FOREST RESIDUE MANAGEMENT AFFECTS SOIL NITROGEN AVAILABILITY AND HUMIC ACID COMPOSITION

N. J. MATHERS*, Z. H. XU

CRC for Sustainable Production Forestry,
Australian School of Environmental Studies, Griffith University,
Kessels Rd, Nathan, Queensland 4111, Australia

and S. E. BOYD

Magnetic Resonance Facility, School of Science, Griffith University,
Kessels Rd, Nathan, Queensland 4111, Australia

ABSTRACT

Soil humic substances are important components of soil organic matter and contain a significant portion of total soil organic carbon (C) and nitrogen (N). Solid-state ^{13}C nuclear magnetic resonance (NMR) with cross-polarisation and magic angle spinning (CPMAS) was applied to humic acids extracted from 0–10 cm soils collected from areas under windrows of harvest residues and those areas between the windrows, 3 years after implementation of residue management in a second-rotation plantation of *Araucaria cunninghamii* Ait. ex D.Don (hoop pine). In addition, nitrogen availability of under-windrow and between-windrow soils was also assayed by anaerobic incubation with either water or ^{15}N-labelled ammonium sulphate solution in the laboratory. The NMR spectra of the humic acids showed that the carbon composition of the under-windrow humic acids was different to that of the between-windrow humic acids. Potentially mineralisable nitrogen of the under-windrow soils was greater than that of the between-windrow soils, as was gross nitrogen mineralisation (ammonification, m_g). Soil potentially mineralisable nitrogen was also positively correlated with humic acid-alkyl and humic acid-O-alkyl carbon (p<0.05), while gross nitrogen mineralisation was positively correlated with humc acid-aromatic carbon (p<0.01). The gross nitrogen mineralisation was 33–45 mg N/kg dry soil as determined by isotope dilution with ^{15}N-labelled ammonium sulphate (100 mg N/kg and 99 atom% ^{15}N excess) and was greater in under-windrow than between-windrow soil after the 7-day anaerobic incubation. In addition, gross ^{15}N immobilised (NH_4^+ consumption, m_i) was positively correlated with humic acid-aromatic carbon (p<0.05). Humic acid-iron content was positively correlated with humic acid-alkyl and O-alkyl carbon (p<0.05).

Keywords: humic acid; ^{13}C cross-polarisation and magic angle spinning; nuclear magnetic resonance; forest soil; harvest residues; soil nitrogen availability; *Araucaria cunninghamii*.

* Present address: Department of Natural Resources and Mines Block B, Natural Resource Sciences 80 Meiers Rd, Indooroopilly, Queensland 4068, Australia

INTRODUCTION

Mineralisation of soil nitrogen is recognised as one of the main pools of "available" nitrogen in many forest systems and is an important part of soil nitrogen cycling (Keeney 1980). An improved understanding of soil nitrogen availability and carbon pools under different harvest residue management regimes is necessary for sustainable management of forest plantations, especially in the context of growing global demand for forest-based products and increasing expectations that forests may act as potential sinks for carbon dioxide, with managed forests having a particularly high potential to sequester large amounts of carbon (Sanchez *et al.* 2003). Soil organic matter (OM) is the principal source and sink of plant nutrients in both natural and managed forests, with 95% of nitrogen in surface soils found in the organic matter (Fisher & Binkley 2000), while plant residues contribute the largest fraction of organic carbon to organic matter (Paul & Clark 1996). The organic matter, or humus, consists of two major types of compounds: non-humic substances and humic substances. Humic substances represent the most active fraction of humus and consist of complex, high-molecular weight, polymeric mixtures referred to as humic acid, fulvic acid, and humin (Stevenson & Cole 1999). The non-humic materials consist of organic compounds including carbohydrates, fats, waxes, and proteins (Stevenson 1994) and may be covalently bonded to humic substances. In situ organic matter is therefore a complicated, intertwined network of humic and non-humic materials adsorbed on to mineral components and containing complexed metal ions (Davies *et al.* 2001; Jansen *et al.* 1996). The low solubility and complex chemical structure of humic substances make them difficult to study in situ (Schnitzer 1991); however, nuclear magnetic resonance spectroscopy is non-destructive and can show differences in the carbon composition of soil humic substances without necessarily displaying an overall difference in total soil organic carbon content (Almendros *et al.* 1992).

Solid-state ^{13}C NMR spectroscopy, using cross-polarisation with magic angle spinning, has been increasingly applied to the study of forest soil humic acids (Zech *et al.* 1997; Dai *et al.* 2001). The limited solubility of humic substances in suitable NMR solvents and the need for longer acquisition times for solution-state ^{13}C NMR spectroscopy have led to the widespread use of solid-state ^{13}C cross-polarisation with magic angle spinning NMR spectroscopy for the study of organic matter and humic substances (Monteil-Rivera *et al.* 2000). Carbon-13 cross-polarisation with magic angle spinning NMR differentiates the ^{13}C nuclei based on their chemical environments, giving an indication of the overall carbon composition. Carbon atoms contained in different chemical environments within the organic matter are differentiated based on chemical shift values, which characterise distinct types of carbon. 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 carbon present in a given sample (Baldock & Preston 1995). The ^{13}C cross-polarisation with magic angle spinning NMR spectra can be divided into chemical shift regions in which the chemistry of the carbon atoms within each region is similar, and by integrating the signal intensity contained within each chemical shift region, the proportion of a given type of carbon can be calculated. However, this analytical approach is only semi-quantitative (Mathers *et al.* 2000), but useful when comparing soils of similar origin analysed under identical NMR operating conditions (Baldock & Preston 1995). More recently, Conte *et al.* (2002) revealed that ^{13}C cross-polarisation with magic angle spinning NMR spectroscopy provided a quantitative representation of the whole carbon content in humic substances.

The Queensland Department of Primary Industries-Forestry currently manages approximately 45 000 ha of native hoop pine plantations in south-east Queensland, Australia. Large areas of mature first-rotation hoop pine plantations are currently being harvested and the traditional practice of burning harvest residues (slash materials) has been responsible for a considerable loss in soil fertility between rotations (Holt & Spain 1986; Bubb *et al.* 1999; Pu *et al.* 2001). Forest management practices now require that clearfall harvest residues be retained on-site wherever possible during the inter-rotation period, and the preferred practice is to windrow the harvest residues into heaps about 10–15 m apart (Pu *et al.* 2001). Squire *et al.* (1991) stated that clearfall harvest residue retention, rather than windrow burning, was more likely to maintain or improve soil organic matter quantity and quality, minimise soil compaction, conserve nutrients, and prevent soil degradation due to soil erosion on steep slopes. New hoop pine plantations are increasingly being established on second-rotation sites because fewer new areas are being cleared for first-rotation plantations. However, few studies have examined the effects of site management practices, such as retention or removal of harvest residues, on soil organic matter composition and quality, or nitrogen availability in second-rotation hoop pine plantations (Bubb *et al.* 1998; Mathers *et al.* 2003; Blumfield 1998). The objective of this study was to examine the impacts of harvest residue management regimes on soil nitrogen availability assayed by anaerobic incubation methods, and the chemical composition of soil humic acids, using ^{13}C cross-polarisation with magic angle spinning NMR spectroscopy, in a hoop pine plantation 3 years after the adoption of harvest residue management practices in south-east Queensland, Australia.

MATERIALS AND METHODS
Experimental Site and Soil Sampling

The experimental site for this study was located in the Imbil State Forest, south-east Queensland, Australia (26°31'S, 152°38'E), and has been described previously by Prasolova *et al.* (2000), Pu *et al.* (2001), and Mathers *et al.* (2003). Briefly, the site is situated about 100–300 m above sea level in the coastal highlands of the upper Mary River. The mean annual rainfall is 1200 mm, and the region is characterised by a subtropical climate, having a cool dry winter and a warm wet summer. The second-rotation site was planted with native hoop pine seedlings between windrows (*ca* 2.5 m wide, spaced 10 m apart) of harvest residues retained from the clearfall harvest of the first-rotation hoop pine plantation. Windrows were created immediately after the clearfall harvest of the first-rotation, 3 years prior to sample collection. The two areas used for this NMR study (under windrows and between windrows) were chosen on the assumption that there might be differences in soil humic acid composition because the large patches of bare ground between the windrows would create different soil conditions from those under the windrows. These differences might affect the amount of soil nitrogen available to the hoop pine seedlings during the early years of plantation establishment.

Soil samples were collected from nine transects (1.2 m apart) by bulking four augured cores (internal diameter = 7.5 cm) from the 0–10 cm soil layer in April 1998 as described by Mathers *et al.* (2003). Transects were 10 m in length, beginning at one windrow and ending at the next so as to include the inter-windrow space, and the transects were 1.2 m apart. Therefore, two bulked samples (one directly beneath the windrow and one between

the two windrows) were taken in each of the nine transects, creating nine replicates. The soil is classified as a Ferrosol under the Australian Classification System (Isbell 1996) or Oxisol (Soil Survey Staff 1999). The site was part of a larger experiment and plots included two windrows (Blumfield 1998; Pu *et al.* 2001).

Sample Preparation, and Chemical and Statistical Analyses

After collection, soil samples were placed in airtight polyethylene bags and transported back to the laboratory where they were air-dried immediately. The soil samples for each harvest residue treatment were sieved (<2 mm) for anaerobic incubations. Sub-samples were taken and ground to pass through a 0.14-mm sieve before soil chemical analysis. Available soil phosphorus (extracted with 0.1 M sulphuric acid and then analysed by the molybdate blue spectroscopic method), cation exchange capacity, pH (1:5 H_2O), and total phosphorus and potassium were analysed as described by Xu *et al.* (1995). Soil organic carbon was determined by the Walkley-Black method (Rayment & Higginson 1992). Total nitrogen content was determined by isotope ratio mass spectrometry on a Roboprep CN (7001)/Tracermass System (9001) manufactured by Europa Scientific as reported by Bubb *et al.* (1999). Soil particle size was analysed by the method of Gee & Bauder (1986).

Soil potential mineralisable nitrogen was determined by the 7-day anaerobic incubation method as reported by Waring & Bremner (1964). Briefly, four portions (5 g) of soil were weighed into 50-ml polypropylene Falcon centrifuge tubes, to which 25 ml of water were added. Two tubes were mixed and the soil was extracted with 25 ml of 4 M KCl less than 0.5 h after the addition of water, shaken end-over-end for 1 h, centrifuged at 2000 rpm for 10 min, and filtered through Whatman 42 filter paper. The other two tubes were mixed, sealed tightly, and incubated at 40°C for 7 days. After incubation, the soil-solution mix was also extracted with 25 ml of 4 M KCl as described previously. Exchangeable NH_4^+-N (mineral nitrogen) in extracts was determined by flow injection analysis using a Lachat QuikChem8000 automated ion analyser (QuikChem method 10-107-064-D for NH_4^+). This procedure was duplicated using ^{15}N-labelled ammonium sulphate solution (100 mg N/kg and 99 atom% ^{15}N) instead of water. The potential mineralisable nitrogen was determined as the difference in mineral nitrogen of the potassium chloride extracts before and after incubation. Soil gross nitrogen mineralisation and immobilisation rates in the 0–10 cm soil were measured using ^{15}N isotope dilution techniques (Hart *et al.* 1994) and steam distillation (Keeney & Nelson 1982; Chen *et al.* 2002). The distillates were transferred into 8 × 5-mm tin (Sn) capsules and dried at 60°C, before determination of $^{15}NH_4^+$-N on an Isoprime isotope ratio mass spectrometer with a Eurovector elemental analyser (Isoprime-EuroEA 3000). Duplicate samples of the incubated soils were extracted for their humic acids.

Soil humic acid was extracted in duplicate with 0.5 M NaOH following the International Humic Substances Society method (Swift 1996). Humic acids were de-ashed (purified) during the extraction procedure with a hydrochloric acid/hydrofluoric acid solution, which has been proven to successfully reduce ash in humic acids without significantly modifying their chemical composition or structure (Sánchez-Monedero *et al.* 2002). The humic acid total nitrogen, total carbon, and ^{15}N analyses were determined before and after incubation on an Isoprime isotope ratio mass spectrometer with a Eurovector elemental analyser (Isoprime-EuroEA 3000), but only the incubated results are presented in this paper.

Chemical analyses were performed on all replicates, but humic acids were bulked to provide enough material for solid-state ^{13}C cross-polarisation with magic angle spinning NMR. In order to detect paramagnetic materials that might interfere with the cross-polarisation process in the NMR experiment, hydrochloric acid-extractable aluminium and iron (Schmidt *et al.* 1997) were determined on soil humic acids after extracting *ca* 0.5 mg oven-dry humic acid overnight with 25 ml 1 M HCl, by flame atomic absorption spectrophotometry on a Varian SpectrAA 10/20 spectrophotometer (Mathers *et al.* 2002). All statistical analyses, including Pearson linear correlations, were performed using the STATISTICA software package (StatSoft 1999).

NMR Spectroscopy

Solid-state ^{13}C cross-polarisation with magic angle spinning NMR spectra of water-incubated and ammonium sulphate-incubated soil humic acids were obtained on a Varian Unity Inova400 spectrometer operating at a frequency of 100.6 MHz. Samples were packed in a silicon nitride rotor (outside diameter = 7 mm) and spun at 5 kHz at the magic angle. Single contact times of 2 ms were applied (as determined by variable contact time experiments), with an acquisition time of 14 ms and a recycle delay of 2 s. Approximately 20 000 transients were collected for each humic acid and a line-broadening value of 50 Hz was applied to all spectra. An attempt was made to remove spinning sidebands with the total suppression of sidebands pulse sequence (Dixon 1985), but the spectra (not shown here) were unreadable. Therefore, corrections were incorporated into the standard ^{13}C cross-polarisation with magic angle spinning NMR spectra for the appearance of spinning sidebands from the carbonyl carbon spectral region after integration. Carbon-13 chemical shift values were referenced externally to hexamethylbenzene at 132.1 ppm, which is equivalent to tetramethylsilane at 0 ppm.

The ^{13}C cross-polarisation with magic angle spinning NMR spectra were divided into the four common chemical shift regions: alkyl carbon (0–50 ppm), O-alkyl carbon (50–118 ppm), aromatic carbon (118–165 ppm), and carbonyl carbon (165–220 ppm), and relative intensities for each region were determined by integration using the Varian NMR software package (Version 6.1c, Varian Inc., CA). In some instances it was necessary to further divide some chemical shift regions, these were: O-alkyl carbon into methoxyl carbon (50–65 ppm), carbohydrate carbon (65–100 ppm), and di-O-alkyl carbon (100–118 ppm); aromatic carbon into aryl carbon (118–144 ppm) and phenolic carbon (144–165 ppm); and carbonyl carbon into carboxyl, amide, and ester carbon (165–185 ppm) and ketone/aldehyde carbon (185–220 ppm). The carboxyl and ketone spectral regions both produced spinning sidebands, which appear on the low-field side of the centreband at approximately 223 and 240 ppm respectively. Because spinning sidebands of equal intensity are known to appear on either side of the originating centreband (i.e., also at 123 and 140 ppm), the visible spinning sideband was integrated and the aromatic, carboxyl, and ketone/aldehyde spectral regions were all corrected for the presence of spinning sidebands.

An index of the extent of decomposition, the alkyl carbon/O-alkyl carbon ratio (A/O-A, Equation 1), has been recommended by Baldock & Preston (1995) after concluding that the aromaticity index proposed by Hatcher *et al.* (1981) did not necessarily increase as the extent of decomposition increased, but that the alkyl carbon spectral region increased concomitantly with a decrease in the O-alkyl carbon spectral region. Webster *et al.* (2001)

have also suggested that the overall resource quality of soil carbon as a substrate for heterotrophic microbes may be inversely related to the A/O-A ratio.

$$A/O\text{-}A = \frac{\text{Alkyl C region intensity (0--50 ppm)}}{\text{O-alkyl C region intensity (50--118 ppm)}} \qquad (1)$$

Aromaticity (Equation 2) has been used to characterise the extent of humification of soil organic matter, under the assumption that soil organic matter becomes aromatic during decomposition (Dai *et al*. 2001). Carbons in carboxylic groups (-COOH) are omitted from the aromaticity calculation because it is not known how many -COOH groups are bonded to aromatic structures and how many to aliphatic structures (Schnitzer 1991).

$$\text{Aromaticity (\%)} = \left[\frac{\text{Aromatic carbon (118--165 ppm)}}{\text{Aromatic carbon + Alkyl carbon + O-alkyl carbon (0--165 ppm)}} \right] \times 100 \qquad (2)$$

However, recent solution ^{13}C NMR studies have shown that humic acid, the most important stable soil organic matter pool exists with a highly aliphatic nature as well as an aromatic nature (Conte *et al.* 1997; Guggenberger & Zech 1999). Therefore, both of these decomposition indices were used in this study.

RESULTS

Soil Chemical and Biological Properties

The initial soil chemical and physical properties before ^{15}N-labelling, anaerobic incubation, or humic acid extraction for soil organic matter from under-windrow and between-windrow soils are given in Table 1. Organic carbon and the carbon/nitrogen ratio were both greater in under-windrow soil as reported by Mathers *et al.* (2003), but other chemical and physical properties were similar in the contrasting treatments. Biological and chemical properties of the whole soil and the extracted soil humic acids are shown in Table 2. Potential mineralisable nitrogen, as determined by anaerobic incubation, was

TABLE 1–Chemical and physical properties of surface soils (0–10 cm depth) under two harvest residue management regimes in the 3-year-old hoop pine plantation.

	Under windrows	Between windrows
Chemical properties		
pH (1:5 H$_2$O)	5.57	5.24
Organic C (g/kg)	39.5	34.6
Total N (g/kg)	2.50	2.64
C/N ratio	15.8	13.1
Total K (mg/kg)	4275	5188
Total P (mg/kg)	643	663
Available P (mg/kg)	52.5	52.2
Cation exchange capacity (cmol/kg)	30.7	26.7
HCl-extractable Fe (mg/kg)	5338	5775
Physical properties		
Clay (%)	32	34
Silt (%)	28	28
Coarse sand (%)	20	21
Fine sand (%)	20	17

TABLE 2–Potential mineralisable nitrogen (PMN), gross nitrogen mineralisation (m_g) and immobilisation (m_i) of 0–10 cm soil samples, and ^{15}N enrichment, total nitrogen, and total carbon of soil humic acids under two contrasting residue management regimes in a 3-year-old hoop pine plantation. Mean ± standard errors, n=2.

Residue management treatment*	PMN (mg N/kg)	PMN (%)	m_g (mg N/kg)	m_i (mg N/kg)	HA-atom% non-incubated	^{15}N excess incubated	HA-Total N (g/kg)	HA-Total C (g/kg)
Incubation with water								
UW	144.8±4.6	5.79±0.18	–	–	0	0	30.5±3.5	450.6±49.1
BW	89.4±0.9	3.39±0.03	–	–	0	0	35.5±1.8	498.8±7.5
Incubation with ^{15}N-labelled ammonium sulphate solution								
UW	130.5±15.3	5.22±0.61	45.3±1.2	32.4±1.2	1.39±0.11	2.32±0.12	28.8±0.1	492.2±7.6
BW	104.3±10.0	3.95±0.38	33.4±6.8	38.1±11.8	1.06±0.40	1.85±0.06	32.6±0.8	497.0±25.3

* UW: soil samples collected from areas under the windrows of hoop pine harvest residues; BW: soil samples from areas between the windrows.

greater in under-windrow (131–145 mg N/kg) than between-windrow soil (89–104 mg N/kg) for both incubation methods. Humic acids treated briefly (*ca* 0.5 h) with ^{15}N-labelled ammonium sulphate produced an excess of ^{15}N, with the ^{15}N enrichment greater in humic acids from under-windrow (1.40 atom% ^{15}N excess) than between-windrow humic acids (1.06 atom% ^{15}N excess). This trend repeated itself in the incubated humic acids (2.32 atom% ^{15}N excess and 1.85 atom% ^{15}N excess for under windrow and between windrow, respectively), and the ^{15}N enrichment of both samples was greater than the non-incubated samples (Table 2).

The chemical properties of extracted humic acids after incubation with either water or ammonium sulphate are given in Table 2. The total nitrogen content was consistently less in the humic acids from under windrows than humic acids from between windrows, while total carbon showed no trends with incubation treatment. Gross ^{15}N mineralisation (m_g) was greater in under-windrow soil (45 mg N/kg) than between-windrow soil (33 mg N/kg); yet gross ^{15}N immobilisation (m_i) was greater in between-windrow soil (38 mg N/kg) than under-windrow soil (32 mg N/kg). Total hydrochloric acid-extractable aluminium and iron contents are given in Table 3 for soil humic acid from both harvest residue management treatments after incubation with either water or ammonium sulphate. Hydrochloric acid-extractable iron and aluminiun contents were similar for both under windrow and between windrow in the water-treated humic acids, even after de-ashing with hydrochloric acid/ hydrofluoric acid. In the ammonium sulphate-treated humic acids, hydrochloric acid-extractable iron content was greater in the under windrow than in the between windrow. The reverse was true for hydrochloric acid-extractable aluminium with between windrow having a greater aluminium content than under windrow (Table 3).

TABLE 3–Total chloroform-extractable aluminium and iron contents in soil humic acids from the two harvest residue management treatments. Mean ± standard error, n=2.

Residue management treatment*	HCl-extractable Fe (mg/kg)	HCl-extractable Al (mg/kg)
Incubation with water		
UW	1370±420	2985±1125
BW	1040±30	2635±25
Incubation with ^{15}N-labelled ammonium sulphate solution		
UW	1140±10	2190±30
BW	950±20	3280±60

* UW: soil samples collected from areas under the windrows of hoop pine harvest residues; BW: soil samples from areas between the windrows.

Carbon-13 cross-polarisation magic angle spinning NMR spectroscopy

Water-treated humic acid

Integration data from the ^{13}C cross-polarisation and magic angle spinning NMR spectra of the water-treated hunic acids extracted from under-windrow and between-windrow soils are displayed in Table 4 and the spectra in Fig. 1. The incubated humic acid from under-windrow soil had a greater proportion of aliphatic (alkyl and O-alkyl carbon) structures than that from between-windrow soil. This was supported by the reduced aromaticity (16%) of

TABLE 4–Influences of harvest residue management on the relative intensities (%) for ^{13}C cross-polarisation and magic angle spinning NMR spectral regions (after integration and correction for spinning sidebands), A/O-A ratio*, and aromaticities[†] of soil humic acids.

NMR chemical shift regions		Incubation with water		Incubation with ^{15}N-labelled ammonium sulphate	
		Under windrows	Between windrows	Under windrows	Between windrows
Main regions	**Sub-regions**				
Alkyl C		38.2	28.6	33.9	30.1
O-alkyl C	Methoxyl C	6.6	0.0	6.2	4.4
	Carbohydrate	11.7	16.3	10.4	11.7
	Di-O-alkyl C	1.3	-0.1	2.0	0.0
Aromatic C	Aryl C	9.3	9.4	13.8	13.9
	Phenolic C	1.4	2.5	3.8	2.6
Carbonyl C	Carboxyl C	14.2	28.1	17.2	18.4
	Ketone/aldehyde C	15.7	15.6	12.8	19.4
NMR indices of decomposition / humification					
A/O-A ratio*		2.0	1.8	1.8	1.9
Aromaticity[†]		15.6	21.0	25.1	26.4

* A/O-A ratio is the alkyl C/O-alkyl carbon ratio (Baldock *et al.* 1997), where O-alkyl carbon includes the methoxyl, carbohydrate, and di-O-alkyl carbon regions.
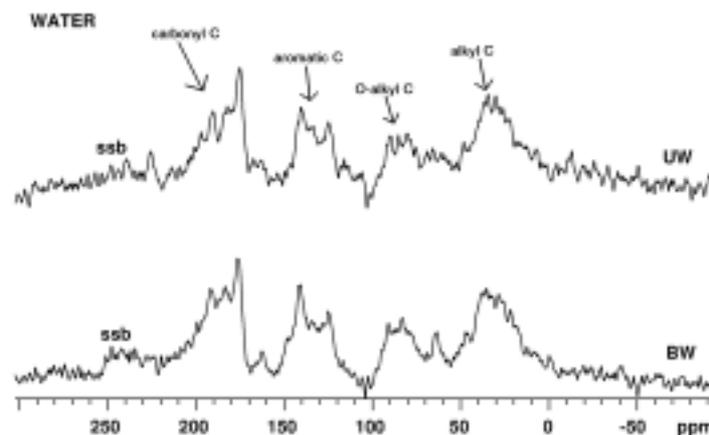[†] Aromaticity (%) was determined following Hatcher *et al.* (1981) and Schnitzer (1991).

FIG. 1–Carbon-13 cross polarisation and magic angle spinning NMR spectra of soil humic acid extracted from under the windrows (UW) and between the windrows (BW), incubated with water (ssb = spinning sidebands).

under-windrow humic acid compared with between-windrow humic acid (21%), although there was little difference in aromatic carbon intensity (Table 4). The between-windrow humic acid was dominated by the carbonyl carbon intensity, with 28% carbon attributed to carboxyl, amide, and ester carbon and 16% to ketone and aldehyde carbon (Table 4). The under-windrow humic acid had much less intensity in the carboxyl, amide, and ester region (14%) than between-windrow humic acid and this region may be associated with the alkyl carbon region.

Ammonium sulphate-treated humic acid

Integration data from the ^{13}C cross-polarisation and magic angle spinning NMR spectra of the ammonium sulphate-treated humic acids extracted from under-windrow and between-windrow soils are also displayed in Table 4 and the spectra in Fig. 2. The incubated under-windrow humic acid had a greater proportion of aliphatic structures than between-windrow humic acid (Table 4). The ketone/aldehyde spectral regions showed an increase in relative intensity in between-windrow humic acid compared with under-windrow humic acid (Table 4). The aromaticity and A/O-A ratio both showed little difference between humic acid taken from either under-windrow or between-windrow soils (Table 4).
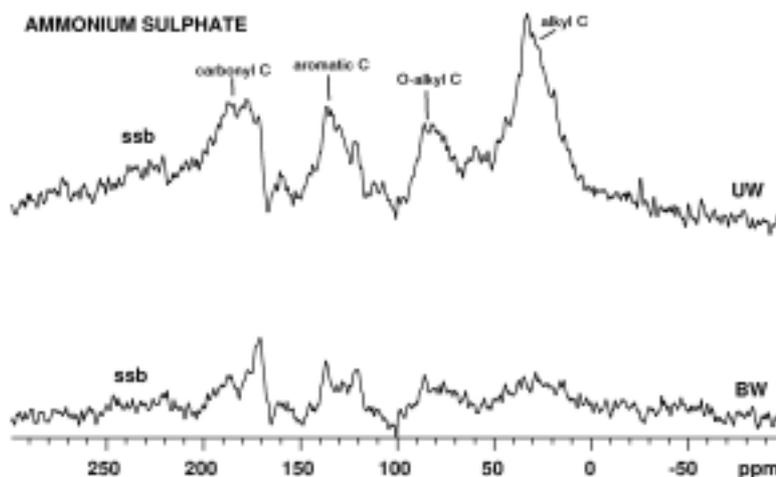

FIG. 2–Carbon-13 cross polarisation and magic angle spinning NMR spectra of soil humic acid extracted from under the windrows (UW) and between the windrows (BW), incubated with ammonium sulphate (ssb = spinning sidebands).

Correlations Between the Variables Measured

Simple linear correlations were performed on all ammonium sulphate- and water-treated humic acids, and a simplified matrix is shown in Table 5. Humic acid-aromatic carbon was positively correlated to gross soil nitrogen mineralisation ($p<0.01$) and immobilisation ($p<0.05$). Soil potentially mineralisable nitrogen was positively correlated to humic acid-alkyl and humic acid-O-alkyl carbon ($p<0.05$), but negatively correlated to humic acid-carbonyl carbon ($p<0.05$). The humic acid-O-alkyl carbon was positively correlated with hydrochloride-extractable iron ($p<0.05$), as was the humic acid-alkyl carbon spectral region ($p<0.05$).

DISCUSSION

Major cellulose and carbohydrate peaks that dominate the whole soil NMR spectra (Mathers *et al.* 2003) were rarely visible in the humic acid spectra, because these are non-humic materials and would not normally be extracted with the humic acid (Stevenson 1994). In both incubation methods (water and ammonium sulphate), the under-windrow

TABLE 5—Simple linear correlation matrix of humic acid ^{13}C cross-polarisation and magic angle spinning NMR spectral regions, and soil chemical and biological data.

	Potentially mineralisable nitrogen	m_g	m_i	Total humic acid-carbon	Total humic acid-nitrogen	Alkyl carbon	O-alkyl carbon	Aromatic carbon	Carbonyl carbon	A/O-A ratio	Aromaticity (%)
m_g	-0.154										
m_i	-0.301	0.963*									
Total HA-C	-0.865	0.590	0.646								
Total HA-N	-0.847	-0.326	-0.115	0.467							
Alkyl C	0.979*	-0.352	-0.482	-0.942	-0.734						
O-alkyl C	0.957*	-0.279	-0.469	-0.841	-0.817	0.964*					
Aromatic C	-0.067	0.990**	0.965*	-0.494	-0.370	-0.267	-0.226				
Carbonyl C	-0.966*	-0.107	0.051	-0.716	0.937	-0.893	-0.891	-0.191			
A/O-A Ratio	0.748	-0.345	-0.304	-0.876	-0.361	0.782	0.589	-0.209	-0.662		
Aromaticity (%)	-0.445	0.938	0.987*	0.759	0.015	-0.614	-0.586	0.921	0.202	-0.433	
HCl-extractable Fe	0.889	-0.556	-0.699	-0.932	-0.602	0.956*	0.952*	-0.500	-0.749	0.657	-0.798

* Correlation significant at $p<0.05$
** Correlation significant at $p<0.01$

humic acid displayed increased intensity in the alkyl carbon region (Table 4) compared to that for between-windrow humic acid. This suggests that the under-windrow humic acid alkyl carbon may be composed of plant biomacromolecules, such as cutin, suberin, and waxes, from the retained harvest residues. It has been suggested previously that selective preservation of resistant aliphatic biomacromolecules by soil microbes might be responsible for the increase in alkyl carbon during decomposition and humification of soil organic matter (Theng *et al*. 1992), but an increase in cross-linking of long-chained alkyl structures during humification has also been reported for this increase (Zech *et al*. 1992). Jansen *et al*. (1996) reported that chemical crosslinks can be formed in humic acid from oxidation and condensation chemistry and may require metal co-ordination at certain chemically active sites. More recently, Chen *et al*. (2003) observed a significant negative relationship between alkyl carbon and chloroform-released carbon, indicating that increased microbial biomass reduced the proportion of alkyl carbon in the NMR spectra; this is in contrast to these previous studies. The reduced alkyl carbon intensity in between-windrow humic acid is most likely the result of a lack of readily available plant residues accessible to the soil microbial biomass for decomposition during the incubation period, because no added residues were initially applied in the form of windrows.

The existence of metals such as iron, aluminium, manganese, and magnesium in humic acids has been well documented (Schnitzer 1991; Schulten & Schnitzer 1998), but attempts by Mao *et al*. (2002) to correlate hunic acid nitrate-nitrogen with any of these metal elements failed. Humic acids have high metal-binding capacities and can selectively bind, store, and release metals (Davies *et al*. 2001). The humic acid-alkyl carbon and humic acid-O-alkyl carbon intensities of this study were both correlated with the hydrochloric acid-extractable iron content of the humic acids (*see also* Mathers *et al*. 2002), but these correlations could also be due to a decrease in the aromatic region if iron was preferentially complexed to aromatic moieties. This relationship suggests that iron may be bound to humic acids in carbohydrates and long or short branched-chain polymethylene structures where crosslinks have formed, requiring metal co-ordination (Jansen *et al*. 1996). However, because only 86% of iron could be removed from these soils (Mathers *et al*. 2002), it is possible that the greater iron concentrations for both under-windrow humic acids could result in a greater suppression of the aromatic region leading to proportionally greater alkyl and O-alkyl carbon signals.

The carboxyl/carbonyl carbon region (165–220 ppm) was divided into two regions corresponding to carboxylic, amide, and ester carbon (centred at about 174 ppm), and ketone and aldehyde groups (centred at 190 ppm). The clear separation of these two regions in the ammonium sulphate-treated humic acid may be a result of the applied ^{15}N-labelled fertiliser contributing to increased gross nitrogen mineralisation and increasing the decarboxylation of aliphatic and aromatic components. The unclear separation of the ketone and carboxylic peaks in the water-treated between-windrow humic acid could be due to the occurrence of another peak in this humic acid, between the carboxylic and ketone peaks, centred at 182 ppm. The appearance of this peak may be an indication of increased decomposition and humification, but no general trends were clear.

The ^{13}C cross-polarisation and magic angle spinning NMR spectra of water-incubated humic acid from both under-windrow and between-windrow soils displayed resonances at

approximately 124 and 142 ppm. These signals most probably correspond to by-products of lignin decomposition, as lignin is recognised as a precursor to humic acid and coal formation (Stevenson 1994). This suggests that some less-recalcitrant aromatic structures may be utilised by soil microbes in the synthesis and preservation of alkyl carbon structures in the water-treated humic acids, and is supported by a reduction in the aromaticities of the water-treated humic acids compared with those for ammonium sulphate-treated humic acid (Table 4). Preston *et al.* (1994) reported that "young" humic acid from a secondary-growth *Pseudotsuga menziesii* (Mirb.) Franco (Douglas fir) forest showed a relatively sharp alkyl carbon region and some of the original features of lignin. Considering that these were new plantations established on second-rotation sites, the soil organic matter, particularly the humic acid fraction, could begin to undergo influences more similar to those exhibited by cultivated agricultural soils than by unmanaged old- or secondary-growth forests. Chen *et al.* (2003) reported that the A/O-A ratio was negatively correlated to chloroform-released carbon (p<0.01) indicating that higher microbial biomass produced less alkyl carbon, leading to a reduced extent of decomposition. However, in this study of humic acid there was no significant difference between the incubation treatments and the residue treatments in the A/O-A ratios.

Nitrogen mineralisation is the release of ammonium-nitrogen during the decomposition of organic matter by soil microbes (Powlson & Barraclough 1993). The gross nitrogen mineralisation results (Table 2) indicated that nitrogen turnover was faster where added harvest residues were formed into windrows, suggesting an increased microbial activity. This was supported by the relationship between gross nitrogen mineralisation and humic acid-aromatic carbon (p<0.01). With increased decomposition and humification, accumulation of aromatic carbon is expected, and these results support this assumption. This may be the result of a number of factors due to the incubation, including increased stimulation of microbes with the addition of ^{15}N in the anaerobic incubation and a different microbial community structure in under-windrow soil compared to between-windrow soil because of the differences in soil temperatures due to a lack of protection. The gross nitrogen mineralisation rates in this study were lower, particularly in between-windrow soil, than those reported by Chen *et al.* (2002) for a 13-year-old hoop pine plantation that was also located in a different climatic region. The correlation matrix in Table 5 indicates that gross nitrogen immobilisation was positively correlated to humic acid-aromatic carbon and the aromaticity index (p<0.05) and this may also be an indication of increased mineralisation and humification by the microbial community. The potentially mineralisable nitrogen was always greater in under-windrow than between-windrow soil, but these values were greater in both areas than those reported by Blumfield (1998). The potentially mineralisable nitrogen was also significantly correlated to the humic acid-alkyl carbon and humic acid-O-alkyl carbon, as was chloroform-extractable iron, indicating that potentially mineralisable nitrogen may also play a role in binding metals to humic acid, although potentially mineralisable nitrogen was not directly correlated to the chloroform-extractable iron. Gross mineralisation and immobilisation rates formed a positive relationship (p<0.05, Table 5), which has also been reported in a number of recent mineralisation studies (Verchot *et al.* 2001; Compton & Boone 2002), but the rates of gross nitrogen immobilisation may be over-estimated by this method because of substrate stimulation (Hart *et al.* 1994). The humic acid-aromatic carbon region could also be directly related to gross nitrogen mineralisation as suggested by Guinto *et al.* (1999).

CONCLUSIONS

Incubation method and subsequent extraction of soil humic acid by the traditional fractionation scheme indicated that carbon composition of soil humic acid was altered due to the contrasting harvest residue management regimes imposed on the soil. In under-windrow soil where harvest residues were retained, soil potentially mineralisable nitrogen increased and decomposition processes, as determined by the A/O-A ratio, declined due to the addition of labile organic matter, as compared with between-windrow soil. Solid-state ^{13}C cross-polarisation and magic angle spinning NMR spectroscopy of the most stable soil organic matter component, humic acid, revealed that differences existed in its chemical composition under contrasting residue treatments, regardless of incubation method. Gross nitrogen mineralisation was greater in under-windrow than between-windrow soil, but the reverse was true for immobilisation. Both gross mineralisation and immobilisation were positively related to humic acid-aromatic carbon, while potentially mineralisable nitrogen was positively related to humic acid-alkyl carbon and humic acid-O-alkyl carbon, but negatively correlated to humic acid-carbonyl carbon. Generally, the retention of harvest residues in young hoop pine plantations is likely to increase soil fertility and nitrogen availability in the next rotation.

ACKNOWLEDGMENTS

REFERENCES

ALMENDROS, G.; GONZÁLEZ-VILA, F.J.; MARTÍN, F.; FRÜND, R.; LÜDEMANN, H.-D. 1992: Solid state NMR studies of fire-induced changes in the structure of humic substances. *Science of the Total Environment 117/118*: 63–74.

BALDOCK, J.A.; PRESTON, C.M. 1995: Chemistry of carbon decomposition processes in forests as revealed by solid-state carbon-13 nuclear magnetic resonance. Pp.89–117 *in* McFee, W.W.; Kelly, J.M. (Ed.) "Carbon Forms and Functions in Forest Soils". Soil Science Society of America, Madison, WI.

BLUMFIELD, T.J. 1998: The impact of residue management techniques in hoop pine plantations of south east Queensland. Honours Thesis, Griffith University, Brisbane, Queensland, Australia.

BUBB, K.A.; XU, Z.H.; SIMPSON, J.A.; SAFFIGNA, P.G. 1998: Some nutrient dynamics associated with litterfall and litter decomposition in hoop pine plantations of southeast Queensland, Australia. *Forest Ecology and Management 110*: 343–352.

——1999: Growth response to fertilisation and recovery of N-15-labelled fertiliser by young hoop pine plantations of subtropical Australia. *Nutrient Cycling in Agroecosystems 54*: 81–92.

CHEN, C.R.; XU, Z.H.; HUGHES, J.M. 2002: Effects of nitrogen fertilisation on soil nitrogen pools and microbial properties in a hoop pine (*Araucaria cunninghamii*) plantation in southeast Queensland, Australia. *Biology and Fertility of Soils 36*: 276–283.

CHEN, C.R.; XU, Z.H.; MATHERS, N.J. 2004: Soil carbon pools in adjacent natural and plantation forests of subtropical Australia. *Soil Science Society of America Journal 68*: 282–291.

COMPTON, J.E.; BOONE, R.D. 2002: Soil nitrogen transformations and the role of light fraction organic matter in forest soils. *Soil Biology and Biochemistry 34*: 933–943.

CONTE, P.; PICCOLO, A.; VAN LAGEN, B.; BUURMAN, P.; DE JAGER, P.A. 1997: Quantitative differences in evaluating soil humic substances by liquid- and solid-state ^{13}C-NMR spectroscopy. *Geoderma 80*: 339–352.

CONTE, P.; PICCOLO, A.; VAN LAGEN, B.; BUURMAN, P.; HEMMINGA, M.A. 2002: Elemental quantification of natural organic matter by CPMAS ^{13}C NMR spectroscopy. *Solid State NMR 21*: 158–170.

DAI, K.H.; JOHNSON, C.E.; DRISCOLL, C.T. 2001: Organic matter chemistry and dynamics in clear-cut and unmanaged hardwood forest ecosystems. *Biogeochemistry 54*: 51–83.

DAVIES, G.; GHABBOUR, E.A.; STEELINK, C. 2001: Humic acids: Marvellous products of soil chemistry. *Journal of Chemical Education 78*: 1609–1614.

DIXON, W.T. 1985: Pulse sequence for spinning sideband suppression in spectra of quadrupolar nuclei. *Journal of Magnetic Resonance 64*: 332–333.

FISHER, R.F.; BINKLEY, D. 2000: "Ecology and Management of Forest Soils", third edition. John Wiley and Sons, Inc., New York, USA. 155 p.

GEE, G.W.; BAUDER, J.W. 1986: Particle-size analysis. Pp.383–411 *in* Klute, A. (Ed) "Methods of Soil Analysis, Part 1: Physical and Mineralogical Methods". Soil Science Society of America, Madison, WI.

GUGGENBERGER, G.; ZECH, W. 1999: Soil organic matter composition under primary forest, pasture, and secondary forest succession, Región Huetar Norte, Costa Rica. *Forest Ecology and Management 124*: 93–104.

GUINTO, D.F.; SAFFIGNA, P.G.; XU, Z.H.; HOUSE, A.P.N.; PERERA, M.C.S. 1999: Soil nitrogen mineralisation and organic matter composition revealed by ^{13}C NMR spectroscopy under repeated prescribed burning in eucalypt forests of south-east Queensland. *Australian Journal of Soil Research 37*: 123–135.

HART, S.C.; STARK, J.M.; DAVIDSON, E.A.; FIRESTONE, M.K. 1994: Nitrogen mineralisation, immobilisation and nitrification. Pp.985–999 *in* Weaver, R.W.; Angle, J.S.; Bottomley, P.S. (Ed.) "Methods of Soil Analysis. Part 2. Microbiological and biochemical properties". Soil Science Society of America, Madison, WI.

HATCHER, P.G.; SCHNITZER, M.; DENNIS, L.W.; MACIEL, G.E. 1981: Aromaticity of humic substances in soils. *Soil Science Society of America Journal 45*: 1089–1094.

HOLT, J.A.; SPAIN, A.V. 1986: Some biological and chemical changes in a north Queensland soil following replacement of forest with *Araucaria cunninghamii* (Coniferae: Araucariaceae). *Journal of Applied Ecology 23*: 227–237.

ISBELL, R.F. 1996: "The Australian Soil Classification". CSIRO Publishing, Australia.

JANSEN, S.; PACIOLLA, M.; GHABBOUR, E.; DAVIES, G.; VARNUM, J.M. 1996: The role of metal complexation in the solubility and stability of humic acid. *Materials and Science Engineering C4*: 181–187.

KEENEY, D.R. 1980: Prediction of soil nitrogen availability in forest ecosystems: a literature review. *Forest Science 26*: 159–171.

KEENEY, D.R.; NELSON, D.W. 1982: Nitrogen — inorganic forms. Pp.643–698 *in* Page, A.L.; Miller, R.H.; Keeney, D.R. (Ed.) "Methods of Soil Analysis. Part 2: Chemical and Microbial Properties". Soil Science Society of America, Madison, WI.

MAO, X.A.; XU, Z.H.; LUO, R.S.; MATHERS, N.J.; ZHANG, Y.H.; SAFFIGNA, P.G. 2002: Nitrate in soil humic acids revealed by nitrogen-14 nuclear magnetic resonance spectroscopy. *Australian Journal of Soil Research 40*: 717–726.

MATHERS, N.J.; XU, Z.H.; BERNERS-PRICE, S.J.; PERERA, M.C.S.; SAFFIGNA, P.G. 2002: Hydrofluoric acid pre-treatment for improving ^{13}C CPMAS NMR spectral quality of forest soils in south-east Queensland, Australia. *Australian Journal of Soil Research 40*: 655–674.

MATHERS, N.J.; XU, Z.H.; BLUMFIELD, T.J.; BERNERS-PRICE, S.J.; SAFFIGNA, P.G. 2003: Composition and quality of harvest residues and soil organic matter under windrow residue management in young hoop pine plantations as revealed by solid-state ^{13}C NMR spectroscopy. *Forest Ecology and Management 175*: 467–488.

MATHERS, N.J.; MAO, X.A.; XU, Z.H.; SAFFIGNA, P.G.; BERNERS-PRICE, S.J.; PERERA, M.C.S. 2000: Recent advances in the application of ^{13}C and ^{15}N NMR spectroscopy to soil organic matter studies. *Australian Journal of Soil Research 38*: 769–787.

MONTEIL-RIVERA, F.; BROUWER, E.B.; MASSET, S.; DESLANDES, Y.; DUMONCEAU, J. 2000: Combination of X-ray photoelectron and solid-state ^{13}C nuclear magnetic resonance spectroscopy in the structural characterisation of humic acids. *Analytica Chimica Acta 414*: 243–255.

PAUL, E.A.; CLARK, F.E. 1996: "Soil Microbiology and Biochemistry", second edition. Academic Press, San Diego. 152 p.

POWLSON, D.S.; BARRACLOUGH, D. 1993: Mineralization and assimilation in soil-plant systems. Pp.209–242 *in* Knowles, R.; Blackburn, T.H. (Ed.) "Nitrogen Isotope Techniques". Academic Press, San Diego.

PRASOLOVA, N.V.; XU, Z.H.; SAFFIGNA, P.G.; DIETERS, M.J. 2000: Spatial-temporal variability of soil moisture, nitrogen availability indices and other chemical properties in hoop pine (*Araucaria cunninghamii*) plantations of subtropical Australia. *Forest Ecology and Management 136*: 1–10.

PRESTON, C.M.; HEMPFLING, R.; SCHULTEN, H.-R.; SCHNITZER, M.; TROFYMOW, J.A; AXELSON, D.E. 1994: Characterization of organic matter in a forest soil of coastal British Columbia by NMR and pyrolysis-field ionisation mass spectrometry. *Plant and Soil 158*: 69–82.

PU, G.X.; SAFFIGNA, P.G.; XU, Z.H. 2001: Denitrification, leaching and immobilization of ^{15}N-labelled nitrate in winter under windrowed harvesting residues in hoop pine plantations of 1–3 years old in subtropical Australia. *Forest Ecology and Management 152*: 183–194.

RAYMENT, G.E.; HIGGINSON, F.R. 1992: "Australian Laboratory Handbook of Soil and Water Chemical Methods". Inkata Press, Melbourne, Australia.

SANCHEZ, F.G.; CARTER, E.A.; KLEPAC, J.F.; 2003: Enhancing the soil organic matter pool through biomass incorporation. *Biomass and Bioenergy 24*: 337–349.

SÁNCHEZ-MONEDERO, M.A.; CEGARRA, R.J.; BERNAL, M.P.; PAREDES, C. 2002: Effects of HCl-HF purification treatment on chemical composition and structure of humic acids. *European Journal of Soil Science 53*: 375–381.

SCHMIDT, M.W.I.; KNICKER, H.; HATCHER, P.G.; KÖGEL-KNABNER, I. 1997: Improvement of ^{13}C and ^{15}N CPMAS NMR spectra of bulk soils, particle size fractions and organic material by treatment with 10% hydrofluoric acid. *European Journal of Soil Science 48*: 319–328.

SCHNITZER, M. 1991: Soil organic matter — the next 75 years. *Soil Science 145*: 448–454.

SCHULTEN, H.R.; SCHNITZER, M. 1998: The chemistry of soil organic nitrogen: A review. *Biology and Fertility of Soils 26*: 1–15.

SOIL SURVEY STAFF 1999: "Soil Taxonomy: A Basic System of Soil Classification for Making and Interpreting Soil Surveys", second edition. USDA Soil Conservation Services, Washington.

SQUIRE, R.O.; FLINN, D.W.; CAMPBELL, R.G. 1991: Silvicultural research for sustained wood production and biosphere conservation in the pine plantations and native eucalypt forests of southeastern Australia. *Australian Forestry 54*: 120–133.

STATSOFT 1999: "STATISTICA". StatSoft, Tulsa, Oklahoma.

STEVENSON, F.J. 1994: "Humus Chemistry: Genesis, Composition, Reactions", second edition. John Wiley and Sons, New York.

STEVENSON, F.J.; COLE, M.A. 1999: "Cycles of Soil. Carbon, Nitrogen, Phosphorus, Sulfur, Micronutrients", second edition. John Wiley and Sons, New York.

SWIFT, R.S. 1996: Organic matter characterisation. Pp.1011–1069 *in* "Methods of Soil Analysis. Part 3: Chemical methods". SSSA Book Series No. 5. Soil Science Society of America and American Society of Agronomy, Madison, WI.

THENG, B.K.G.; TATE, K.R.; BECKER-HEIDMANN, P. 1992: Towards establishing the age, location and identity of the inert soil organic matter of a spodosol. *Journal of Plant Nutrition and Soil Science 155*: 181–184.

VERCHOT, L.V.; HOLMES, Z.; MULON, L.; GROFFMAN, P.M.; LOVETT, G.M. 2001: Gross vs. net rates of N mineralisation and nitrification as indicators of functional differences between forest types. *Soil Biology and Biochemistry 33*: 1889–1901.

WARING, S.A.; BREMNER, J.M 1964: Ammonium production in soil under waterlogged conditions as an index of nitrogen availability. *Nature 201*: 951–952.

WEBSTER, E.A.; HOPKINS, D.W.; CHUDEK, J.A.; HASLAM, S.F.I.; ŠIMEK, M.; PÎCEK, T. 2001: The relationship between microbial carbon and the resource quality of soil carbon. *Journal of Environmental Quality 30*: 147–150.

XU, Z.H.; SIMPSON, J.A.; OSBORNE, D.O. 1995: Mineral nutrition of slash pine in subtropical Australia. I. Stand growth response to fertilization. *Fertiliser Research 41*: 93–100.

ZECH, W.; ZIEGLER, F.; KÖGEL-KNABNER, I.; HAUMAIER, L. 1992: Humic substances distribution and transformation in forest soils. *Science of the Total Environment 117/118*: 155–174.

ZECH, W.; SENESI, N.; GUGGENBERGER, G.; KAISER, K.; LEHMANN, J.; MIANO, T.M.; MILTNER, A.; SCHROTH, G. 1997: Factors controlling humification and mineralization of soil organic matter in the tropics. *Geoderma 79*: 117–161.