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Changes in organic components for fallen logs in old-growth Douglas-fir forests monitored by ^{13}C nuclear magnetic resonance spectroscopy

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^{13}C cross-polarization magic-angle spinning nuclear magnetic resonance (CPMAS NMR) spectroscopy was used to characterize heartwood from decaying fallen boles of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), and western red cedar (*Thuja plicata* Donn). The sample decay classes I to V had been previously assigned based on field observations. Solid-state ^{13}C CPMAS NMR spectra were analyzed to determine the proportion of C of the following chemical types: carbohydrate, lignin, aliphatic, and the sum of carboxyl plus carbonyl. For both Douglas-fir and western hemlock, the proportion of carbohydrate C increased slightly in the early stages of decay. This was followed by a substantial increase in lignin C, while carbohydrate C declined to about 10% of total C. By contrast, the spectra for western red cedar generally showed little change with increasing decay class. One exceptional sample of western red cedar class IV was highly decomposed, indicating complete loss of carbohydrate C, and some loss of lignin side-chain C. For all three species, signals from alkyl and carbonyl C were weak, but tended to increase slightly with decomposition, most likely because of the selective preservation of waxes and resins (alkyl C), and oxidation. Accumulation of chitin was not observed, and there was little evidence for lignin decomposition or for formation of humic polymers. ^{13}C CPMAS NMR offers a simple and information-rich alternative to wet chemical analyses to monitor changes in organic components during decomposition of woody litter.

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La méthode du transfert de polarisation et de l'angle magique de la spectroscopie de résonance magnétique nucléaire du ^{13}C (RMN CPMAS) a été utilisée pour caractériser les grumes en décomposition sur le sol des espèces suivantes : sapin de Douglas (*Pseudotsuga menziesii* (Mirb.) Franco), pruche occidentale (*Tsuga heterophylla* (Raf.) Sarg.) et thuja géant (*Thuja plicata* Donn). Les échantillons ont été classés sur le terrain suivant cinq classes (I à V) de décomposition avant les analyses. Les spectres obtenus au moyen de la spectroscopie de RMN CPMAS au ^{13}C à l'état solide ont été analysés pour déterminer la contribution des glucides, de la lignine, des composés aliphatiques et des groupes carboxyles et carbonyles pris ensemble à la quantité totale de C. Chez le sapin de Douglas et la pruche occidentale, la proportion de C glucidique s'est accrue légèrement dans les premiers stades de décomposition. Les stades ultérieurs ont montré une augmentation considérable de la proportion de C de lignine et une baisse du C glucidique jusqu'à 10% du C total. Par ailleurs, les différences dans les proportions de C des differents composés étaient peu marquées entre les classes de décomposition du thuja géant. Cependant, un échantillon de thuja géant de classe IV était très décomposé, indiquant une disparition complète de C glucidique et une diminution de la proportion de C des chaînes latérales de lignine. Peu de C des groupes alkyles et carbonyles a été détecté chez les trois espèces, mais la proportion de ce type de C avait tendance à augmenter légèrement avec le degré de décomposition, probablement à cause de l'oxydation et de la préservation sélective des cires et des résines (C d'alkyle). Il ne semble pas y avoir eu d'accumulation de chitine et il y a très peu de raison de croire qu'il y ait eu décomposition de la lignine et formation de polymères humiques. La spectroscopie de RMN CPMAS au ^{13}C est une technique simple fournissant beaucoup d'informations et qui peut avantageusement remplacer les analyses par voie humide mesurer les modifications de la composition et matières organiques de la fraction ligneuse de la litière en décomposition.

Introduction

Recent developments in nuclear magnetic resonance (NMR) spectroscopy have made it possible to obtain well-resolved, chemically informative ^{13}C NMR spectra of solid samples. The cross-polarization magic-angle spinning (CPMAS) method uses a combination of techniques to overcome the problems of broadening and low signal intensity for ^{13}C NMR of solids (Fyfe 1984). Line narrowing is achieved with high-power decoupling to remove ^{13}C–^{1}H dipolar interactions, plus magic-angle spinning to eliminate broadening due to chemical shift anisotropy. (In the latter, the sample is rotated at several kilohertz at an angle of 54.7° with respect to the magnetic field.) Signal intensity is increased by cross-polarization, in which magnetization is transferred to the ^{13}C spin population from the more abundant, faster relaxing, and more highly polarizable ^{1}H spins. The technique is carried out nondestructively on dry, powdered samples; and for samples with high C content (>20%), spectra can normally be obtained in a few hours, providing a "fingerprint" of the organic C in the sample.

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CPMAS NMR is well suited for the investigation of complex, insoluble, and heterogeneous materials encountered in studies of biological decomposition. It has been applied to decomposition in peats (Hammond *et al.* 1985; Orem and Hatcher 1987*a*; Preston *et al.* 1987, 1989), forest litter, and organic horizons (Hempfling *et al.* 1987; Wilson *et al.* 1983; Zech *et al.* 1987). Previous NMR studies of wood decomposition have focussed on coalification (Hatcher 1987, 1988; Hatcher *et al.* 1981; Hedges *et al.* 1985) rather than forest ecosystems.

Decaying fallen logs, which are a prominent feature of the forest floor in old-growth forests of the Pacific Northwest, may constitute a significant portion of the organic matter, are important components of the nutrient cycle, and function as sites for seedling germination and habitat for small mammals (Bingham and Sawyer 1988; Grier 1978; Harmon *et al.* 1986; Sachs and Sollins 1986; Sollins 1982; Sollins *et al.* 1980). The decay process in fallen logs and large woody debris has been investigated with respect to changes in density, moisture content, mineralizable N, concentrations of nutrient elements, and some classes of organic components (Fahey 1983; Hope 1987; Lambert *et al.* 1980; Means *et al.* 1985; Sollins *et al.* 1987). The information available on changes in the major organic components, however, is still very limited, the analytical procedures are laborious, and the interpretation of the operationally defined chemical types is often unclear, especially for highly decomposed or humified samples.

The aim of this study was to investigate the potential of CPMAS ^{13}C NMR spectroscopy to characterize and monitor changes in the organic components in large woody debris decaying on the forest floor. We report a CPMAS ^{13}C NMR investigation of heartwood in fallen boles decomposing on the forest floor for three species from old-growth forests of the Pacific Northwest.

Methods and materials

Samples

The study sites, sampling, and decay classification of the fallen logs have been described previously (Sollins *et al.* 1987). Briefly, logs were sampled from seven old-growth sites on the west slope of the Cascade Range in Oregon and Washington. Logs were classified in the field according to the following system: class I, logs freshly fallen, bark and all wood sound, current-year twigs attached; class II, sapwood decayed but present, bark and heartwood mainly sound, twigs absent; class III, logs still support own weight, sapwood decayed but still structurally sound; class IV, logs do not support own weight, sapwood and bark mainly absent, heartwood not structurally sound, branch stubs can be removed; class V, heartwood mainly fragmented, forming ill-defined elongate mounds on the forest floor sometimes invisible from surface. The species studied were Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco.), western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), and western red cedar (*Thuja plicata* Donn).

For the study reported here, a subset of heartwood samples from a previous study (Sollins *et al.* 1987) was randomly selected to include all of the decay classes and species. The highly decayed samples of class V Douglas-fir from three different sites were designated as follows: V_A, H. J. Andrews Experimental Forest; V_S, Squaw Creek; and V_R, Mount Rainier. Three samples were examined from western red cedar class IV; one of these was much more highly decomposed than the other two and was designated western red cedar IV′. Samples that had previously been air dried and ground in a Wiley mill to a fine powder were used without further treatment for NMR spectroscopy.

Samples representing the most advanced state of decay for each species were subjected to two-stage hydrolysis with H_2SO_4. In this procedure, which is the last stage of preparation of Klason lignin from fresh wood (Cyr *et al.* 1988; Leary *et al.* 1986), we used 15 mL of 72% H_2SO_4 and 1.0 g of sample from Douglas-fir class V_R, western hemlock class IV, and western red cedar class IV′.

Density and N contents of the heartwood samples had been previously determined (Sollins *et al.* 1987). Nitrogen contents of the three Klason lignins, and of the samples from which they were prepared, were determined using the semimicro Kjeldahl method essentially as described by Bremner and Mulvaney (1982) but with mercuric oxide as the catalyst. Carbon contents of the samples used in the present study were determined by an automatic combustion method using a Leco model CR 12 carbon determinator.

NMR spectroscopy

^{13}C NMR spectra were obtained on a Bruker MSL 100 spectrometer operating at 25.18 MHz for ^{13}C at 2.35 T. Samples were spun at 4 kHz in an aluminum oxide rotor of 7 mm outside diameter. Most spectra were acquired with 1 ms contact time, 1.5 s recycle time, and 12 000–50 000 scans and were processed using 15 Hz line-broadening and base-line correction. Dipolar dephased spectra were generated by inserting a delay period of 40–100 μs without ^1H decoupling between the cross-polarization and acquisition portions of the CPMAS pulse sequence (Opella and Frey 1979). Chemical shifts are reported relative to tetramethylsilane (TMS) at 0 ppm. (NMR chemical shifts are measured in parts per million of frequency from a reference. For ^{13}C at 2.35 T, 1 ppm corresponds to a shift of 25.18 Hz from TMS.) The high C contents of the samples, from 462 to 577 $mg \cdot g^{-1}$ (Table 1), meant that spectra with good signal to noise could usually be obtained in 5–10 h of acquisition time. Because the spectra were run at low field (2.35 T), there was no distortion of intensity due to generation of satellite lines known as spinning side bands.

Spectral analysis

Spectra were divided into chemical shift regions corresponding to chemical types of C as follows: *A*, aliphatic 0–50 ppm; *B*, methoxyl 50–60 ppm; *C*, *O*-alkyl 60–96 ppm; *D*, di-*O*-alkyl and aromatic 96–141 ppm; *E*, phenolic 141–159 ppm; *F*, carboxyl 159–185 ppm; and *G*, aldehyde and ketone 185–210 ppm. It should be noted that in the context of this paper the term aromatic C is used to designate specifically the nonoxygenated aromatic C occurring at 96–141 ppm, and phenolic, the oxygen-substituted carbons at 141–159 ppm. These divisions are illustrated in Figs. 1A and 1B. Areas of the chemical-shift regions were measured by cutting and weighing and were expressed as percentages of total area (relative intensity).

With the spectra divided into these chemical-shift regions, proportions of lignin and carbohydrate C were then determined using the procedure outlined below. It is similar to that described by Hemmingson and Newman (1985) but without their corrections for intensity distortion caused by

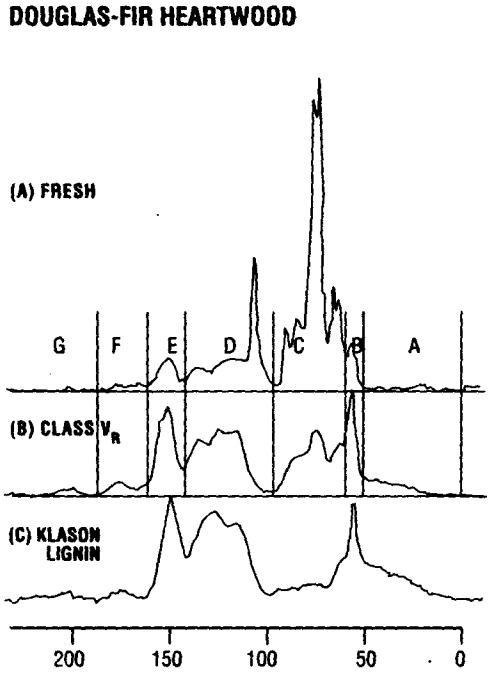


FIG. 1. ^{13}C CPMAS NMR spectra of selected Douglas-fir samples showing chemical shift regions used for analysis. (A) Fresh heartwood. (B) Highly decomposed Douglas-fir class V_R sample. (C) Klason lignin from Douglas-fir class V_R.

spinning side bands, which were not a problem at the much lower magnetic field used in our study. The relative intensity of the 141–159 ppm region (area E) arises almost entirely from the phenolic carbons C_3 and C_4 of the guaiacyl lignin unit (Fig. 2A), which predominates in softwoods. Therefore, the percentage of total C due to the sum of the four aromatic (C_1, C_2, C_5, C_6) and two phenolic (C_3, C_4) carbons of lignin monomer units can be calculated as $3E$, and that due to the three carbons of the lignin side chain (C_α, C_β, C_γ), as 1.5E. The 50–60 ppm region (area B) arises largely from the single methoxyl C of guaiacyl units; thus total lignin C is given by

$$[1] \quad \text{lignin C} = 4.5E + B$$

The 60–96 ppm region (area C) arises from C_2 to C_6 of cellulose and hemicellulose monomer units (Fig. 2B), which predominate in spectra of fresh wood, as well as from the three side-chain carbons of lignin. Including intensity due to the anomeric C_1, the total contribution of carbohydrate C is then calculated from

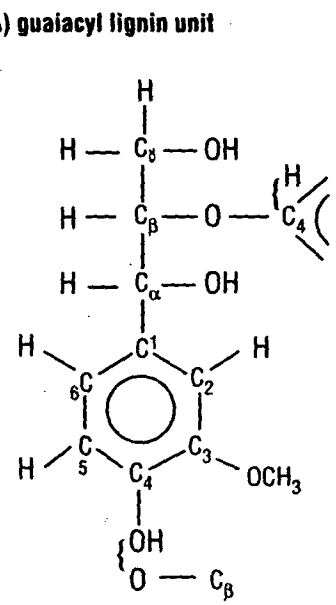
$$[2] \quad \text{carbohydrate C} = 1.2(C - 1.5E)$$

When this calculation was done for the sample of highly decomposed western red cedar (decay class IV′), the value of $1.5E$ was greater than that of area C. Therefore, for this sample carbohydrate C was assumed to be zero, and the whole region from 60 to 159 ppm (areas B–E) was attributed to lignin C.

The ratio of carbohydrate to lignin monomer units (C_mL_m) was calculated from

$$[3] \quad \frac{C_m}{L_m} = \frac{1.2(C - 1.5E)}{3E}$$

An alternative calculation was made of aromatic lignin C (Ar*), by correcting area D for the intensity due to carbohydrate anomeric C:



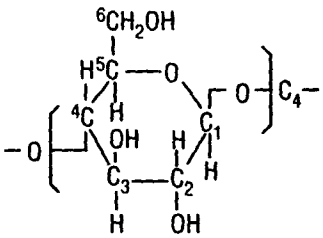


FIG. 2. Repeating unit of (A) guaiacyl and (B) cellulose structural units of softwood lignin.

$$[4] \quad \text{Ar*} = D - 0.2(C - 1.5E)$$

This value was used in the alternate calculation of the ratio of carbohydrate to lignin monomer units

$$[5] \quad \frac{C_m^*}{L_m} = \frac{1.2(C - 1.5E)}{E + \text{Ar*}}$$

To assess further the validity of analyzing the NMR spectra in this way, three other ratios were calculated from NMR intensities attributed to lignin. Based on the structural unit of guaiacyl lignin, the ratio of aromatic to phenolic lignin C should be 2; this is calculated from

$$[6] \quad \frac{\text{aromatic C}}{\text{phenolic C}} = \frac{\text{Ar*}}{E}$$

The ratio of aromatic plus phenolic to methoxyl C should be six, and this was calculated in two ways:

$$[7a] \quad \frac{\text{aromatic C + phenolic C}}{\text{methoxyl C}} = \frac{3E}{B}$$

$$[7b] \quad \frac{\text{aromatic C + phenolic C}}{\text{methoxyl C}} = \frac{E + \text{Ar*}}{B}$$

Some uncertainties are inherent in analyzing the NMR spectra in this way. Intensity distributions in CPMAS spectra may be distorted (Alemany *et al.* 1983; Fyfe 1984), although the conditions used in the present study have been shown

TABLE 1. Selected physical and chemical properties, ratios of carbohydrate to lignin monomer units (C_m/L_m), and intensity ratios (as defined earlier) for the samples examined by ^{13}C CPMAS NMR

Sample	N^a	C (mg·g^{-1})	N (mg·g^{-1})	C_m/L_m (eq. 3)	C_m/L_m* (eq. 5)	Aromatic*, E (eq. 6)	3E, OMe (eq. 7a)	E + aromatic*, OMe (eq. 7b)
Douglas-fir								
Fresh	3	464	0.3	3.22	2.89	2.34	4.22	4.71
Class I	1	476	0.9	3.80	3.37	2.39	3.67	4.14
Class II	1	473	1.0	3.74	3.57	2.15	4.55	4.77
Class III	2	547	1.0	0.17	0.16	2.13	5.72	5.97
Class IV	2	512	2.4	0.22	0.20	2.22	5.13	5.51
Class V_A	1	532	3.2	0.16	0.15	2.10	6.08	6.28
Class V_S	1	499	4.7	0.30	0.26	2.49	5.97	6.96
Class V_R	1	537	3.8	0.025	0.025	1.97	6.33	6.26
Klason lignin		577	1.5					
Western hemlock								
Fresh	2	462	0.2	2.22	2.21	2.02	4.76	4.79
Class I	1	485	0.8	2.45	2.49	1.95	5.44	5.34
Class II	2	479	1.1	1.69	1.70	1.98	5.14	5.10
Class III	2	523	1.2	0.20	0.22	1.73	6.55	5.97
Class IV	2	516	1.8	0.30	0.30	1.95	5.54	5.44
Klason lignin		543	1.0					
Western red cedar								
Fresh	2	467	0.4	2.24	2.18	2.08	4.36	4.49
Class I	1	487	1.1	2.05	1.87	2.29	4.27	4.68
Class II	3	474	1.4	2.14	2.13	2.01	4.55	4.56
Class III	3	471	1.7	2.07	2.04	2.04	4.54	4.60
Class IV	2	473	2.1	1.71	1.60	2.20	5.00	5.34
Class IV′	1	547	2.5				5.96	5.15
Klason lignin		555	1.4					

[a]Sample size.

to produce quantitatively reliable spectra for wood (Hatcher 1988; Haw *et al.* 1984). Consistent with this, we found no significant difference in intensity distribution between spectra of Douglas-fir class V obtained using 1 vs. 2 ms cross-polarization time. Also, there are problems of peak overlap and lack of completely specific chemical shift regions, for which the use of vertical divisions and correction factors cannot fully compensate. Thus, it was not possible to separate hemicellulose from cellulose, and the analysis of lignin signals was based only on the guaiacyl structural unit, which is the major component of softwood lignin. Nonetheless, useful comparisons can be made of relative contributions of different classes of C compounds for the different species and decay classes, and the internal checks on the validity of the procedure gave reasonable values, as discussed later.

As is often the case with NMR studies of this type, it was not possible to carry out many replicate measurements. This was due mainly to time constraints, as each spectrum required several hours of acquisition time. For this reason, we first ran one spectrum of each sample type, and then followed up with replicates where these were considered most essential. The number of samples run for each species and decay class is given in Table 1. Where replicate spectra were obtained, there was good agreement (within 5%) of the relative areas for samples from the same species and decay class. The exception was western red cedar of decay class IV, where two samples showed little decomposition, but one (designated IV′) was highly decomposed. (It should be pointed out that the time constraints arose largely from having to run the spectra on borrowed time in a chemistry department, a common situation for agroforestry research. There is in fact, no NMR instrument available for forestry research in Canada, despite the heavy utilization of NMR in other applied areas.)

Results and discussion

Characterization of fresh heartwood

The chemical shift assignments of the spectra are based on previous studies of wood (Barron *et al.* 1985; Hatcher *et al.* 1981; Haw *et al.* 1984; Hemmingson and Newman 1985; Kolodziejski *et al.* 1982), cellulose (Earl and VanderHart 1981; Maciel *et al.* 1982; VanderHart and Atalla 1984), and lignin (Barron *et al.* 1985; Hatcher 1987; Hatcher *et al.* 1981; Hatfield *et al.* 1987; Leary *et al.* 1986). The spectra for fresh heartwood of the three species were similar. As illustrated for Douglas-fir (Fig. 1A), the spectra are dominated by signals due to cellulose in the 60–110 ppm region. These include the crystalline (65 ppm) and noncrystalline (62 ppm) components of C_6, the C_2, C_3, and C_5 ring carbons (72 and 75 ppm), the crystalline (89 ppm) and noncrystalline (84 ppm) components of C_4, and the anomeric C_1 (105 ppm). The splitting in the intense $C_{2,3,5}$ peak, with higher intensity for the component at 72 ppm, is also diagnostic of a crystalline cellulose component. Weak signals for the acetyl methyl (22 ppm) and carbonyl (173 ppm) groups of hemicellulose could be observed for all three species, but were of lower intensity for western red cedar. Other carbons of hemicellullose occur in the same chemical shift region as those of cellulose. In particular, hemicellulose contributes to the intensity at 84 ppm, and also at 103 ppm, where it produces a slight shoulder on the signal for the anomeric C of cellulose at 105 ppm.

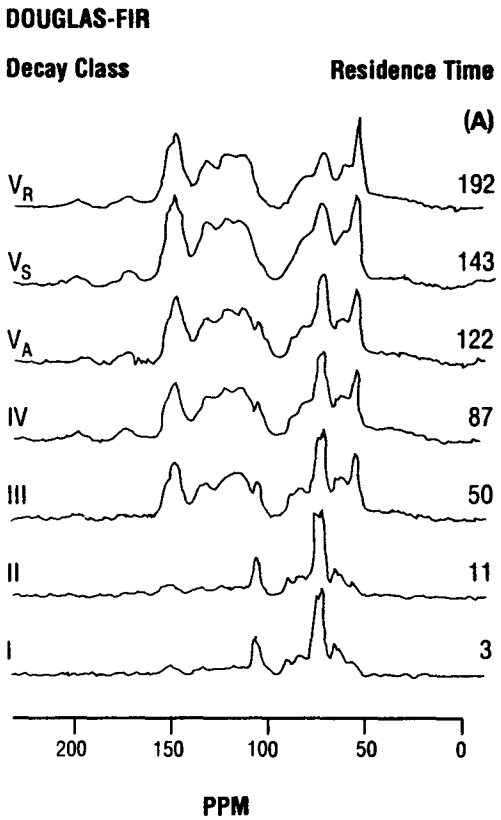


FIG. 3. Stacked plots of ^{13}C CPMAS NMR spectra of Douglas-fir of decay classes I–V.

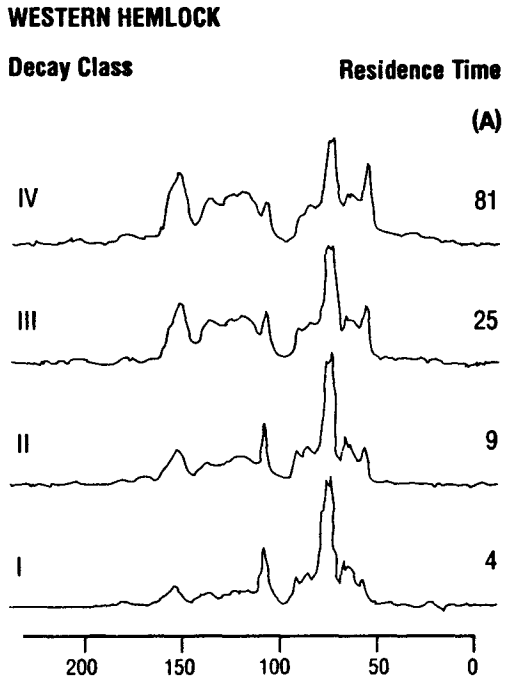


FIG. 4. Stacked plots of ^{13}C CPMAS NMR spectra of western hemlock of decay classes I–IV.

Signals from lignin are seen at 56 ppm for methoxyl C, and from 110 to 160 ppm for aromatic and phenolic C. In the phenolic region from 141 to 159 ppm, guaiacyl C_3 occurs at 148 ppm, while the signal from the guaiacyl C_4 includes free C_4-OH at 146 ppm and C_4 participating in C_β-O-C_4 ether linkages at 153 ppm (Leary *et al.* 1986). This results in a broad phenolic signal with a slight shoulder at 153 ppm. The poorly resolved aromatic region has two rather broad maxima. One occurs at 132 ppm and is assigned to guaiacyl C_1. The second, assigned to C_2, C_5, and C_6 of guaiacyl units, occurs at 115 ppm for western red cedar and Douglas-fir. For western hemlock the maximum is closer to 120 ppm for fresh heartwood but at the normal shift of 115 ppm for decayed samples. There is some uncertainty in the detailed assignment of carbons in this region; the chemical shift of guaiacyl C_2, C_5, and C_6 is usually reported as 115 ppm, although Hatfield *et al.* (1987) reported a lower field value for C_6, 123–125 ppm.

Spectra from samples of fresh heartwood of the three species were generally similar to each other and to published spectra for softwoods, including spruce (Hatcher *et al.* 1981; Leary *et al.* 1986), lodgepole pine (Kolodziejski *et al.* 1982), radiata pine (Hemmingson and Newman 1985), hoop pine (Barron *et al.* 1985), and redwood (Leary *et al.* 1986). The Douglas-fir heartwood differed from the other two species in having a higher ratio of carbohydrate to lignin C (Table 1).

Effects of decomposition

The ^{13}C CPMAS NMR spectra for samples of increasing decay class are shown in Fig. 3, 4, and 5 for Douglas-fir, western hemlock, and western red cedar, respectively. The graphs in Fig. 6 illustrate the changes with increasing decay class for the distribution of C of the following chemical types: aliphatic (area *A*, 0–50 ppm); carboxyl and carbonyl (areas *F* + *G*, 159–210 ppm); total lignin [1]; and total carbohydrate [2]. Ratios derived from areas of chemical-shift regions [3, 5, 6, 7*a*, 7*b*] are shown in Table 1.

In general, the changes observed in the NMR spectra with increasing decay class and residence time (Figs. 3–5) are consistent with loss of cellulose and hemicellulose and with concentration of more resistant lignin, waxes, and resins. There also appears to be some oxidation, as indicated by small increases of relative intensity due to carbonyl C (Fig. 6). There are some small changes in the aromatic region of the lignin spectra, but as discussed later, these do not seem consistent with major structural alterations of lignin with decomposition. For western hemlock and Douglas-fir of decay class III and higher, the broad region around 115 ppm shows some separation into incompletely resolved components at 115 and 122 ppm, with the intensity of the latter generally being higher for the more decomposed samples; this was also seen for the well-decomposed western red cedar sample IV′. The spectrum for Douglas-fir V_R is similar to a previously published spectrum from a highly decayed Douglas-fir log, also from Mount Rainier (Hatcher 1987); it showed a shoulder at 122 ppm rather than a resolved maximum. This change in the aromatic region may be due to reduction in structural heterogeneity as the nonlignin and the more easily decomposable lignin units are removed, although further investigation of this point would be useful.

As found previously (Sollins *et al.* 1987), western hemlock passed through the stages operationally defined by the decay classes more quickly than Douglas-fir. The changes in the organic components, however, followed a similar pattern for both species (Figs. 6A, 6B). In the first stage, from fresh heartwood to Douglas-fir class II and western hemlock class I, there was a small increase in the concentration of

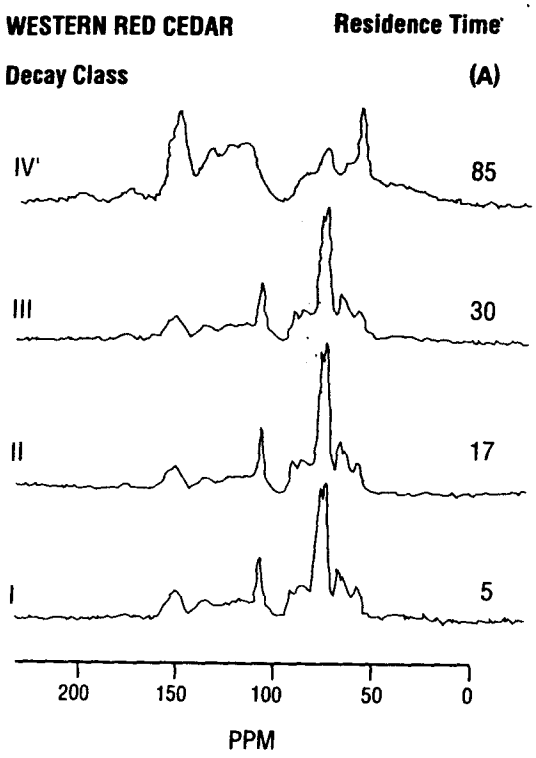


FIG. 5. Stacked plots of ^{13}C CPMAS NMR spectra of western red cedar of decay classes I, II, III, and IV'.

carbohydrate C. This likely reflects an enhancement of the cellulose content, as hemicellulose is removed preferentially in the early stages of decay (Kirk and Highley 1973). Increases in cellulose content in the early stages of decay have also been observed for logs of *Abies amabalis* (Hope 1987) and *Abies balsamea* (Lambert *et al.* 1980).

In the second stage (Douglas-fir classes II and III and western hemlock classes I–III), there was extensive loss of carbohydrate, with increasing concentration of lignin C. At the same time, the relative intensities of aliphatic and carbonyl C increased slightly. For alkyl C, this is probably due to the selective preservation of waxes and resins for alkyl C, while oxidation contributes to the increase in carbonyl C. The third stage is represented by Douglas-fir classes III–V_S and western hemlock classes III and IV. Changes in the spectra were small and inconsistent, with carbohydrate content remaining at about 10% of total C.

Both methods of assessing the ratio of carbohydrate to lignin monomers [3 and 5] gave similar results (Table 1). For both species, C_m/L_m increased initially, then declined to 0.2–0.3 for most samples of decay classes III–V. For the oldest Douglas-fir sample (V_R), however, loss of cellulose had advanced further, and C_m/L_m was reduced to 0.025. Spectral features indicating a crystalline component of cellulose, as described earlier, could be observed through class III for all species.

Most spectra for western red cedar showed little change from fresh heartwood to class IV, with C_m/L_m decreasing from 2.2 to 1.6 (Table 1). In contrast, physical appearance (Sollins *et al.* 1987) and density (Fig. 6C) of the western red cedar logs changed considerably over this sequence. Slow fungal colonization of western red cedar heartwood, relative to Douglas-fir and western hemlock, may explain the lack

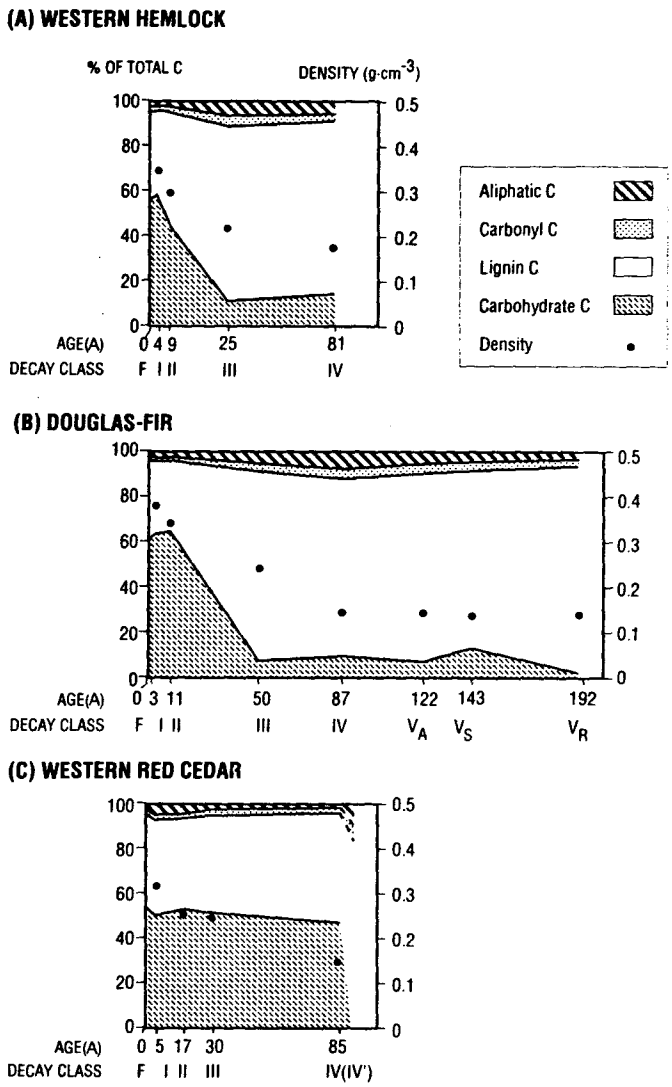


FIG. 6. Changes in classes of organic carbon (as percentage of total spectral area) for increasing decay class and residence time for (A) western hemlock, (B) Douglas-fir, and (C) western red cedar. The mean density for each decay class (right-hand axis) is also shown (density data from Sollins *et al.* 1987). F, fresh heartwood.

of change in chemical properties. In a previous study, western red cedar was the only species that showed no evidence of fungal rhizomorphs at any stage of decay (Sollins *et al.* 1987). Western red cedar heartwood is also unusually resistant to bacterial and fungal growth because of the presence of thujaplicins (Nault 1988).

The results obtained from one sample of the class IV western red cedar samples were exceptional. Designated as western red cedar IV' and shown separately in Figs. 5 and 6C and in Table 1, it appeared to have lost all carbohydrate C, while the intensity from 60 to 96 ppm was less than 1.5*E*, insufficient to account for the three side-chain carbons of the lignin monomer unit. The contrast in the NMR spectra between this and the other two class IV western red cedar samples is also consistent with the differences in C content (Table 1). Carbon content of western red cedar samples showed little change from 467 $mg \cdot g^{-1}$ for fresh heartwood to 473 $mg \cdot g^{-1}$ for decay class IV, but

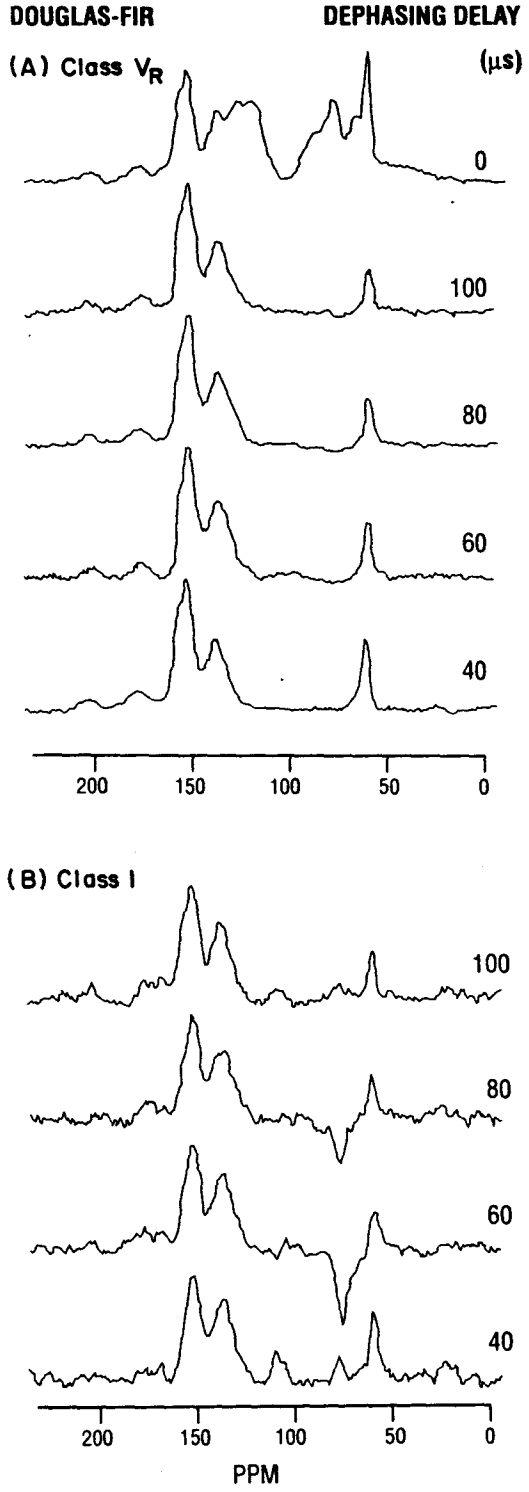


FIG. 7. Dipolar-dephased ^{13}C CPMAS NMR spectra with increasing dephasing time for (A) Douglas-fir decay class V_R and (B) Douglas-fir decay class I.

was 546 $mg \cdot g^{-1}$ for the IV′ sample, closer to the values found for classes III–V for the other two species.

The preferential loss of carbohydrate seen for Douglas-fir and western hemlock is typical of that due to brown-rot fungi (Jurgensen *et al.* 1987; Kirk and Highley 1973). However, despite the role of insects and fungi in decomposition (Edmonds and Eglitis 1989; Harmon *et al.* 1986; Jurgensen *et al.* 1987; Kirk and Highley 1973; Seastedt and Tate 1981), there was no NMR evidence for accumulation of chitin (poly-*N*-acetyl-D-glucosamine) in any of the three species studied. The chemical shifts of chitin are largely coincident with those of cellulose, hemicellulose, and the lignin methoxyl (Preston *et al.* 1986; Saitô *et al.* 1981). However, an accumulation of chitin during decomposition would be accompanied by increasing intensity of the two acetyl signals at 23 and 175 ppm (Preston *et al.* 1986). Lack of NMR evidence for chitin is consistent with reports that chitin decomposes within a few weeks in the soil (Gould *et al.* 1981; Okafor 1966), and also that the concentration of fungal material in decayed wood, apart from portions where mycelium is present, has been estimated to be quite low, about 2% (Cowling 1961 as cited by Kirk 1975). To be detected by NMR in these samples, chitin would probably have to constitute 5% of total C. In addition to biological mechanisms, slow chemical autohydrolysis may also have contributed to loss of carbohydrate (Hedges *et al.* 1985).

There was also no accumulation of aliphatic intensity at 0–50 ppm, in contrast with that found with increasing decomposition in peats (Hammond *et al.* 1985; Orem and Hatcher 1987a; Preston *et al.* 1987, 1989) and organic horizons of forest soils (Hempfling *et al.* 1987; Wilson *et al.* 1983; Zech *et al.* 1987). The broad weak signal observed in the 0–50 ppm region in some of the more decomposed samples is most likely due to recalcitrant resins and waxes from the original wood (Hatcher 1987, 1988; Hatcher *et al.* 1981).

Investigation of lignin structure

Three approaches were used to assess whether structural alterations of lignin had occurred during decomposition. First, examination of the three intensity ratios in Table 1 (from eqs. 6, 7*a*, and 7*b*) suggested that lignin had persisted with little structural alteration. The ratio of aromatic to phenolic C [6] remained close to 2 for all decay classes and species. The ratio of aromatic plus phenolic C to methoxyl C [7*a*, 7*b*] should be 6. In most cases the ratio was less than 6, but tended to increase with increasing decomposition to approximately 5.5–6. The ratios found for the more decayed samples probably reflect more accurately the lignin structure; measurements of methoxyl C intensity in the less decomposed samples would tend to be exaggerated because the peak is not resolved from the region of high carbohydrate intensity and also because there would be some contribution from hemicellulose methoxyl. These interferences would tend to lower the ratio; but with progressive removal of hemicellulose and cellulose, the ratios generally approach 6, indicating little change in the basic guaiacyl structural unit with decomposition.

Second, samples representing the most advanced states of decomposition of the three species (Douglas-fir class V_R, western hemlock class IV, and western red cedar class IV′) were hydrolysed with H_2SO_4, corresponding to the last stage of isolation of Klason lignin. Figure 1C shows the spectrum obtained for Klason lignin from the Douglas-fir sample; those from the western hemlock and western red cedar samples were similar. The spectra were similar to published spectra of Klason lignin prepared from fresh wood (Cyr *et al.* 1988; Haw *et al.* 1984; Kolodziejski *et al.* 1982; Leary *et al.* 1986) and indicate that the hydrolysis removed all of the residual cellulose. Hydrolysis causes other changes

in the spectra; there was an increase of the relative intensity at 0–50 ppm, while the aromatic region showed broadening and loss of intensity of the 132 ppm peak, which was reduced to a shoulder of the peak at 125 ppm. Apart from removal of cellulose, chemical changes caused by H_2SO_4 hydrolysis are not well understood, but are most likely due to some de-etherification at C_4 (Leary *et al.* 1986) and condensation reactions that form C—C bonds (Cyr *et al.* 1988). Incidental to this preparation of Klason lignin, we obtained values for the proportion of N hydrolysable by H_2SO_4. The percentage of N lost upon hydrolysis was 70% for Douglas-fir, 67% for western hemlock, and 60% for western red cedar.

Finally, a sample of Douglas-fir V_R was examined using dipolar dephasing (Fig. 7A) with a series of increasing dephasing times (Opella and Frey 1979; Hatcher 1987, 1988). In this pulse sequence, intensity is lost more quickly from carbons that have strong dipolar interactions with protons. The dipolar interaction is weakened in two cases: for nonprotonated C by the longer separation from hydrogen nuclei, and for methyl C by methyl group rotation, which occurs even in solids (Fyfe 1984; Opella and Frey 1979). The dipolar dephased spectra in Fig. 7A show features consistent with unaltered lignin, namely peaks for methoxyl at 56 ppm, phenolic at 148 ppm with a shoulder at 153 ppm, and nonprotonated aromatic C (guaiacyl C_1) at 132 ppm. Only the weaker carboxyl (172 ppm) and carbonyl (195 ppm) peaks indicate that some oxidation has occurred.

Thus there appeared to be little structural alteration of the remaining heartwood lignin during decay. The NMR spectra showed slight evidence of oxidation, and there appeared to be some loss from the three-carbon side chain for one well-decomposed sample of western red cedar (IV′). This contrasts with the structural alterations of lignin that have been found in incubation studies of wood with white-rot fungi, and to a lesser extent with brown-rot fungi (Enoki *et al.* 1988; Kirk 1975; Kirk and Chang 1975; Nilsson *et al.* 1989), and with the indications of lignin degradation and humification in litter and soil (Hatcher *et al.* 1989; Hempfling *et al.* 1987; Kögel 1986).

The persistence of lignin in these large logs may be due in part to the greater resistance to decay of guaiacyl-based lignin than the syringyl-based lignin of angiosperms (Hedges *et al.* 1985; 1988; Nilsson *et al.* 1989). In addition, moisture may be inhibiting lignin decay. With increasing residence time on the forest floor, the moisture content of the logs increased to approximately 350% in winter and 250% in summer for decay class IV (Sollins *et al.* 1987). Fungal decomposition of lignin, which is primarily an oxidative process, should be restricted if conditions became anaerobic due to waterlogging (Hedges *et al.* 1985, 1988; Kirk 1975; Kirk and Chang 1975; Orem and Hatcher 1987*b*). Limitation of sunlight at the forest floor might also restrict the potential for photodegradation of lignin (Hemmingson and Morgan 1989). Nutrient limitations, especially of N (Sollins *et al.* 1987; Jurgensen *et al.* 1987), and lack of mechanisms for fragmentation and mixing with the soil may also limit decomposition.

Figure 7B illustrates complications that arise with the use of dipolar dephasing for a sample with high cellulose content, Douglas-fir class I. Unlike the spectra for the well-decomposed Douglas-fir class V_R and previously reported dipolar-dephased spectra of lignin (Hatcher 1987), the intensity due to CH and CH_2 carbons does not decay to zero after 40 μs of dephasing time, and oscillatory behaviour is observed for the cellulose peak at 72 ppm. A similar oscillatory behaviour was seen for spectra taken with a constant dephasing time of 60 μs and spinning speeds varying from 2000 to 5000 Hz (spectra not shown). This phenomenon can occur when dipolar interactions are weakened by molecular motion and can also be caused by the magic-angle spinning itself when the spinning is done at high speeds (Alemany *et al.* 1983; Newman 1990).

Conclusions

Solid-state ^{13}C NMR can elucidate patterns of decomposition in the organic components of woody litter decaying on the forest floor. For Douglas-fir and western hemlock logs, the major process appears to be loss of cellulose, with concomitant concentration of the more resistant lignin and resins, and a small amount of oxidation. The process was nearly twice as fast in western hemlock as in Douglas-fir. The pattern of decomposition for western red cedar was completely different; the NMR spectra generally indicated little change in the relative abundance of organic components despite loss of density and other indicators of decomposition. Thus, samples of western red cedar examined in this study showed little evidence of chemical change, despite mass loss and physical collapse in the older logs. For all three species, there appeared to be little degradation of lignin structures. Throughout the decay series, the NMR spectra can be interpreted for the most part by changes in the proportions of the original components in the wood. There was no evidence for significant accumulations of chitin, humic substances, or aliphatic products of microbial activity. The decay processes were consistent with activity by insects, brown-rot fungi, and chemical autohydrolysis, with further decomposition of the highly ligneous residue apparently restricted by unfavourable environmental conditions.

The aims of this investigation were essentially qualitative, i.e., to attempt to elucidate in broad terms the changes in organic components during decomposition, and to evaluate the ^{13}C CPMAS NMR technique for studies of decomposition in large woody litter. The information provided by NMR can be further used to select the most appropriate chemical analyses of organic components, or to design further studies to answer the questions raised in this preliminary survey. In general, it should also be possible to apply the information available from NMR analysis to develop a better understanding of the chemical and biological mechanisms controlling decay and nutrient release in woody litter.

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