the Henongjiang Mollisol. (a) 53–2000 μm diameter, $\leqslant 2.0$ Mg m^{-3}, (b) 20–53 μm diameter, $\leqslant 2.0$ Mg m^{-3}, (c) 2–20 μm diameter, $\leqslant 2.0$ Mg m^{-3}, (d) 0.2–2 μm diameter, whole fraction, and (e) < 0.2 μm diameter, whole fraction.

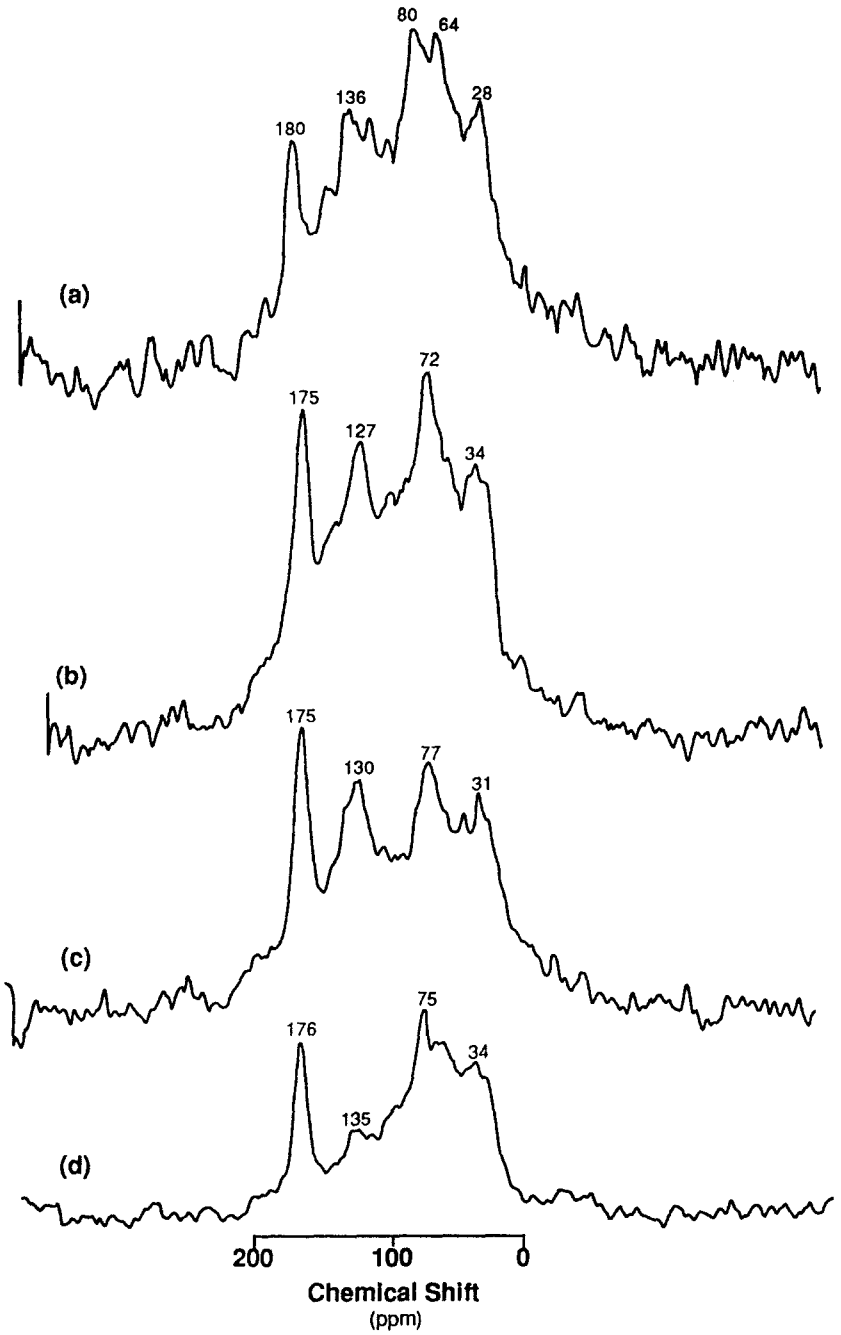

Fig. 3. Solid-state CP/MAS ^{13}C NMR spectra acquired for the Mount Schank Andosol. (a) 250–2000 μm diameter, $\leqslant 1.0$ Mg m^{-3}, (b) 53–250 μm diameter, $\leqslant 1.0$ Mg m^{-3}, (c) 2–20 μm diameter, whole fraction, and (d) < 2 μm diameter, whole fraction.

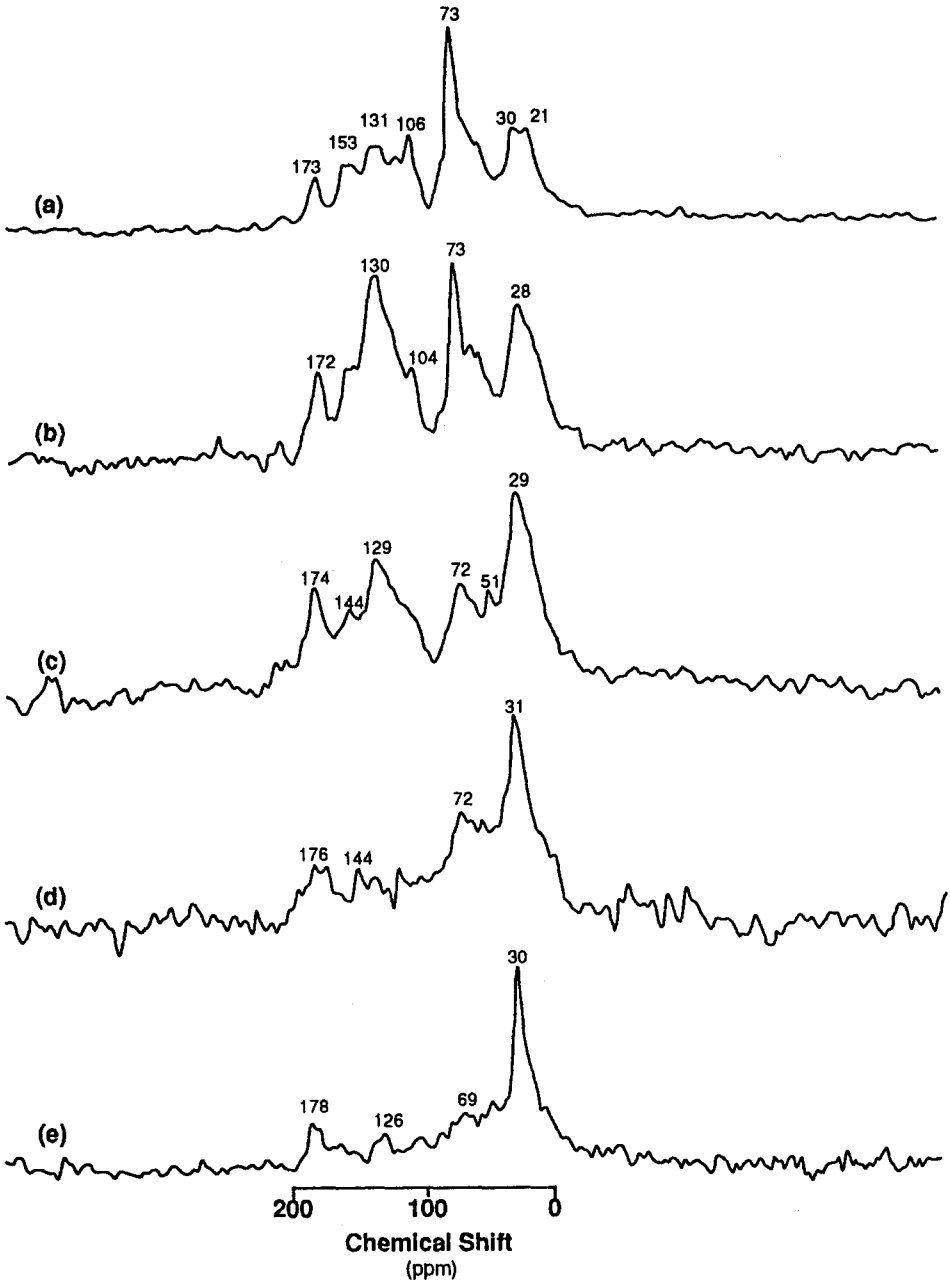

Fig. 4. Solid-state CP/MAS ^{13}C NMR spectra acquired for the Malanda Oxisol. (a) 53–2000 μm diameter, $\leqslant 2.0$ Mg m^{-3}, (b) 20–53 μm diameter, $\leqslant 2.0$ Mg $^{-3}$, (c) 2–20 μm diameter, $\leqslant 2.0$ Mg m^{-3}, (d) 0.2–2 μm diameter, whole fraction, and (e) < 0.2 μm diameter, whole fraction.

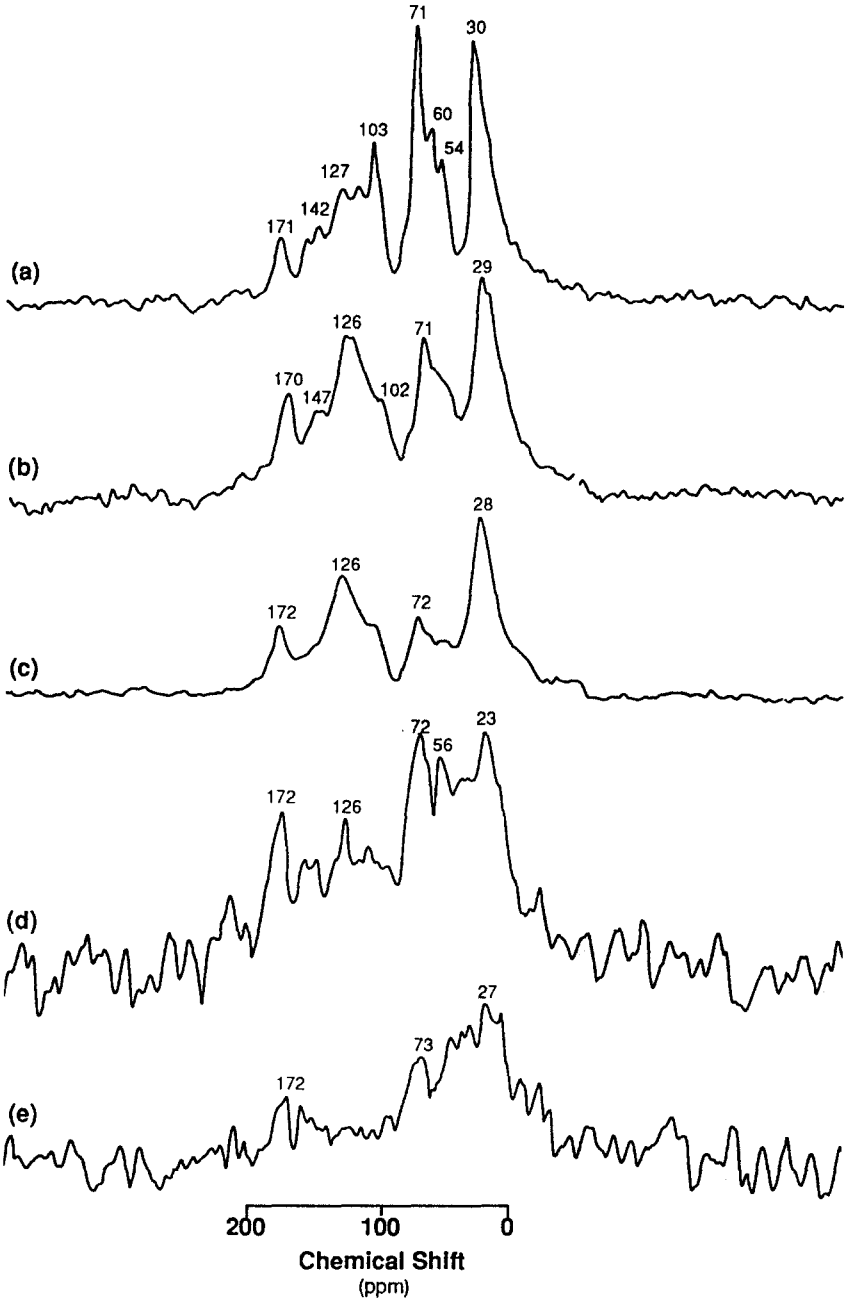

Fig. 5. Solid-state CP/MAS ^{13}C NMR spectra acquired for the Guangdong Oxisol. (a) 53–2000 μm diameter, $\leqslant 2.0$ Mg m^{-3}, (b) 20–53 μm diameter, $\leqslant 2.0$ Mg m^{-3}, (c) 2–20 μm diameter, $\leqslant 2.0$ Mg m^{-3}, (d) 0.2–2 μm diameter, whole fraction, and (e) < 0.2 μm diameter, whole fraction.

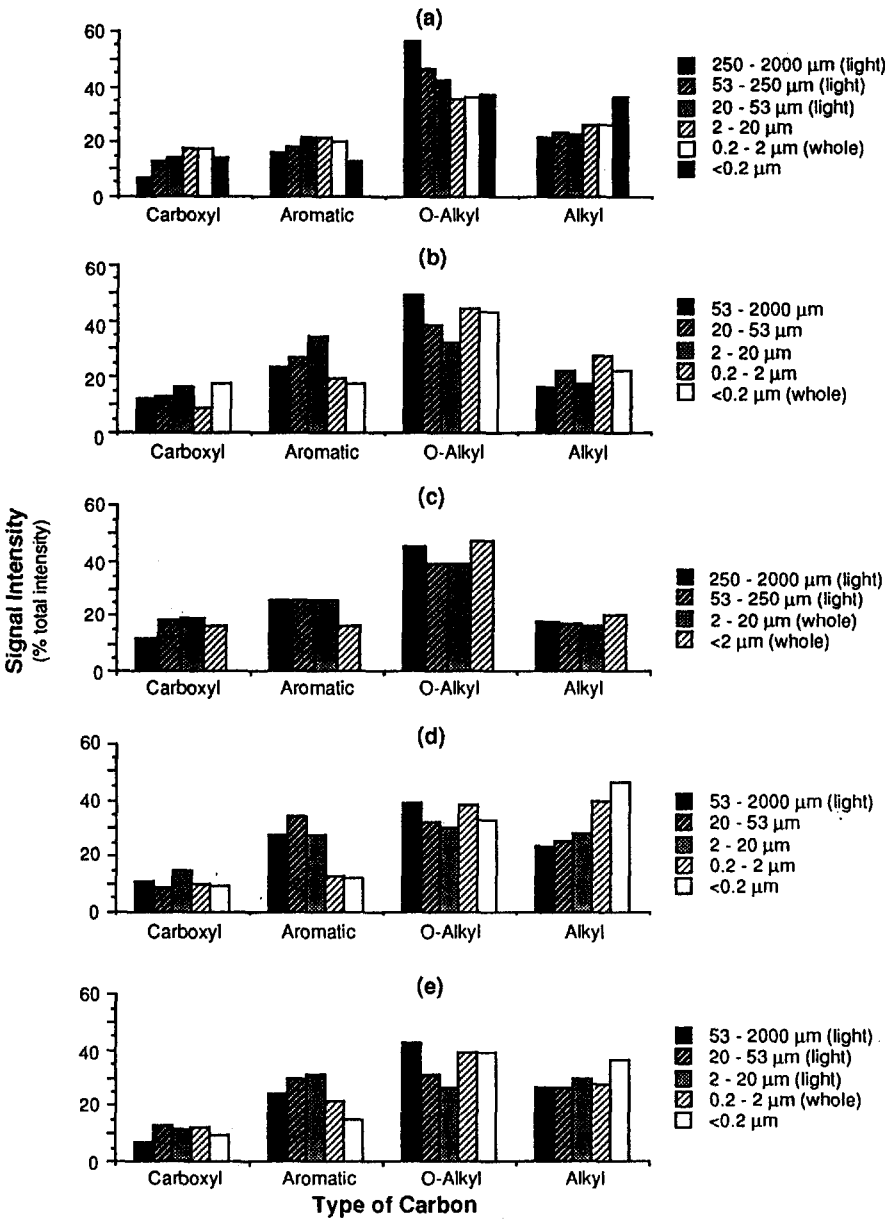

Fig. 6. Contribution of the total acquired CP/MAS ^{13}C NMR signal intensity from each type of carbon for the (a) Millicent Mollisol, (b) Henongjiang Mollisol, (c) Mount Schank Andosol, (d) Malanda Oxisol and (e) Guangdong Oxisol.

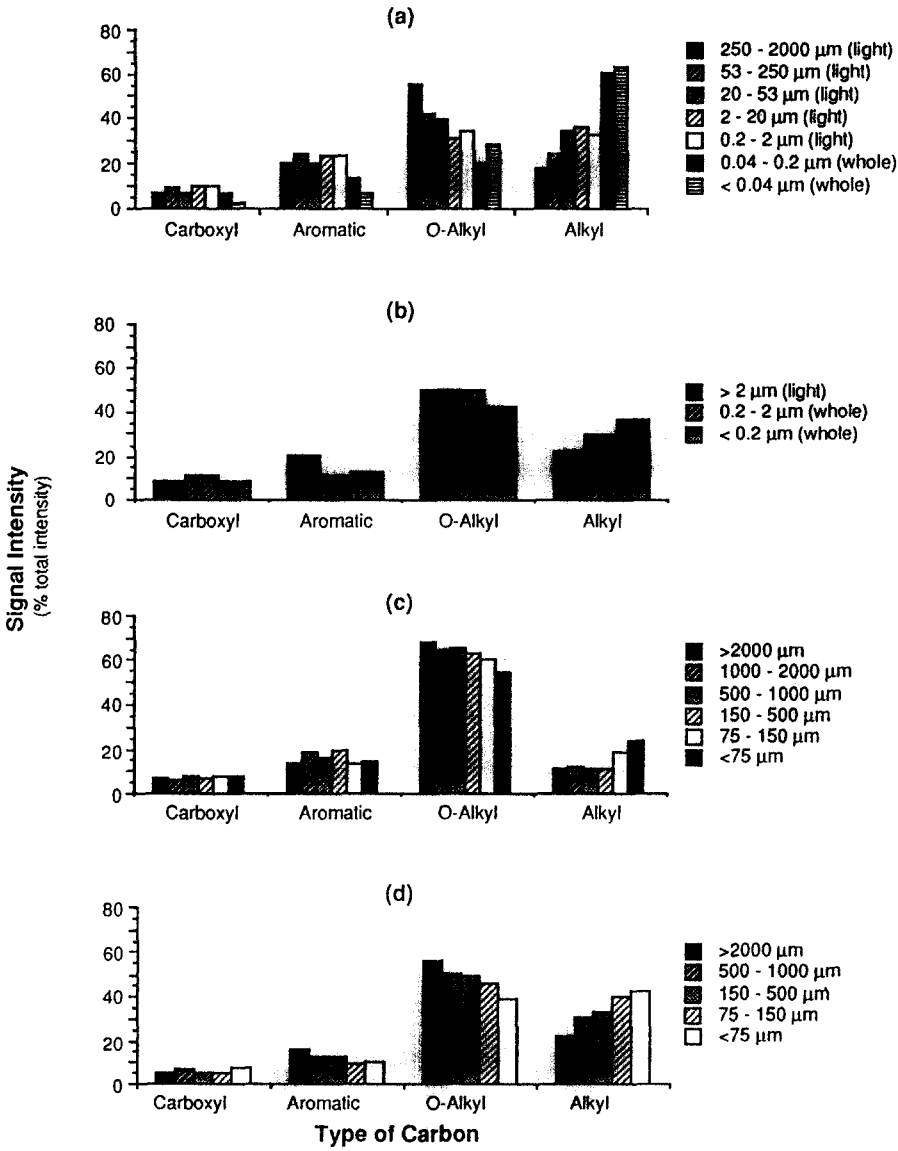

Fig. 7. Contribution of the total acquired CP/MAS ^{13}C NMR signal intensity from each type of carbon for the (a) Urrbrae Alfisol (Oades et al. 1987), (b) Meadows Alfisol (Baldock unpublished), (c) Gatineau Histosol (Preston et al. 1989) and (d) Farnham Histosol (Preston et al. 1989).

be recycled, as carbon is lost through mineralisation during decomposition processes, it is suspected that the increased signal intensity in the 45—60 ppm of the spectra acquired for the clay fractions resulted from a relative accumulation of amine type carbon structures.

An increase in the content of alkyl carbon and a decrease in that of O-alkyl carbon in finer particles, when compared to coarser particles, was also noted by Oades et al. (1987) for an Alfisol (Urrbrae fine sandy loam) and Preston et al. (1989) for a well decomposed Histosol (Farnham) (Fig. 7). For a poorly decomposed peat (Gatineau) and another Alfisol (Meadows fine sandy loam), the changes in the relative signal intensities of the alkyl and O-alkyl spectral regions noted by Preston et al. (1989) and Baldock (unpublished), respectively, were similar but much smaller in magnitude. For the aromatic and carboxyl carbon and changes noted in these previous studies were minor.

The composition of the organic carbon contained in humic acid extracted from particle size fractions of a Mollisol (Bainsville clay loam) was studied by Catroux and Schnitzer (1987) using solution-state ^{13}C NMR. The fine clay (< 0.2 μm) fraction was enriched in alkyl carbon and the relative contribution of aromatic carbon was greatest in the 10—45 μm and 2—10 μm fractions (Fig. 8). Although the proportion of O-alkyl carbon observed in the finer particles was not much smaller than that observed in the larger particles, the results of Catroux and Schnitzer (1987) are consistent with those acquired by solid-state CP/MAS ^{13}C NMR in this and previous studies.

Mount Schank Andosol. The spectra acquired for the Mount Schank Andosol (Fig. 3) indicated that, except for a small accumulation of signal

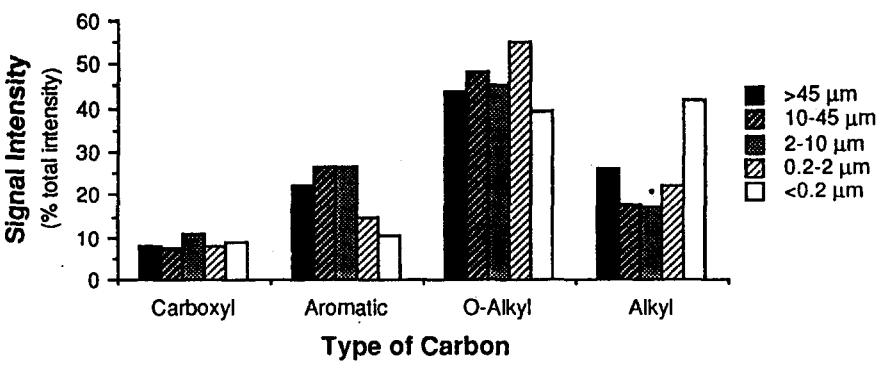

Fig. 8. The composition of the organic carbon contained in humic acid extracted from particle size fractions of a Mollisol as determined by solution state ^{13}C NMR (Catroux and Schnitzer 1987).

intensity in the carboxyl region (175 ppm) of the silt (2—20 μm) and sand (53—250 μm) fractions and a loss of aromatic signal intensity (130 ppm) in the clay fraction, the composition of the organic carbon in the particle size fractions was similar. Quantitation of the signal intensities associated with each spectral region confirmed this observation (Fig. 6).

A consideration of the dynamics of organic carbon cycling in Andosols can provide some insight into why the composition of the organic carbon contained in the various particle size fractions was more constant than was observed for other soils (Figs 6 and 7). Fresh organic residues decompose rapidly in Andosols but the products of decomposition are protected from microbial attack by the formation of stable organic-alumina complexes (Duchaufour 1976). The protection against microbial attack offered to organic materials in allophanic soils is demonstrated clearly by the results of Martin et al. (1982), Zunino et al. (1982) and Monreal and McGill (1989) by measuring the extent of mineralisation of carbon from ^{14}C labelled substrates added to allophanic soils, nonallophanic soils and nonallophanic soils amended with allophane. The presence or addition of allophane decreased the amount of substrate carbon mineralised to carbon dioxide by as much as two thirds of that in the nonallophanic soils. The results of Martin et al. (1982) and Zunino et al. (1982) support the proposal made by Broadbent et al. (1964) that the more recalcitrant products formed during the latter stages of decomposition of organic substrates are stabilised to the greatest extent in allophanic soils. Zunino et al. (1982) suggested that the stabilisation resulted from a strong interaction of active chemical functional groups on the organic materials with allophane through the formation of organic-Al (or Fe)-allophane complexes. Under such conditions, the amount of unaltered fresh plant material in large particle size fractions would be small and changes to the chemical structure of the decomposition products brought about by continual cycling would be minimised. As a result, a large difference in the chemical composition of the organic carbon associated with different particle size fractions would not be expected.

Discussion

Whole soils

An indication of the chemical composition of the entire organic fraction contained in each soil used in this study was calculated by weighting the chemical composition of the organic materials in each particle size fraction (Fig. 6) on the basis of the carbon balance data presented in Appendix

1. In order to complete this calculation several assumptions were made. With respect to the Mollisols and Oxisols it was assumed that the chemical composition of the organic carbon contained in the heavy (> 2.0 Mg m^{-3}) fractions for which no NMR spectra were acquired was equivalent to that in the 0.2—2 μm clay fraction. This assumption appeared reasonable when it was considered that the organic carbon in the heavy fractions must have been adsorbed to soil minerals and was therefore expected to resemble the organic carbon in the clay fraction more closely than the unadsorbed plant debris contained in light fractions. For the Mount Shank Andosol the following two assumptions were made: (1) that the organic carbon contained in the light and heavy material separated in each size fraction had a similar chemical composition, since water (density = 1.0 Mg m^{-3}) was used in place of a heavy solution (density = 2.0 Mg m^{-3}) and (2) that the chemical composition of the organic carbon contained in the 20—53 μm fraction, for which no CP/MAS ^{13}C NMR spectrum could be acquired because of its high Fe content (15.2% Fe_2O_3), was equivalent to that contained in the 53—250 μm fraction. The estimated composition of the entire organic carbon fraction contained in each of the soils included in this study is shown in Fig. 9a, while that estimated for the Urrbrae Alfisol (Oades et al. 1988) and Meadows Alfisol (Baldock unpublished) and the composition of uncultivated and cultivated Ormstown Histosol (Preston et al. 1987) are presented in Fig. 9b.

The composition of organic materials contained in soil is controlled primarily by two factors: (1) the chemical composition of the carbon inputs and (2) the nature and magnitude of decomposition processes. The latter factor may be controlled by the type(s) of organism(s) and their metabolism, while the magnitude would be regulated by environmental conditions (e.g. soil temperature, water content, pH and redox potential) and the extent of any protection offered to the organic materials via an interaction with the inorganic matrix.

Oades et al. (1988) demonstrated that long-term changes in the nature of the organic residues added to the Urrbrae Alfisol did not alter the composition of the soil organic fraction. It was suggested that the composition of soil organic materials was controlled by the activity of the microbial biomass and the interaction of the biomass and its decomposition products with the soil matrix. An indication of the variability associated with the composition of the organic inputs into each soil included in this study was obtained from the solid-state CP/MAS ^{13}C NMR spectra acquired for the light fraction of the largest particle size fraction isolated from each soil (Fig. 10). The organic materials contained in these fractions consisted of easily identifiable plant fragments which had been decom-

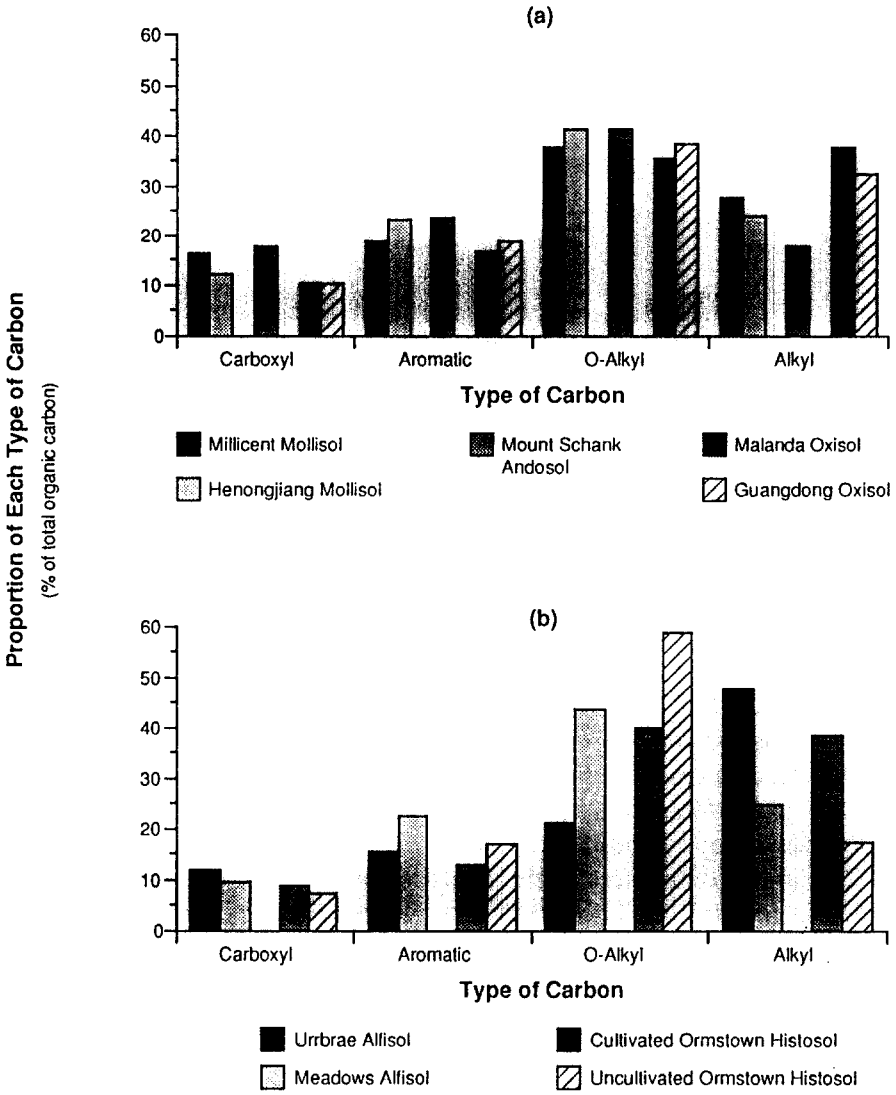

Fig. 9. Estimated chemical composition of the organic matter contained in (a) each of the unfractionated soils included in this study and (b) the Urrbrae Alfisol (Oades et al. 1988), the Meadows Alfisol (Baldock unpublished) and uncultivated and cultivated (15 years) Ormstown Histosol (Preston et al. 1987).

posed only to a limited extent. Although variations in the content of all four types of carbon were noted, the largest changes were associated with the O-alkyl carbon. Relative to the changes noted in the O-alkyl region, those noted in the alkyl region were minor. Since the largest changes noted in the composition of the organic materials contained in each soil

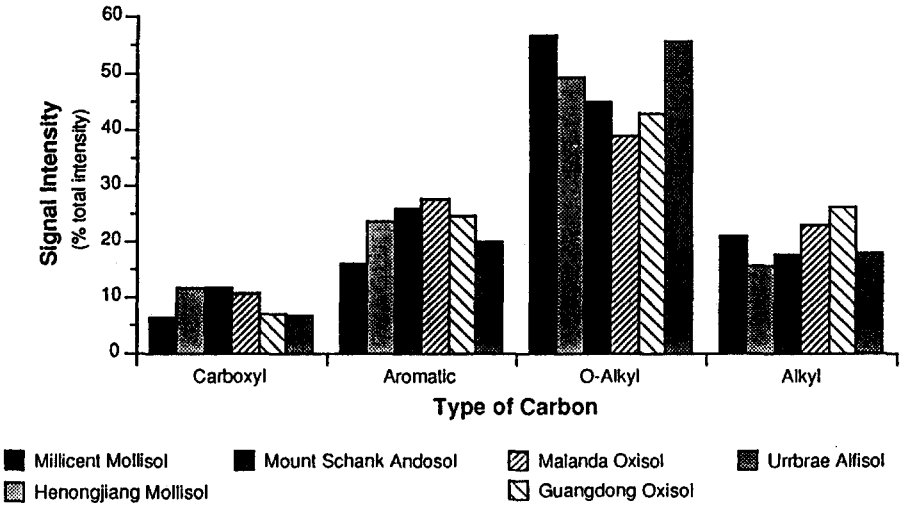

Fig. 10. Composition of the organic materials contained in the light fraction of the largest particle size fraction isolated from each soil and that is isolated from the Urrbrae Alfisol (Oades et al. 1988).

were associated with the alkyl carbon region, it would appear that, at least for the soils included in this study and the Urrbrae Alfisol (Oades et al. 1988), the changes in composition of the carbon associated with each soil resulted from differences in the nature and magnitude of the decomposition processes operating in each soil.

The largest differences in the composition of the organic carbon contained in the soils included in this study were observed between the soil orders. The differences observed within orders were smaller. The largest proportion of the organic carbon contained in the Mollisols was O-alkyl carbon followed by approximately equal contents of alkyl and aromatic carbon and less carboxyl carbon. The Andosol was also dominated by O-alkyl carbon but differed from the Mollisols in its lower alkyl carbon content. The amount of aromatic and carboxyl carbon present in the Andosol was of the same order as that present in the Mollisols. In contrast to the other soils, the organic materials contained in the Oxisols had a greater alkyl carbon content and were thus dominated by both O-alkyl and alkyl carbon. Using liquid state ^{13}C NMR, Lobartini and Tan (1988) also noted a greater content of alkyl carbon in the humic acids extracted from two tropical soils than that extracted from seven temperate soils.

The composition of the organic materials contained in the Urrbrae Alfisol (Oades et al. 1988) and the Meadows Alfisol (Baldock unpublished) was different indicating that relative compositional differences may

exist between soils of the same order (Fig. 9b). The Urrbrae Alfisol contained more alkyl carbon and less O-alkyl and aromatic carbon than the Meadows Alfisol. The changes observed between the two Alfisols were similar to those induced by cultivating a Histosol for 15 years (Preston et al. 1987). Since cultivation has been demonstrated to promote the decomposition of soil organic materials, the changes were presumed to indicate that the organic materials contained in the Urrbrae Alfisol were decomposed to a greater extent than that contained in the Meadows Alfisol. The lower average temperature and prolonged periods of water-logging experienced by the Meadows Alfisol which would reduce the rate of organic matter decomposition are consistent with this observation.

The differences observed in the chemical structure of the organic materials contained in the Andosol, Mollisols, the Oxisols included in this study also resembled those noted by Preston et al. (1987). The organic materials contained in the Andosol appeared to be the least decomposed which is consistent with the slow turnover and cycling of carbon in these soils (Duchaufour 1976; Martin et al. 1982; Zunino et al. 1982; Monreal and McGill 1989). The higher alkyl carbon content of the Oxisols suggested that the extent to which the organic materials contained in these soils were decomposed was greater than that of the organic materials in the other soils. The recalcitrant nature of alkyl carbon in soil (Paul and van Veen 1978) and the rapid turnover of organic materials in tropical environments (Ladd and Amato 1985; Oades 1988) are consistent with this suggestion. However, the greater alkyl carbon content of the 53—2000 μm particle size fractions of the Oxisols relative to the Andosol and Mollisols (Fig. 6 and Appendix 2) suggests that the higher estimated alkyl carbon content of the Oxisols may also be related to differences in the chemical composition of plant residues from which the soil organic materials were derived.

Chemical changes associated with the decomposition of plant materials

Changes in the C:N ratio of the fractions for which CP/MAS ^{13}C NMR spectra were acquired suggested that organic materials contained in finer particles were more decomposed than those contained in the larger particles. The results of research reviewed by Paul and van Veen (1978) demonstrated that the rate of decomposition of known organic materials in soils depended on their chemical composition. Based on the rate constants presented by Paul & van Veen (1978), proteinaceous and carbohydrate carbon would be utilised and either mineralised or assimilated at a faster rate than the more recalcitrant aromatic and alkyl carbon during decomposition processes. Therefore, as decomposition proceeds,

the relative concentration of the more recalcitrant forms of carbon should increase. In progressing from the coarser to finer particle size fractions the initial increase in aromatic carbon content, the continual increase in alkyl carbon content and the continual decrease in O-alkyl carbon content are consistent with the proposal that the organic carbon contained in the finer particle size fractions was more decomposed than that contained in the coarser fractions. The results obtained by Anderson and Paul (1984) for the radiocarbon age of the organic carbon contained in the particle size fractions of a soil support this suggestion.

Solid-state CP/MAS ^{13}C NMR had been used previously in numerous studies to examine the chemical changes associated with the decomposition of the natural organic materials contained in peats (Preston et al. 1987, Hammond et al. 1985, Hatcher et al. 1986, Zech et al. 1985, Dereppe et al. 1983), litter layers of mull, mor and moder type forest soils (Kögel et al. 1987; Kögel-Knabner et al. 1988; Hempfling et al. 1987), decomposing fallen logs (Preston et al. 1990) and buried wood (Hatcher et al. 1981, 1989; Bates et al. 1991; Hedges et al. 1985; Spiker and Hatcher 1987; Stout et al. 1988). As observed in this study, a decrease in the relative intensity of O-alkyl carbon resonances and an increase in the alkyl carbon resonances as the extent of decomposition increased was noted in all cases. The changes associated with the carboxyl and aromatic carbon were variable and much smaller than those associated with the alkyl and O-alkyl carbon.

Hatcher et al. (1983) suggested that the increase in the proportion of alkyl carbon present as decomposition proceeds is the result of the utilisation of the easily decomposable carbohydrates by microorganisms and a selective preservation of the more recalcitrant alkyl (polymethylene) carbon associated with the original plant materials. Baldock et al. (1989, 1990a, b) demonstrated that the utilisation of carbohydrate carbon (glucose) by soil microorganisms resulted in the synthesis of a significant quantity of alkyl carbon. The synthesis of alkyl carbon by microorganisms in peat was also demonstrated by Harvey et al. (1989), and Ellwardt et al. (1981) showed that alkyl structures may be formed during lignin biodegradation. Therefore the accumulation of alkyl carbon as the extent of decomposition of organic materials increases is likely to result from two processes: (1) a selective preservation of alkyl carbon derived from both the original material and microorganisms, and (2) an *in situ* synthesis by microorganisms utilising the carbohydrate and/or aromatic fractions of the organic material.

Selective preservation versus in situ synthesis

The greater contents of carboxyl and aromatic carbon in the intermediate

particle size fractions and of alkyl carbon in intermediate and finer particle size fractions (Fig. 6) may have arisen as a result of selective preservation and/or *in situ* synthesis, as O-alkyl carbon was decomposed and either assimilated into various microbial products or lost through mineralisation to carbon dioxide. The ability of the process of selective preservation to account for the observed changes in the chemical composition of the soil organic carbon associated with the particle size fractions was assessed by adjusting the composition of the organic materials contained in the particle size fractions for the loss of O-alkyl carbon noted in progressing from coarser to finer particle size fractions. To complete this adjustment the following three assumptions were required:

(1) the organic materials contained in the coarsest particle size fraction represented the initial substrate from which the organic materials contained in all finer fractions were derived,
(2) the extent of decomposition of the organic materials contained in the particle size fractions increased in progressing from coarser to finer particles, and
(3) only O-alkyl carbon was mineralised during decomposition processes.

Of these three assumptions, the third is the most tentative, as carbon would undoubtedly be mineralised from carbon structures other than O-alkyl during decomposition. However, given the dynamic nature of O-alkyl carbon (carbohydrate structures) in soil, the majority of the mineralised carbon would be expected to have originated from O-alkyl materials, at least during the initial stages of decomposition. It is also important to note the adjustment and subsequent discussion focus on the net changes in chemical composition as decomposition proceeds. Where no net change in composition was observed, *in situ* synthesis and/or selective preservation may be occurring, but are offset by mineralisation.

The content of O-alkyl carbon (expressed as a fraction of the total organic carbon) in the coarsest particle size fraction of each soil, OA_c, and that associated with each of the finer particle size fractions, OA_f, were obtained from the quantitation of acquired CP/MAS ^{13}C NMR spectra. For every 100 carbon atoms contained in the coarsest fraction, the decomposition of the O-alkyl carbon can be described by equation (1),

$$100\,OA_c - x = y \qquad (1)$$

where x is the number of O-alkyl carbons lost due to decomposition processes (that mineralised plus that assimilated and converted to other structures) and y is the number of residual O-alkyl carbons. Since we assume that only O-alkyl carbon was mineralised, for every 100 carbon

atoms present initially, (100 − x) atoms will remain after decomposition and y carbon atoms will be O-alkyl carbon in the residue. Therefore, since the selectively preserved organic carbon contained in the finer particle size fractions is presumed to be derived from that contained in the coarsest fraction, the content of O-alkyl carbon in the finer fractions, OA_f, can be expressed as a function of x and y using equation (2).

$$OA_f = \frac{y}{100 - x} \tag{2}$$

By substituting equation (1) into equation (2) and rearranging, the number of O-alkyl carbons lost from the coarsest fraction, x, can be obtained using equation (3).

$$x = \frac{100(OA_c - OA_f)}{1 - OA_f} \tag{3}$$

The number of residual O-alkyl carbons remaining in the fine particle size fraction, y, can then be calculated using equation (1).

To adjust the relative contents of each type of carbon for selective preservation, a correction factor, CF, was calculated according to equation (4).

$$CF = \frac{100\ OA_f}{y} \tag{4}$$

Dividing the contents of each type of carbon obtained from the CP/MAS ^{13}C NMR data acquired for each particle size fraction by the correction factor calculated for that fraction, will adjust the contents for enrichment due to selective preservation (Fig. 11). It should be noted that in addition to the assumptions about the dynamics of the carbon associated with changes in particle size, this analysis also assumes that the different types of carbon are all detected by the NMR spectrometer with the same efficiency, which has yet to be demonstrated for the materials contained in mineral soils.

Values indicative of the change in content of the various types of carbon due to selective preservational enrichment were obtained by subtracting the composition obtained from the largest particle size fraction from the adjusted composition obtained for a given particle size fraction (Fig. 12). Positive values indicate a net synthesis of carbon and negative values indicate a net loss of carbon, presumably through decomposition (mineralisation to carbon dioxide and conversion to other forms of carbon through assimilation). Given the nature of the assumptions made in these calculations and the error associated with the CP/MAS ^{13}C NMR analyses, only the major changes are considered worthy of comment.

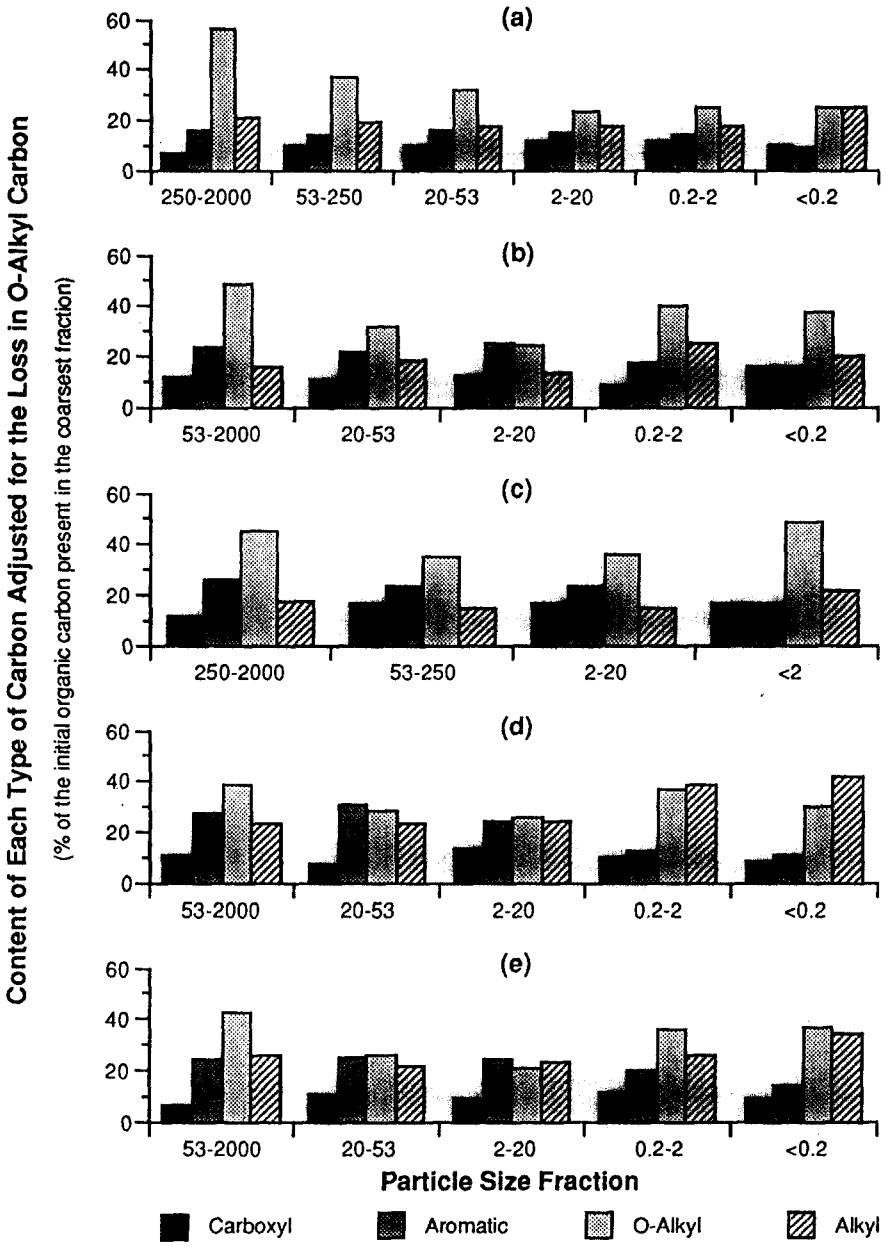

Fig. 11. Content of each type of carbon in the particle size fractions adjusted for the loss of O-alkyl carbon for the (a) Millicent Mollisol, (b) Henongjiang Mollisol, (c) Mount Schank Andosol, (d) Malanda Oxisol and (e) Guangdong Oxisol.

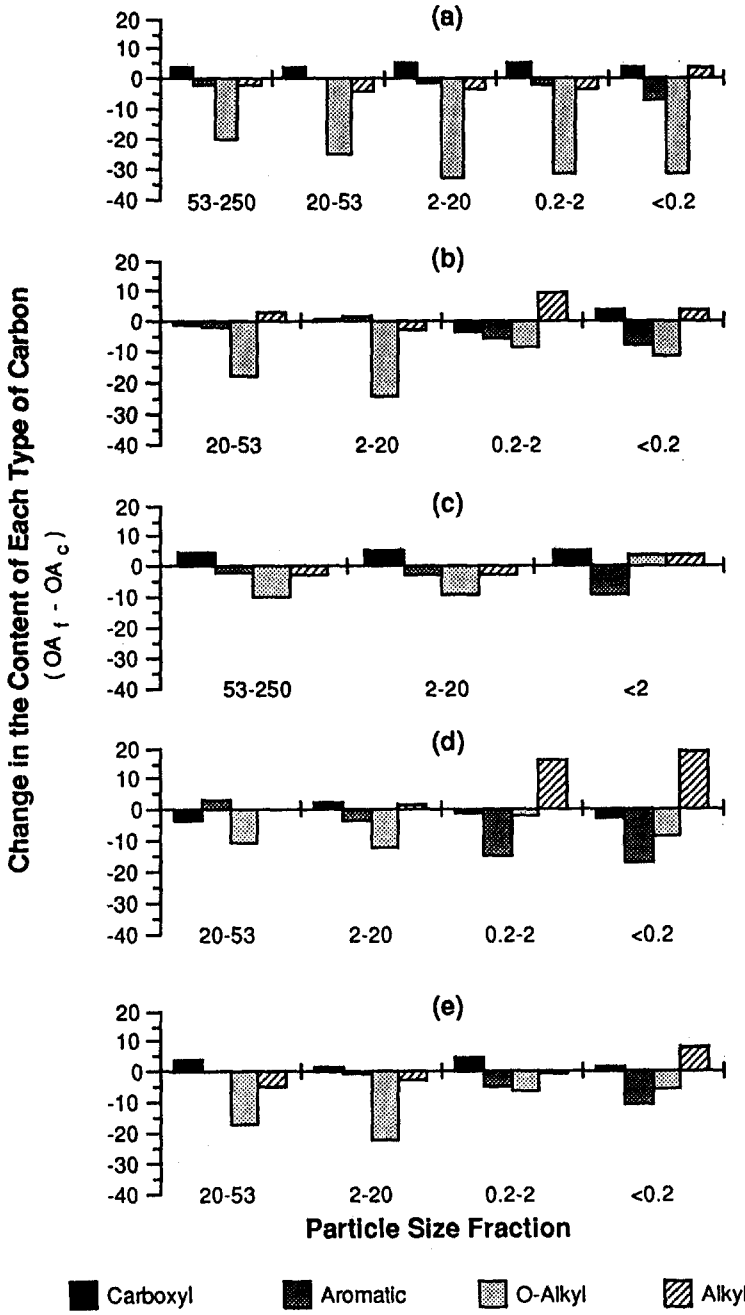

Fig. 12. Differences in the content of each type of carbon contained in the largest particle size fraction and all smaller fractions for each soil after adjusting for the loss of O-alkyl carbon as particle size became finer.

The data presented in Fig. 12 proposes that there is no net synthesis of aromatic carbon during the decomposition of O-alkyl carbon. The increased aromatic carbon content, noted for the organic materials contained in the intermediate particle size fractions (Fig. 6), could be explained on the basis of an enrichment due to selective preservation of aromatic structures presumably present in the lignin of the original plant residues or synthesised by microbes. Losses of aromatic carbon as a result of decomposition were only noted for particle sizes < 2 μm. The initiation of a detectable amount of aromatic carbon decomposition was presumed to arise as a result of two processes: 1) a removal of carbohydrate (O-alkyl) material from the plant residues resulting in the exposure of the lignin molecule, and 2) the increased surface area of lignin structures available to decomposition in the finer particles. The initial net increase in alkyl carbon also appeared to be a result of selective preservation (Fig. 12). However, in the finer particle size fractions (< 2 μm diameter), selective preservation could not explain the net accumulation of alkyl carbon completely and it is suggested that a significant portion of the increased alkyl carbon content in the clay fractions of the soils studied resulted from an *in situ* synthesis and accumulation. Changes in the net carboxyl carbon content of the fractions tended to be more variable and smaller in magnitude than those associated with the alkyl and aromatic carbon; however, there is some indication that carboxyl carbon was synthesised as O-alkyl carbon was decomposed.

A model of the oxidative decomposition of plant materials in mineral soils

Based on the differences noted in the chemical structure of the organic materials contained in the particle size fractions of the soils and models presented by Waksman (1936) and Hatcher and Spiker (1988), a model describing the oxidative decomposition of plant materials in mineral soils by microorganisms has been formulated (Fig. 13). It is proposed, as suggested by Oades (1981), that the extent of decomposition of organic materials in soils follows a continuum from fresh plant residues in the large particle size fractions (> 20 μm diameter) through partially degraded residues in intermediate fractions (2—20 μm diameter) to degraded residues in the finest fractions (< 2 μm diameter).

During the initial stages of the decomposition of plant materials, carbohydrate (cellulose and hemicellulose) and protein structures are degraded. A portion of the degraded carbohydrate and protein carbon is mineralised to carbon dioxide while the remainder is assimilated and converted into microbial tissues and metabolites. The mineralisation process accounts for the decrease in the amount of O-alkyl carbon observed in the CP/MAS

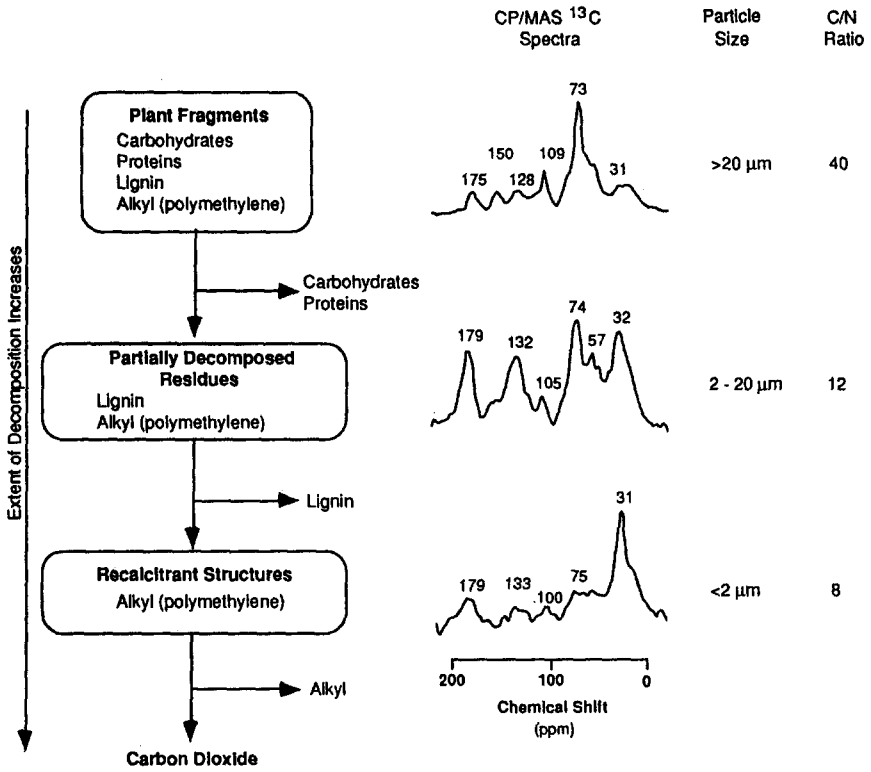

Fig. 13. A simple model describing the oxidative decomposition of plant materials in mineral soils using the data acquired for the Millicent Mollisol.

^{13}C NMR spectra in progressing from coarse to fine particles. Since appreciable quantities of microbially derived O-alkyl carbon (carbohydrate and/or protein) are synthesized, as carbohydrate carbon is utilised by soil microorganisms (Baldock et al. 1989, 1990a), a complete disappearance of O-alkyl carbon as the extent of decomposition increases is unlikely. However, a conversion from plant derived O-alkyl carbon to microbially derived O-alkyl carbon would be expected. The increase observed in the content of alkyl and aromatic carbon during this initial stage of decomposition could be completely explained on the basis of selective preservation of these more recalcitrant organic structures.

Once the O-alkyl carbon associated with the plant materials has been degraded, the second stage in the decomposition process is initiated. Lignin molecules, previously surrounded by O-alkyl structures (e.g. cellulose and hemicellulose), are exposed to microbial decomposition and a reduction in the content of aromatic carbon results.

The most recalcitrant organic carbon found in soils is that contained in alkyl carbon structures. The chemical shift values associated with these materials indicate that polymethylene type structures are an important component; however, little is known about their actual structure. Dipolar dephasing CP/MAS ^{13}C NMR experiments have indicated that a significant portion of the alkyl carbon is contained in structures exhibiting molecular motion — more so than normally experienced in solids (Baldock et al. 1989). The accumulation of alkyl carbon in this third stage of decomposition could not be explained on the basis of selective preservation alone and it is therefore proposed to result from both selective preservation and *in situ* synthesis.

The extent to which organic materials progress through the three stages of decomposition outlined in the model will be controlled by their bioavailability. Bioavailability can be viewed as a function of both the chemical structure of the organic materials and the ability of the soil to protect the organic materials against microbial attack via organo-mineral interactions. In the case where no organo-mineral interactions occur, bioavailability is controlled completely by the chemical structure of the organic materials, and the extent of decomposition can continue through to the ultimate end product, carbon dioxide, provided the suite of microorganisms required to decompose the various organic materials are present and active. However, where bioavailability of the organic materials is reduced by an interaction with inorganic soil components (e.g. clays and polyvalent cations; see Oades (1988)), the extent to which the organic materials are decomposed may be limited.

The organic materials contained in the Oxisols had a lower O-alkyl, aromatic and carboxyl carbon content than that of the Mollisols or Andosol. The labile nature of O-alkyl carbon and the recalcitrant nature of alkyl carbon suggest that the bioavailability of the plant residues added to the Oxisols was high allowing decomposition to progress through the first two stages of the proposed model. The accumulation of alkyl carbon indicated that its bioavailability was low. The low bioavailability of the alkyl carbon resulted presumably from a combination of its chemical structure and its adsorption onto clay size particles.

The chemical structure of the organic materials contained in the Mollisols indicated that they were decomposed to a lesser extent that those contained in the Oxisols. The lower alkyl and higher aromatic carbon content suggested that decomposition was limited during the second stage of the proposed model. The high base status and the presence of active calcium carbonate in Mollisols leads to a stabilisation of organic materials against microbial attack via the formation of Ca^{2+}-organic complexes (Duchaufour 1976; Oades, 1988). Removing Ca^{2+}

from an organic soil has been shown to enhance the mineralisation of carbon and nitrogen (Gaiffe et al. 1984) and addition of calcium to a soil (as gypsum or agricultural lime) has been shown to decrease the proportion of ^{14}C-labelled organic substrate carbon mineralised (Muneer and Oades 1989a, b).

The extent of decomposition of the organic materials contained in the Andosol appeared to be more limited than that contained in the Mollisols. The decreased bioavailability of the organic materials in the Andosol has been attributed to the formation of Al^{3+}-organic complexes as discussed earlier.

On the basis of the solid-state CP/MAS ^{13}C NMR results acquired for the soils included in this study, a tentative ranking of the extent of decomposition of the soil organic fractions would be:

Andosol < Mollisols < Oxisols.

Conclusions

Solid-state CP/MAS ^{13}C NMR spectroscopy proved to be a useful technique for obtaining information about the chemical structure of the organic materials contained in mineral soils. The particle size and density fractionation scheme employed concentrated soil organic materials into specific fractions successfully.

In progressing from the coarsest particle size fraction to the finest, the following changes in the chemical structure of the organic materials contained in each fraction were noted for the Mollisols and Oxisols:

(1) carboxyl carbon content was variable with no consistent trend being observed across all of the soils studied,
(2) aromatic carbon content increased to a maximum in the intermediate fractions (2—53 μm diameter) and decreased in the clay fractions (< 2 μm diameter),
(3) O-alkyl carbon content decreased, and
(4) alkyl carbon content increased.

The structural changes were most pronounced for the Millicent Mollisol and resembled those acquired in other studies where CP/MAS ^{13}C NMR was used to monitor the chemical changes associated with the decomposition of natural organic materials. The changes in chemical composition and C:N ratio indicated that the organic materials contained in the finer particle size fractions were decomposed to a greater extent than those contained in the coarser fractions. A proposed mechanism of

selective preservation of the more recalcitrant aromatic and alkyl carbon, as O-alkyl carbon was decomposed by soil microorganisms, was able to account completely for the changes in the chemical structure of the organic materials in progressing from the coarse to intermediate particle size fractions. The further increase in alkyl carbon content in the clay fractions could not be explained on the basis of selective preservation alone. It is suggested that the accumulation of alkyl carbon in the soil clay fractions resulted from a combination of its selective preservation and *in situ* synthesis by microogranisms utilising the more labile organic structures. For the Andosol, the chemical structure of the organic materials contained in the particle size fractions was similar, indicating that the organic materials present in all particle size fractions were decomposed to a similar extent.

The chemical structure of the entire organic fraction contained in the soils included in this study was obtained by weighting the CP/MAS ^{13}C NMR results acquired for the particle size fractions on the basis of the carbon balance data collected from the fractionation procedure. The largest differences in chemical structure were noted between soil orders with the differences noted within orders being much smaller. However, previous studies indicated that the chemical structure of organic materials contained in different soils from the same order could differ significantly. The Mollisols and the Andosol were both dominated by O-alkyl carbon but the Andosol had a lower alkyl carbon content. The Oxisols were dominated by both O-alkyl and alkyl carbon.

The CP/MAS ^{13}C NMR results acquired in this study confirmed early proposals made by Waksman (1936) that the decomposition of plant materials involved an initial loss of carbohydrate (hemicellulose and cellulose) followed by the slow transformation of the aromatic structures of lignin molecules. Omitted from Waksman's proposal was the highly recalcitrant nature of alkyl carbon which, until recently, was not considered to be present in significant quantities in soils. Based on the results obtained from this study and material presented by Oades (1981) and Hatcher and Spiker (1988), a model of the oxidative decomposition of plant materials in soils which includes the following three successive stages of decomposition is proposed:

(1) loss of O-alkyl carbon (hemicellulose, cellulose and protein),
(2) exposure and subsequent decomposition of aromatic carbon (lignin), and
(3) a loss of the highly recalcitrant alkyl carbon.

The stage to which plant materials are decomposed in soils is controlled by the ability of the soil to limit the bioavailability of the plant

materials through the formation of organo-mineral complexes. The influence of soil order and particle size on the chemical structure of soil organic materials noted in this and previous studies could be explained in terms of bioavailability and the extent to which the organic materials were decomposed.

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Appendix 1. Distribution of soil particles and organic carbon in the particle size and density fractions isolated from the five soils included in this study.

Soil	Particle size (μm)	Particle density (Mg m^{-3})	Mass balance (%)	Organic[2] carbon content (%)	Organic[3] carbon balance (%)	C:N ratio
Millicent Mollisol	250—2000	≤2.0	0.2	30.0	2.0	39.6
		>2.0	2.6	1.0	0.7	16.8
	53—250	≤2.0	1.2	21.1	7.0	20.4
		>2.0	4.6	0.5	0.6	10.9
	20—53	≤2.0	0.1	13.9	0.6	17.1
		>2.0	4.8	0.4	0.5	13.5
	2—20	≤2.0	2.7	24.4	17.7	11.5
		>2.0	21.9	3.2	19.0	8.8
	0.2—2	N.A.[1]	18.9	5.5	28.1	8.1
	<0.2	N.A.[1]	26.1	2.6	18.5	7.5
	Recovery		83.2		94.7	
Henongjiang Mollisol	250—2000	≤2.0	0.1	36.0	1.1	18.3
		>2.0	0.4	0.8	0.1	13.0
	53—250	≤2.0	0.4	27.1	3.4	16.4
		>2.0	2.1	0.6	0.4	12.4
	20—53	≤2.0	1.0	7.6	2.4	15.2
		>2.0	29.4	0.1	1.3	9.3
	2—20	≤2.0	4.1	18.2	23.4	20.1
		>2.0	32.7	1.8	18.6	10.1
	0.2—2	N.A.[1]	19.6	5.6	34.2	10.7
	<0.2	N.A.[1]	9.4	3.9	11.6	9.9
	Recovery		99.2		96.4	
Mount Schank Andosol	250—2000	≤1.0	0.2	19.2	0.7	15.7
		>1.0	17.5	0.9	3.0	16.5
	53—250	≤1.0	0.1	20.4	0.4	11.3
		>1.0	23.7	2.5	11.1	13.7
	20—53	N.A.[1]	10.5	2.7	5.2	11.9
	2—20	N.A.[1]	33.3	8.7	53.4	13.5
	<2	N.A.[1]	12.6	10.9	25.5	10.1
	Recovery		97.8		99.4	
Malanda Oxisol	250—2000	≤2.0	0.5	48.9	4.6	30.5
		>2.0	1.5	2.3	0.6	15.1
	53—250	≤2.0	0.8	42.3	5.8	26.5
		>2.0	3.5	2.8	1.8	14.4
	20—53	≤2.0	0.2	34.4	1.3	29.0
		>2.0	5.6	2.0	2.1	18.9

Appendix 1 (Continued)

Soil	Particle size (μm)	Particle density (Mg m^{-3})	Mass balance (%)	Organic[2] carbon content (%)	Organic[3] carbon balance (%)	C:N ratio
	2—20	≤2.0	2.1	31.8	12.4	23.0
		>2.0	19.8	5.2	18.9	14.5
	0.2—2	N.A.[1]	14.5	6.3	17.0	10.7
	<0.2	N.A.[1]	41.1	3.1	23.9	11.7
	Recovery		89.5		88.5	
Guangdong Oxisol	250—2000	≤2.0	0.1	41.6	1.5	46.2
		>2.0	2.0	0.3	0.2	17.5
	53—250	≤2.0	0.2	38.8	2.6	27.5
		>2.0	11.1	0.9	4.6	15.3
	20—53	≤2.0	0.2	17.4	1.3	22.0
		>2.0	4.0	0.8	1.5	14.2
	2—20	≤2.0	0.5	23.6	5.4	24.8
		>2.0	11.5	2.6	13.8	14.7
	0.2—2	N.A.[1]	20.7	1.9	17.6	11.7
	<0.2	N.A.[1]	49.8	2.4	53.4	14.8
	Recovery		100.1		102.0	

[1] N.A. = not applicable since no density fractionation was performed on these fractions.

[2] Organic carbon contents refer to those measured after fractionation but prior to dithionite extraction.

[3] Organic carbon balance values refer to the percentage of the total soil organic carbon contained in each of the isolated fractions.

Appendix 2. Distribution of the total signal intensity collected in the carboxyl, aromatic, O-alkyl and alkyl spectral regions of the CP/MAS ^{13}C NMR spectra acquired for the particle size and density fractions analysed.

Soil	Fraction		Distribution of signal intensity (% total signal intensity)			
			Carboxyl	Aromatic	O-Alkyl	Alkyl
Millicent Mollisol	250—2000 μm,	< 2.0 Mg m^{-3}	6.3	16.0	56.7	21.0
	53—250 μm,	< 2.0 Mg m^{-3}	12.6	17.9	46.1	23.4
	20—53 μm,	< 2.0 Mg m^{-3}	13.7	21.4	42.1	22.8
	2—20 μm,	< 2.0 Mg m^{-3}	17.0	21.8	35.3	25.9
	0.2—2,	whole	17.4	20.1	36.6	26.0
	< 0.2,	whole	14.3	12.7	36.7	36.3
Henongjiang Mollisol	53—2000 μm,	< 2.0 Mg m^{-3}	11.6	23.6	49.1	15.6
	20—53 μm,	< 2.0 Mg m^{-3}	12.7	26.7	38.2	22.3
	2—20 μm,	< 2.0 Mg m^{-3}	16.3	33.8	32.3	17.5
	0.2—2,	whole	8.8	19.4	44.2	27.6
	< 0.2,	whole	17.3	17.9	42.6	22.2
Mount Schank Andosol	250—2000 μm,	< 2.0 Mg m^{-3}	11.7	25.8	45.0	17.5
	53—250 μm,	< 2.0 Mg m^{-3}	18.2	26.1	39.1	16.6
	2—20,	whole	18.6	25.8	39.4	16.2
	< 2,	whole	16.1	16.1	47.0	20.8
Malanda Oxisol	53—2000 μm,	< 2.0 Mg m^{-3}	10.5	27.7	38.8	23.0
	20—53 μm,	< 2.0 Mg m^{-3}	8.2	34.5	31.8	25.6
	2—20 μm,	< 2.0 Mg m^{-3}	14.7	27.5	30.0	27.8
	0.2—2,	whole	9.7	12.9	37.8	39.6
	< 0.2,	whole	8.9	11.9	33.1	46.2
Guangdong Oxisol	53—2000 μm,	< 2.0 Mg m^{-3}	6.8	24.6	42.8	26.2
	20—53 μm,	< 2.0 Mg m^{-3}	12.8	30.0	31.0	26.1
	2—20 μm,	< 2.0 Mg m^{-3}	11.3	30.9	26.6	29.9
	0.2—2,	whole	12.4	21.4	38.9	27.3
	< 0.2,	whole	9.2	14.9	39.3	36.6