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Recent advances in the application of ^{13}C and ^{15}N NMR spectroscopy to soil organic matter studies

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Abstract

Nuclear magnetic resonance (NMR) spectroscopy has been applied to many studies in soil science, geochemistry, and environmental science. In recent years, the study of soil organic matter (SOM) using NMR techniques has progressed rapidly. NMR spectroscopy has been used to study chemical changes of SOM during decomposition, and also of soil extract fractions such as humic acid and fulvic acid. NMR spectroscopy of soils has improved rapidly in recent years with the introduction of pre-treatment and particle-size fractionation. In addition to routine liquid- and solid-state ^{13}C NMR applications, ^{15}N NMR spectra of natural abundant samples have been reported, but ^{15}N-enriched material is more convenient to use due to the low natural abundance of ^{15}N. Some newly developed NMR techniques have also been utilised, such as 2-dimensional NMR spectroscopy and improved ^{1}H NMR techniques. These are reviewed and commented on in this paper.

Additional keywords: new NMR techniques, soil carbon pools, residue decomposition, magnetic materials, soil organic nitrogen.

Introduction

Nuclear magnetic resonance (NMR) spectroscopy has developed rapidly since the early 1960s. Recent advancements in NMR spectroscopy have allowed for the application of its versatile techniques to intact soils as well as fractionated samples (Stevenson and Elliott 1989; Preston 1996). Preston (1996) attributed the first application of NMR to soil organic matter (SOM) study to Barton and Schnitzer (1963), who ran ^{1}H NMR of a methylated humic acid. Early coverage of NMR applications to SOM studies can be found in Wershaw and Mikita (1987), Wilson (1987), Pfeffer and Gerasimowicz (1989), and Preston and Rusk (1990), and more recently in Fründ *et al.* (1994) and Preston (1996).

NMR spectroscopy can provide structural information about SOM by various techniques. Early NMR studies of SOM utilised solution techniques applied to humic materials (Anderson and Russell 1976; González-Vila *et al.* 1976; Wilson *et al.* 1978; Ogner 1979), but in these studies the largest fraction of SOM, the insoluble humin fraction, was by necessity ignored (Skjemstad *et al.* 1997). Fractionation of SOM into humic acid, fulvic acid, and humin involves lengthy extraction procedures and biological approaches, which generally extract only part (approximately 60–70%) of the SOM (Anderson *et al.* 1974; Kononova 1975). Also, with highly weathered tropical soils, only a small proportion of SOM is likely to be extracted because of the strong bonds between SOM and iron and aluminium oxides (Fox 1980; Lessa *et al.* 1996). Solid state NMR techniques, with which more information about SOM can be obtained, were first applied to SOM studies in the 1970s and have achieved great progress in recent years (Preston 1996) with the development of superconducting magnets, Fourier transform (FT) techniques, and cross-polarisation magic angle spinning (CP/MAS). Nevertheless, solution NMR is still useful, as advanced techniques can overcome old problems. Thus, the scope of NMR applications encompasses solutions, solids, and intermediate physical states, pure materials and mixtures, and a wide range of nuclei suitable for probing both organic and inorganic materials (Preston 1996).

This review is focused on the progress in ^{13}C and ^{15}N NMR studies of SOM in recent years. Our purpose is not to give a comprehensive review but, rather, a critical review. Emphasis is given to the applications of newly developed NMR techniques. For convenience, some important works before 1990 are also mentioned. Before the survey of the literature, fundamental concepts for SOM as well as NMR are briefly described.

Soil organic matter

SOM influences soil physical, chemical, and biological properties and processes, and is a major determinant of soil fertility, acting as a source and reservoir of nutrients for the biota, particularly in highly weathered soils of the tropics (Duxbury *et al.* 1989; Stevenson and Elliott 1989; Lessa *et al.* 1996). SOM and associated sediments comprise the major repository for organic carbon (C) and nitrogen (N) on the earth's surface (Stevenson and Kelley 1985), which, once converted by microbial decomposer communities, become the major nutrient source for plants. The relative size of the various SOM pools is determined by the nature of the vegetation, soil, environmental factors such as climate, topography, and physiology, and land-use practices (Anderson *et al.* 1974; Lessa *et al.* 1996).

SOM, by definition, consists of partially decayed plant residues that are no longer recognisable as plant material, microorganisms, and the byproducts of decomposition (Paul and Clark 1996). These byproducts undergo a process called humification to form the material known as humus. During the humification and mineralisation process, the starting plant material, which consists mainly of insoluble lignocellulosics (Knicker and Lüdemann 1995), is thoroughly converted by microorganisms. Simultaneously, stabilisation of the degradation products occurs, leading to the formation of recalcitrant SOM, which resists chemical modification for hundreds to several thousands of years (Hsieh 1992). This process may include the formation of aromatic heterocycles (Stevenson and Kelley 1985; Anderson *et al.* 1989). Numerous textbooks provide detailed information on the nature of SOM (Kononova 1966; Schnitzer and Khan 1972; Aiken *et al.* 1985; Greenland and Hayes 1985; Stevenson 1994; Paul and Clark 1996; Swift 1996).

Decomposition is defined as the alteration of the original chemical structure of an organic compound or tissue (Baldock *et al.* 1997) and, in terms of SOM, is characterised by gradual changes in C functional groups as determined by NMR spectroscopy. Changes can be detected as a function of decomposition time, down a gradient of litter and soil horizons, or as a function of size and density fractions. NMR also provides information on the carbon skeleton. Decomposition processes can be influenced by environmental factors such as temperature, nutrient status, and water content (Paul and Clark 1996; Lessa *et al*. 1996). Temperature and nutrient status exert their major influence by controlling decomposition rates, but also have been shown to affect lignin decomposition by fungi (Baldock and Preston 1995).

The major changes that occur in an NMR spectrum after decomposition of SOM are a decrease in carbohydrates, an increase in the relative proportion of alkyl (aliphatic chains) and carboxyl C, and partial breakdown of lignin (Baldock and Preston 1995). Mineralisation of inorganic nutrient elements also occurs as SOM decomposes, although relationships between these elements and ^{13}C NMR spectroscopy are not well researched. McColl and Gressel (1995) have reported that although there is clear evidence of differential changes in total phosphorus (P), organic P, and microbial P as litter decomposes, little is known of the relationship between changes in P species and changes in associated functional C groups as measured by ^{13}C NMR spectroscopy. However, Guinto *et al*. (1999) recently reported that changes in C functional groups determined by ^{13}C NMR spectroscopy are related to N mineralisation of native forest soils subjected to periodical fuel reduction burning.

NMR spectroscopy

NMR spectroscopy is a highly advanced technique that can be used to characterise SOM. The theory behind the NMR technique is not presented here since general accounts of the NMR theory for soil science and geochemistry applications can be found in Axelson (1985) and Wilson (1987, 1991). Stejskal and Memory (1994) also give a detailed account of the theory for high resolution NMR in the solid state, focused on CP/MAS. Many other textbooks on the general NMR theory are widely available such as Breitmaier and Voelter (1987), Ernst *et al.* (1987), Sanders and Hunter (1988), Werhli *et al.* (1988), and Mao (1996). Instead, a simplified explanation is presented below.

NMR is a form of spectroscopy that utilises the magnetic property, called spin, of a nucleus in an atom and an associated magnetic moment so that a resonance occurs at a characteristic frequency. Because every nuclear spin in a molecule senses also the small magnetic fields of its nearest neighbours, it is possible to separate the signals from different atomic surroundings, and these individual signals can be used to determine the structure of the molecule. In addition, the frequency of the pulse, which has to be in resonance with the spin system for energy transfer, is dependent on the nucleus to be investigated. Molecules may have magnetic nuclei precessing at slightly different frequencies representative of their different chemical environments. Thus, it is easy to choose the nucleus for study simply by changing the frequency of the pulse. The resulting time-dependent NMR signal is complex, but a Fourier transformation and computer processing of the data provide a NMR spectrum containing structural information.

So far in the application of NMR to SOM studies, simple 1-dimensional (1D) spectra have been measured and chemical shifts have been discussed. Chemical shifts are the most easily measured NMR parameters and carry important structural information (Wijmenga and van Buuren 1998). However, NMR spectroscopy gives not only the

chemical shift information, but also atomic connectivity (through J coupling) and dynamical behaviour (through relaxation and molecular self-diffusion). These properties should find application equal to chemical shift for SOM studies in the near future. Indeed, preliminary application has already commenced (Wang *et al.* 1998), while sophisticated methods, such as 2-dimensional multiple quantum spectroscopy (Wang *et al.* 1998) and solid state spectral editing (Mao *et al.* 1998), have also been used in humic acids and forest leaf litter investigations. These advanced techniques provide further structural information on the sample that simple 1D spectra are unable to reveal.

NMR application to SOM studies

^{13}C-NMR spectroscopy

Carbon atoms contained in different chemical structures within SOM are differentiated on the basis of chemical shift values. Each chemically distinct type of C has a characteristic chemical shift value. The signal intensity observed for a given chemical shift value, expressed as a fraction of the total signal intensity acquired, represents the proportion of that type of C present in a sample (Baldock and Preston 1995). Spectra can be roughly divided into chemical shift regions in which the chemistry of the C atoms within each region is similar. By integrating the signal intensity contained within each chemical shift region, the proportion of a given type of C can be calculated. Baldock and Preston (1995) give a review of chemical shift assignments for SOM of forest ecosystems.

For spectra with poor signal-to-noise ratios, it may be possible to delineate 4 such chemical shift regions corresponding to alkyl (0–50 ppm), O-alkyl (50–110 ppm), aromatic (110–160 ppm), and carbonyl (160–200 ppm) C atoms as shown in Fig. 1.
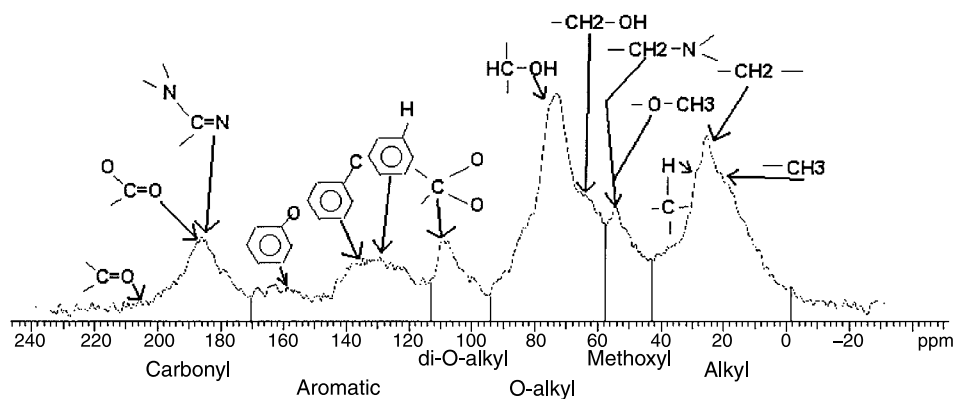

Fig. 1.   Chemical shift assignments for a ^{13}C CP/MAS NMR spectrum.

The name given to each region is general, and thought to be indicative of the dominant form of C present. It should be apparent that the proportions of each type of C obtained by this approach are only approximate, as there would undoubtedly be some overlap between adjacent regions. It should be noted that in solid state spectra of SOM, peak heights do not accurately reflect intensities of each type of C, because peaks can vary in width and shape. Also, peaks having equal integration area do not necessarily indicate the same number of spins, because the efficiency of cross-polarisation is not uniform for all molecular species.

Solution ^{13}C NMR spectroscopy

Solution NMR has not been widely utilised for SOM studies since the early 1980s because of the rapid advancement of solid state NMR techniques, and the relative insolubility of whole soils. Chemical fractionation of SOM into humic acid and fulvic acid can provide reasonably soluble fractions for solution NMR studies, despite not all SOM being contained within these 2 fractions. But Hatcher *et al.* (1981) commented that meaningful spectra of humic acids were difficult to obtain because of the limited solubility of these fractions in common NMR solvents. Therefore, solution ^{13}C NMR spectroscopy may not be quantitatively reliable in predicting the composition of SOM.

Common solvents used in liquid state ^{13}C NMR are NaOH and NaOD, and these appear to display no absorptions. The DMSO-d$_6$ provides a sharper spectrum, but the 30–40 ppm region is obscured unless the solvent is depleted in ^{13}C (Swift 1996). However, DMSO-d$_6$ has recently been used as a solvent for proton NMR studies (Wang *et al.* 1998) with encouraging results. There are a number of problems associated with liquid state ^{13}C NMR, such as the amount of sample required (100–200 mg), insolubility of some compounds in suitable solvents, interference from water in ^{1}H NMR, and the long analysis time required. In some cases, the dissolution process interferes with the analysis.

Humic fractionation schemes based on solubility have suggested that SOM is highly aromatic in nature (Hatcher *et al.* 1981). Although soils containing highly aromatic humus are recognised (Calderoni and Schnitzer 1984; Zech *et al.* 1989; Mahieu *et al.* 1999), most soils and soil fractions have been shown by solid state ^{13}C NMR spectroscopy to be relatively low in aromaticity (Wilson *et al.* 1981*a*, 1981*b*; Preston and Ripmeester 1982; Zech *et al.* 1992). By using ^{13}C CP/MAS NMR spectroscopy after photo-oxidation of some Australian soils in conjunction with scanning electron microscopy, Skjemstad *et al.* (1996) found that the majority of the aromatic peak at 130 ppm could be attributed to charcoal. This was found for both particle size fractions and the humic acid fraction. Soils with no charcoal content tended to be much lower in aromaticity.

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

Recent developments in solid state ^{13}C CP/MAS NMR spectroscopy have allowed for the application of this technique to whole soils as well as fractionated samples (Stevenson and Elliott 1989; Baldock *et al.* 1992; Preston 1996; Guinto *et al.* 1999; Mahieu *et al.* 1999; Nelson *et al.* 1999; Xing and Chen 1999; Xu *et al.* 1999). Solid state ^{13}C CP/MAS NMR spectroscopy has become an important tool for examining the chemical structure of natural organic materials (Mao *et al.* 1998) and chemical changes associated with decomposition (Baldock *et al.* 1997) of plant material and SOM. Unlike many other analytical techniques, solid state ^{13}C CP/MAS NMR spectroscopy is non-destructive, and does not depend on sample solubility, allowing for the examination of insoluble soil fractions as well as bulk soil samples. It is now common for soil samples to be fractionated based on particle size or density before solid state ^{13}C CP/MAS NMR spectra are obtained. Mahieu *et al.* (1999) conducted a statistical analysis of published ^{13}C CP/MAS NMR spectra of 311 whole soils, physical fractions (25 clay, 43 silt, and 52 sand-sized fractions), and chemical extracts (208 humic acids and 66 fulvic acids), covering a wide range of land use, climate, fertiliser, or manure application, and using different spectrometer characteristics and experimental conditions. From this comprehensive statistical analysis, Mahieu *et al.* (1999) found that functional groups in

the whole soils are always in the same abundance order: O-alkyls (mean 45%, increasing with soil C content), followed by alkyls (mean 25%), aromatics (mean 20%), and finally carbonyls (mean 10%, decreasing with soil C content); humic acids and fulvic acids contain much smaller proportions of O-alkyls (mean 26%) compared with the whole soils; and clay-size fractions differ significantly from the whole soils in terms of O-alkyl content, while sand-size fractions are generally similar to the whole soils.

However, there are a number of limitations associated with solid state ^{13}C CP/MAS NMR spectroscopy, including the low natural abundance of ^{13}C (about 1.1%) in SOM (Skjemstad *et al.* 1997), the low recoveries of many fractionation techniques, quantitation difficulties, and the presence of magnetic materials. An important factor, which must be considered when interpreting solid state ^{13}C NMR spectra, is whether or not the spectra reflect quantitatively the actual distribution of C types present in the sample (Baldock and Preston 1995). Paramagnetic species, including iron, copper, and nickel, can decrease the signal decay rate ($T_{1\rho}$H), leading to a loss of signal intensity, and quantitation is compromised if this loss of signal is not equal for all resonances in the spectrum (Smernik and Oades 1999). The ^{13}C CP/MAS NMR spectra are only quantitative when the following conditions are met (Wilson 1987; Pfeffer and Gerasimowicz 1989):

 (i) the rate of sample spinning is fast enough that no spinning sidebands are produced;

 (ii) the recycle delay period inserted between repetitive pulses is long enough to allow all protons to fully relax;

(iii) the rates of signal development (T_{CH}) and decay ($T_{1\rho}$H) during the contact time are similar for all types of C in the sample; and

(iv) $T_{CH} \ll T_{1\rho}$H.

In order to obtain quantitative data, it is essential that the contact time be optimised for each type of nuclei present. In practice, this is rarely possible and a compromise is made between build up (T_{CH}) and loss of magnetisation ($T_{1\rho}$H) for all nuclei (Skjemstad *et al.* 1997). If these differ profoundly between C functional groups, corrections can be made to the integrals for each C functional group, provided a contact time experiment is performed for each sample (Malcolm 1992). It is also important that all nuclei involved in the measurement be allowed to relax back to their equilibrium states between measurements, and recycle times should be at least 7 times longer than the longest T_1H in the sample (Wilson 1987). For soil and plant material, recycle times of 0.3–1.0 s are usually adequate, although longer recycle times may be sometimes necessary (Skjemstad *et al.* 1997). T. K. Pratum and R. S. Sletten (unpublished data) recently developed a spinning sideband correction technique that allows higher field strength instruments to be used with good signal-to-noise ratios. This eliminates the need for NMR laboratories to acquire a low-field NMR spectrometer with a specialised spinning assembly for solid samples.

In the cross-polarisation experiment, an additional problem is encountered. A C nucleus can only cross-polarise with protons that are in close proximity, probably within 4–5 bond lengths (Alemany *et al.* 1983). Carbon nuclei that are further away from protons cannot undergo cross-polarisation, and therefore cannot be observed in the cross-polarisation experiment (Snape *et al.* 1989). For samples that contain significant amounts of C isolated from protons, such as in charcoal, ^{13}C CP/MAS NMR spectra cannot give quantitative data. In this case, it is necessary to carry out a Bloch decay experiment, which does not utilise cross-polarisation, but magnetises the C nuclei directly (Skjemstad

et al. 1996, 1997). Relaxation now occurs through the ^{13}C spins so that theoretically all of the ^{13}C nuclei are observed. This technique still suffers from problems associated with free radicals and magnetic materials, and requires very long recycle times because of the typically long C T_1 rates. Recycle times of 30–90 s are usually required, leading to the collection of very few transients within a reasonable time frame, or very long accumulation times to acquire an acceptable signal-to-noise spectrum. Bloch decays of charcoal-containing soils have proven that the aromatic C may be underestimated by a factor of 30–50% with the application of the CP/MAS NMR technique (Skjemstad *et al.* 1996, 1997). However, this does not occur for soils that do not contain charcoal, and such errors in cross-polarisation data are then minimal (Skjemstad *et al.* 1997).

Hopkins and Chudek (1997) reported that the application of NMR to mineral soils is restricted by soil iron (Fe), copper (Cu), and manganese (Mn) contents because of their paramagnetic properties, which interfere with the NMR signals (Pfeffer *et al.* 1984; Preston *et al.* 1984) by severely reducing the proton rotating frame relaxation times ($T_{1\rho}H$), thereby reducing the signal-to-noise ratio of a spectrum, and, in severe cases, preventing a spectrum from being obtained at all (Smernik and Oades 1999). Smernik and Oades (1999) conducted cation exchange on samples of a de-ashed (HF-treated) soil with a series of paramagnetic (Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Pr^{3+}, Eu^{3+}) and non-paramagnetic (Na^+, Ca^{2+}, Zn^{2+}) ions. They found that exchange with non-paramagnetic ions does not affect the NMR properties of the SOM, but that a number of NMR properties including signal intensity, broadness, and relaxation time constants ($T_{1\rho}H$, T_1H, T_1C) are affected by the paramagnetic ions. They also showed that while paramagnetic species have been utilised extensively in solution NMR as both relaxation and shift reagents, the effect of paramagnetic species on solid state ^{13}C NMR has not been widely investigated (Aime *et al.* 1996).

There have been many reports on the adverse effects that paramagnetic species have on the NMR spectra (Chacko *et al.* 1983; Pfeffer *et al.* 1984; Vassallo *et al.* 1987; Smernik and Oades 1999), and the rectification of this problem by removing the paramagnetic material with various chemical treatments. The two most common treatments are (i) dithionite over a range of pH (Preston *et al.* 1984; Vassallo *et al.* 1987; Arshad *et al.* 1988; Skjemstad *et al.* 1992), and (ii) hydrofluoric acid (HF) solutions at varying concentrations (Calderoni and Schnitzer 1984; Skjemstad and Dalal 1987; Preston *et al.* 1989; Skjemstad *et al.* 1994; Schmidt *et al.* 1997).

Of these various techniques, Skjemstad *et al.* (1994) showed that treatment with 2% HF acid is the most effective and reliable. They found that this treatment could improve the relative visibility of C in a soil sample by as much as 25 times that of the best dithionite treatment, and it does not induce citrate contamination, unlike the dithionite treatment. Also, the HF treatment does not produce major structural changes in the composition of humic fractions (Preston *et al.* 1989), yet increases the sensitivity by removing a considerable portion of the inorganic matrix, which concentrates the C content of the sample (Skjemstad *et al.* 1997).

Examples from 3 different forest soil types of south-east Queensland (Mathers *et al.* unpublished data), demonstrating the improvement of the ^{13}C CP/MAS NMR spectrum after 2% HF treatment, are shown in Figs 2–4. All 3 figures demonstrate the ability of the HF treatment to increase the signal-to-noise ratios of the ^{13}C CP/MAS NMR spectra, thereby effectively reducing the NMR instrumental time. However, the degree of improvement differs for the 3 soil types due to the differences in the chemical composition of the SOM in the starting material.
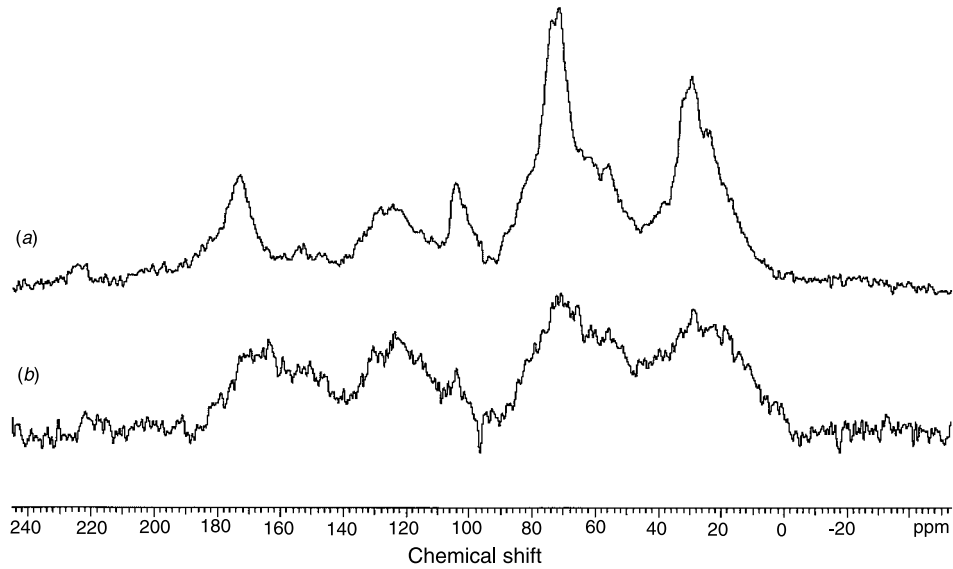
Fig. 2. ^{13}C CP/MAS NMR spectra of a surface soil (0–10 cm depth) from an area of the second-rotation hoop pine (*Araucaria cunninghamii*) plantation in south-east Queensland with the first-rotation tree harvesting residues windrowed: (*a*) HF-treated, 0.5 h, 1500 scans; and (*b*) untreated, 21 h, 100 000 scans.
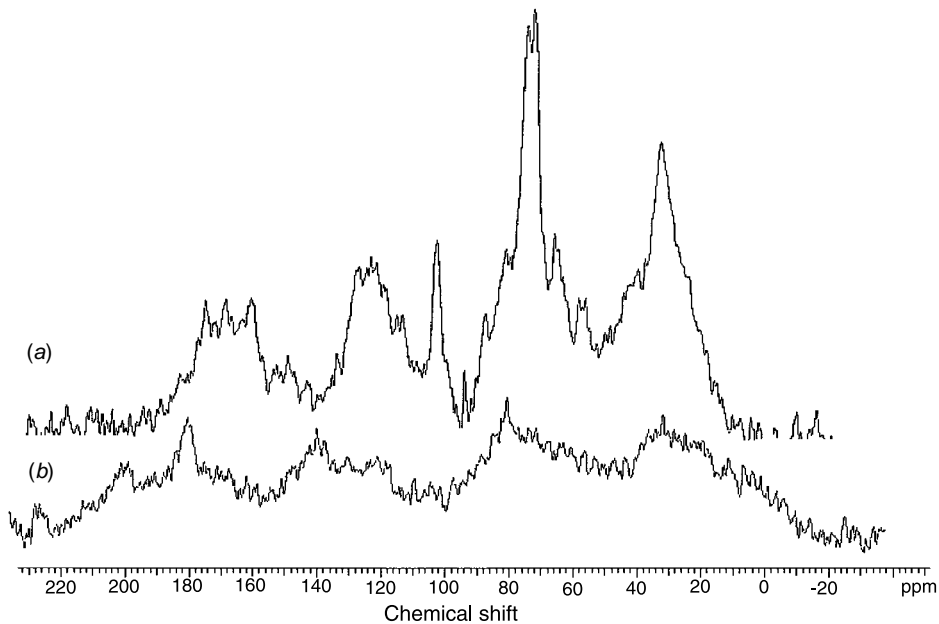

Fig. 3. ^{13}C CP/MAS NMR spectra of a surface soil (0–5 cm depth) from a biennially burnt site of the Blackbutt (*Eucalyptus pilularis*) dominated natural forest in south-east Queensland: (*a*) HF-treated, 5 h, 15 000 scans; and (*b*) untreated, 21 h, 18 000 scans.
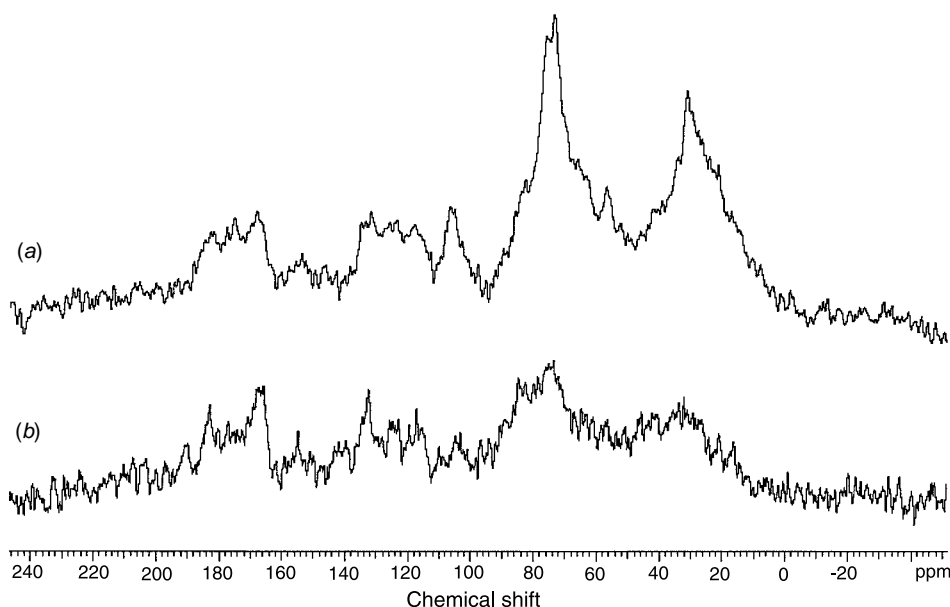
Fig. 4. ^{13}C CP/MAS NMR spectra of a surface soil (0–10 cm depth) from an area of the second-rotation exotic pine plantation of the F_1 hybrid between slash pine (*Pinus elliottii*) and Caribbean pine (*Pinus caribaea*) in south-east Queensland with double the amount of the first-rotation tree harvesting residues applied: (*a*) HF-treated, 5 h, 15 000 scans; and (*b*) untreated, 21 h, 18 000 scans.

Although the HF treatment can be very effective as illustrated above, it must be used with some caution since some soil organic materials are soluble in the HF acid. If this soluble fraction is significant, it could result in a NMR spectrum not representative of the SOM originally present in the sample. In general, the HF treatment (2%) is recommended for all mineral soil samples to be analysed using ^{13}C CP/MAS NMR spectroscopy.

Application of ^{13}C NMR to decomposition studies

Many laboratory studies on decomposition of plant residues have been made using solid state ^{13}C CP/MAS NMR spectroscopy to observe changes in organic chemistry during decomposition (Baldock and Preston 1995; Knicker and Lüdemann 1995; Trofymow *et al.* 1995; Lessa *et al.* 1996; Wershaw *et al.* 1996; Baldock *et al.* 1997; Preston *et al.* 1997; Mao *et al.* 1998). All these studies consistently demonstrate that the first materials to be affected are the polysaccharides, which decompose rapidly, while the alkyl, aromatic (aryl and *o*-aryl), and carbonyl materials decompose more slowly (Skjemstad *et al.* 1997). As reported by Baldock *et al.* (1997), the more readily available (labile) compounds and tissues contained in plant residues are decomposed by soil organisms first, and as decomposition proceeds, compounds with more recalcitrant chemical structures tend to accumulate.

Some studies attribute high alkyl contents in highly decomposed materials to selective preservation (Theng *et al.* 1992). However, Kögel-Knabner *et al.* (1992) and Zech *et al.* (1992) suggested that selective preservation is not the dominant process leading to the high alkyl content in SOM of forest soils, but rather an increase in cross-linking of the long-chained alkyl compounds occurring during humification. Guinto *et al.* (1999) and Xu *et al.* (1999) recently reported that O-alkyl C is rather sensitive to plant residue

management regimes, such as fuel reduction burning in natural forest ecosystems and residue retention in plantation forest ecosystems, and that this may be related to N mineralisation. Baldock and Preston (1995) found that, for forest litter, increases in alkyl C content were always associated with decreases in O-alkyl C (carbohydrate) content, and have thus proposed an index of decomposition (alkyl C/O-alkyl C). This is considered a more sensitive index than the commonly used aromaticity index (content of aromatic C expressed as a fraction of total organic C content). As the magnitude of the alkyl/O-alkyl ratio increases, the extent of decomposition also increases (Baldock and Preston 1995). However, this index should only be used to assess the extent of decomposition of organic materials where the organic materials are known to be derived from starting materials having a similar composition.

Baldock *et al.* (1997) also studied the decomposition of wood materials by ^{13}C CP/MAS NMR spectroscopy. They found that chemical changes associated with the decomposition of wood materials varied considerably, and were highly dependent on a strong interaction between the tree species examined and the species composition of the microbial decomposer community. Baldock and Preston (1995) also studied the decomposition of wood, and found little accumulation of alkyl C during aerobic or anaerobic conditions. This lack of alkyl C accumulation was thought to be a function of the low alkyl content of undecomposed wood and an inability of wood decomposer organisms to synthesise biologically stable alkyl structures. Because of the low contents of alkyl C in wood and differences in chemical changes being related to the species of wood and the microbial decomposer community, both studies concluded that the derivation of a single index for wood decomposition was unlikely.

Recent developments in the ^{13}C CP/MAS spectral editing technique have enabled the NMR spectrum to be edited into C-, CH-, CH$_2$-, and CH$_3$-subspectra according to their attached proton multiplicity (Mao *et al.* 1998). This technique can separate intensities of C functional groups attributed to different structures that are hidden by other C intensities in a normal CP/MAS NMR spectrum. Spectral editing has been performed on a number of materials including de-ashed soil (Smernik and Oades 1999), dried pine leaves (Mao *et al.* 1998), and dairy pond sludge (Newman and Condron 1995).

^{15}N NMR spectroscopy

NMR studies of N are limited because the most abundant N-stable isotope (^{14}N) has a large quadrupole moment, and thus it is almost impossible to obtain high resolution spectra from this nucleus (Witanowski and Webb 1993). Also, application of ^{15}N NMR spectroscopy to SOM studies may be difficult because of the extremely low natural abundance of ^{15}N (about 0.366%) and a small gyromagnetic ratio. Thus, sensitivity of a ^{15}N NMR experiment without sample enrichment is approximately 1/50 of that for a ^{13}C NMR spectrum (Knicker and Lüdemann 1995). This drawback can be used as an advantage to study interactions of N fertilisers or xenobiotic compounds with soil components. Enrichment of the compound of interest with ^{15}N enables good spectral resolution with minimal interference (noise) from background soil N, which is not enriched (McColl and Gressel 1995). By combining ^{15}N NMR with quantitative analyses of total N and ^{15}N and their recoveries as conventional forms of N, with pyrolysis, hydrolysis, and incubation experiments, a much needed insight into the nature and availability of organic N in soils should be gained (Preston 1996; Xu *et al.* 1999).

15N CP/MAS NMR spectroscopy

Natural abundance ^{15}N CP/MAS NMR spectra of 6 German soils after accumulation of approximately 1 million scans were obtained by Knicker *et al.* (1993), and also of fungal melanins and SOM (Knicker *et al.* 1995*a*) and coal (Knicker *et al.* 1995*b*). In the first 2 studies, Knicker *et al.* (1993, 1995*a*) found that almost all signal intensity was in the chemical shift region assigned to peptide/amide N. But this was a broad and intense signal of which >70% of the total intensity could cover less intense signals at the low-field side of the main peak originated from indoles, purine bases, and quinone imines. The main peak in the ^{15}N NMR spectrum of coal was assigned to pyrrolic N compounds. Knicker *et al.* (1995*a*) noted that, even under the most favourable conditions, aromatic heterocycles could not contribute more than 15% of the total intensity of fungal melanins, indicating they could not be major contributors to the refractory N in SOM. The absence of heterocyclic N could also be attributed to the lack of attached hydrogens, which diminishes the cross-polarisation process.

The ^{15}N NMR spectra were obtained by Knicker *et al.* (1995*a*) on a Bruker MSL-300 spectrometer at a frequency of 30.4 MHz, by applying a Hartmann–Hahn contact time between 0.7 s and 1 ms, and a pulse delay shorter than 500 ms, as a pulse delay of 500 ms would require approximately 1 week to obtain a solid state ^{15}N CP/MAS NMR spectrum. Unfortunately, many laboratories are unable to run 1 million transients on 1 sample, which could take more than a week, and therefore this technique is not generally available. For coal (Knicker *et al.* 1995*b*), the NMR parameters were a contact time of 1 ms, an acquisition time of 20 ms, and a pulse delay of 100 ms. About 1 500 000 scans were collected in this study, and a line broadening of 150 Hz was used. One major problem with ^{15}N NMR is that there is no universally accepted chemical shift standard and authors do not always report the standard used (Preston 1996). Knicker *et al.* (1995*a*) reported chemical shifts relative to nitromethane (= 0 ppm).

By pretreating the soil with 10% HF, Schmidt *et al.* (1997) were able to obtain reasonable ^{15}N CP/MAS NMR spectra after an accumulation of 500 000–770 000 scans. The enrichment of SOM after HF treatment was accompanied by a relative enhancement of the organic N content, and was sufficient to provide spectra with greatly improved signal-to-noise ratios. However, inorganic N was removed by the HF treatment (Schmidt *et al.* 1997). Other ^{15}N CP/MAS NMR studies have been applied to fossil algal residues (Knicker *et al.* 1996*a*), organic residues (Knicker *et al.* 1997), plant biomass (Knicker and Lüdemann 1995; Knicker *et al.* 1996*b*), and humic substances (Zhuo and Wen 1992, 1993; Zhuo *et al.* 1992), but these have all been conducted with ^{15}N-labelled material. This is considered a more viable option for solid state ^{15}N NMR spectroscopy, as these studies produced similar spectra depicting an amide/peptide peak accounting for more than 80% of the total N. By contrast, Zhuo and Wen (1993) used synthetic humic acids. Knicker and Lüdemann (1995) also discovered the presence of a new signal at 0 ppm, after 250 days of composting the plant material, and they assigned this to nitrate (NO_3^-) ions. They concluded that the decrease in intensity of the main amide peak during the composting period resulted from N mineralisation and the formation of nitrate ions due to nitrification of ammonium N.

It has long been known that a large part of soil organic N exists in an unknown form that is not accounted for by hydrolysis and analysis as ammonium, amino acids, or amino sugars. It may be that much of the unknown N is in amide forms that are resistant to hydrolysis (Preston 1996). This has been shown in a geochemical study of ^{15}N-enriched algaenan isolated from *Scenedesmus quadricauda* (Derenne *et al.* 1993). The [15]N

CP/MAS NMR spectra of the algaenan were very similar to those obtained for soil and soil fractions, with amides being the most abundant form of N (70%) with minor amounts of amines and pyrroles. The amides were largely eliminated by pyrolysis at 400°C, with the formation of *n*-alkylnitriles, and these amide groups were not hydrolysed by the acidic hydrolyses used for algaenan isolation. Stearic hindrance within the macromolecular network was suggested for their resistance to hydrolysis (Derenne *et al.* 1993).

Similar approaches can be applied to soils to monitor the effects of hydrolysis or pyrolysis (Preston 1996). Knicker and Lüdemann (1995) and Zhuo *et al.* (1992) compared the ^{15}N CP/MAS NMR spectra of ^{15}N-enriched SOM (compost and HA, respectively) and its residue after hydrolysis with 6 M HCl. In both cases, the amide peak was reduced substantially by hydrolysis and did not reveal the presence or formation of new heterocyclic N. Zhuo *et al.* (1992) concluded that much of the unknown N was in the form of amide, aromatic and/or aliphatic amines, and pyrrole-type N, with some of this material being resistant to hydrolysis. Of the hydrolysable material, some was released as ammonium or as forms that could not be determined, such as simple amino acids and amino sugars. They also reported that a very small proportion of the hydrolysable N might be transformed into heterocyclic N forms during hydrolysis, but if this is occurring it is too small to be detected in the NMR spectra.

Solution ^{15}N NMR spectroscopy

Solution ^{15}N NMR studies of whole SOM have not been achieved because of the relative insolubility of SOM. However, some studies (Thorn and Mikita 1992; Derenne *et al.* 1993; Knicker and Lüdemann 1995) have been performed on SOM fractions and their extracts. These studies may be inconclusive, and only semi-quantitative, as they will not account for the total N contained in SOM, as is the case for organic C in SOM. Knicker and Lüdemann (1995) obtained high-resolution solution ^{15}N NMR spectra of the NaOH extracts of enriched plant material after measured periods of microbial degradation. These extracts were obtained during the fractionation of the plant material into humin, humic acid, and fulvic acid. The solution spectra were obtained using a 10 mm variable frequency probe head on a Bruker MSL-300 spectrometer at a frequency of 30.4 MHz. The standard pulse program used by Knicker and Lüdemann (1995) was the Ridegate sequence (Gerothanassis 1986), which effectively suppresses the acoustic ringing. The protons were decoupled by the inverse gated broadband decoupling scheme in order to suppress the nuclear Overhauser enhancement (nOe) that could introduce intensity distortions.

In solution ^{15}N NMR studies, magnetic saturation effects cannot be observed if the time between radiofrequency (RF) pulses is larger than 5 times T_1 N-15 (Knicker and Lüdemann 1995). In order to avoid saturation effects in the spectra, the T_1 N-15 was determined by the normal inversion recovery pulse sequence. The spectra of the NaOH extracts, which had lost approximately 80% of the total C and N during incubation, did not show any indication of N-containing peptide/amide structures in heterocyclic compounds as expected. So far, not one ^{15}N NMR study has shown that N might be contained in heterocyclic compounds.

Other NMR techniques

There are numerous other techniques besides ^{13}C and ^{15}N CP/MAS and solution NMR spectroscopy that may be applied to SOM and its fractions to provide further structural

information. These include ^{1}H (proton) and ^{31}P NMR studies. Also, diffusion measurements with pulse field gradients (PFG) and 2D NMR spectroscopy are proving to be useful tools in NMR analysis (Randall *et al.* 1997; Wang *et al.* 1998). By using newly developed solution ^{1}H NMR techniques, more structural information can be deciphered from the soluble fractions of SOM than in previous ^{1}H NMR studies. The ^{1}H NMR spectrum of humic acid is often obscured by the large signal overlap. This is why proton NMR has become less favoured over other nuclei such as ^{13}C and ^{15}N in humic acid studies. However, when an advanced technique such as maximum quantum spectroscopy (MAXY) is used, proton NMR can reveal considerably more structural information (Wang *et al.* 1998) by removing the signal overlap.

Diffusion measurements using pulsed field gradient

The widely used NMR method to measure diffusion coefficients PFG (pulsed field gradient) spin-echo depends on the relationship between the signal attenuation and the square of the gradient area $\gamma g\delta$. Here γ is the gyromagnetic ratio, g is the gradient amplitude, and δ is the gradient duration. The self-diffusion coefficients are measured typically by a series of ^{1}H spectra recorded with a longitudinal eddy-current delay modified bipolar pulse pairs (LED–BPP) pulse sequence (Wang *et al.* 1998) with the corresponding 16-step phase cycling (Wu *et al.* 1995). Using normal 1D ^{1}H NMR spectroscopy on a soil humic acid, Wang *et al.* (1998) observed some sharp peaks over the broad resonances in different regions. They put forward 2 possible explanations for the sharp peaks:

 (i) they are from the flexible side chains in the humic polymers; or

(ii) they are from some specific components with low molecular weight which are not part of the humic structure.

To gain a better understanding of these peaks and possibly their origin, self-diffusion coefficients for the broad resonances in different regions and some typically sharp peaks were measured by Wang *et al.* (1998) using PFG with a LED–BPP sequence. They discovered that the gradient pulse attenuates the sharp signals very quickly, implying that they have relatively larger diffusion coefficients. The large self-diffusion coefficients suggest the existence of some species which are not aggregated with other components (Wang *et al.* 1998). They concluded that (based on the data obtained) the sharp peaks could not be assigned to the side chains of polymers in the humic acids, but may instead be produced from a labile component generated by hydrolysis, possibly assigned to formate ions and acetic ions. This agreed with the conclusions reached by Wilson *et al.* (1983, 1988) and Shin and Moon (1996).

Self-diffusion methods by the conventional PFG spin-echo method based on ^{13}C observations are rather scarce and have actually only been employed in rare instances when ^{13}C was the only possible probe for obtaining such information (Malveau *et al.* 1998). There are two problems encountered when using ^{13}C PFG experiments:

 (i) the low natural abundance which may cause sensitivity limitations, and

(ii) the efficiency of the gradient which, in practice, is lowered by a factor of 4 (with respect to proton measurements), because it acts through the expression γg, where g is the gradient amplitude (independent of the observed nuclei in the case of static field gradients) and γ the gyromagnetic ratio.

To date, ^{13}C PFG spin-echo NMR spectroscopy has not been applied to SOM or its soluble fractions, but would be able to provide enhanced structural and dynamical information.

Two-dimensional spectroscopy

One of the major advances in NMR spectroscopy has been the production of spectra which are in 2 frequency dimensions. These are simply referred to as 2D spectra (Ernst *et al.* 1987). Randall *et al.* (1997) made the first attempt to apply 2D NMR spectroscopy to SOM studies, with high field on a Bruker AMX 600 spectrometer at 14.1 T, but this has been mainly confined to ^{1}H and ^{13}C NMR on samples in solution. Newly developed 2D NMR techniques include totally correlated spectroscopy (TOCSY) and maximum quantum spectroscopy (MAXY). Many assignment problems can be simplified by using 2-dimensional 1-bond heteronuclear correlation spectra in which the 2 frequency coordinates of a resonance are the chemical shifts of a heteronucleus and a proton that are scalar-coupled through 1 bond (Palmer *et al.* 1991). These methods were used for solution proton NMR of a Japanese soil humic acid by Wang *et al.* (1998).

Wang *et al.* (1998) used totally correlated spectroscopy (TOCSY) to analyse a soil humic acid in different NMR solvents. The TOCSY spectra were measured under a spin-locking strength of $\gamma B_{SL}/2\pi \approx 8$ kHz and with a mixing time of about 40 ms. They found that for the sample dissolved in DMSO-d$_6$ many correlation peaks appeared between 4.0 and 8.0 ppm, suggesting the existence of polypeptides. These peaks were not observed in the spectrum of the humic acid dissolved in NaOH. This was attributed to the fact that exchange between mobile protons and water in NaOH solution is so fast that mobile protons cannot be observed. Although the TOCSY method was very useful in determining some structural information of the humic acid, the application of advanced pulse sequences was attempted to extract more information from the ^{1}H NMR spectra (Wang *et al.* 1998).

The newly developed MAXY–COSY (Liu *et al.* 1995) technique, which is based on the possible maximum quantum level in CH$_n$ (n = 1, 2, 3) groups and can be used to discriminate different CH$_n$ groups conveniently, was used by Wang *et al.* (1998) together with MAXY ^{1}H spectral editing. Each spectrum is divided into 3 parts with different diagonals and slopes for single quantum resonances (middle region), double quantum resonances (top region), and triple quantum resonances (lower region). The ^{13}CH$_2$ double quantum resonances appear in the top region, the middle region originates from the single quantum of the ^{13}CH and ^{13}CH$_3$ groups, and the lowest region originates from the 3-quantum resonances of ^{13}CH$_3$ groups (Wang *et al.* 1998). In a soil humic acid sample, they discovered some peaks attributed to methyl and methene that were invisible in normal 1-dimensional spectra because of presaturation.

The biological applications of high resolution NMR spectroscopy continue to be limited by the need to study complex systems such as SOM and its fractions from which only small quantities of sample can be obtained or where samples have low solubilities (Cavanagh and Rance 1990; Palmer *et al.* 1991). Consequently, maximisation of the inherent sensitivity of any NMR experiment is of paramount importance. Sensitivity improvement has been achieved in a number of studies (Cavanagh and Rance 1990; Palmer *et al.* 1991; Grzesiek and Bax 1995; Kover *et al.* 1998; Uhrin *et al.* 1998; Zhu *et al.* 1998), but these techniques have yet to be applied to SOM or their soluble fractions, with the exceptions of Randall *et al.* (1997) and Wang *et al.* (1998). Further research in

the application of 2D NMR spectroscopy is required before these techniques can be effectively applied to SOM studies.

Conclusions

NMR spectroscopy has proved to be a useful tool for the examination of SOM and soil N dynamics by using whole soil and organic matter fractions as well as related organic materials. Solid state ^{13}C and ^{15}N NMR spectroscopy have become the preferred methods of NMR studies because of the recent, rapid advances in the technique, and also because of the relative insolubility of soil and associated sediments for solution studies. To increase the sensitivity and reduce the signal-to-noise ratio of NMR spectra, HF pre-treatment of soil has proved to be the most effective method, as there is no contamination or major structural change of the sample and the SOM is concentrated with the removal of the inorganic matrix. For ^{15}N NMR spectroscopy, ^{15}N-enriched materials are commonly used, as more than 1 week may be needed to obtain a NMR spectrum at natural abundance with a good signal-to-noise ratio. There is still plenty of scope for improvement of N NMR spectroscopy, as ^{14}N NMR spectroscopy has been relatively untouched for SOM studies and the potential of ^{15}N NMR spectroscopy has not been fully exploited. The correlation of C and N NMR studies with conventional chemistry and biological techniques for SOM and related organic materials has also been ignored until recently. Newly developed solution 1D and 2D NMR techniques have been recently applied to the soluble humic acid fraction of SOM to provide structural and dynamical information that was not accessible in previous solution NMR studies.

Acknowledgments

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