Comparison of humus horizons from two ecosystem phases on northern Vancouver Island using ^{13}C CPMAS NMR spectroscopy and CuO oxidation

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deMontigny, L. E., Preston, C. M., Hatcher, P. G. and Kögel-Knabner, I. 1993. **Comparison of humus horizons from two ecosystem phases on northern Vancouver Island using ^{13}C CPMAS NMR spectroscopy and CuO oxidation.** Can. J. Soil Sci. **73**: 9–25. Much forested land in the wetter zones of northern Vancouver Island is characterized by thick humus layers, with two distinct ecosystem phases: the younger "HA" phase arising from disturbance is productive after clearcutting, but in the old-growth "CH" phase, seedlings suffer growth check after 5–8 yr, with reinvasion of the ericaceous shrub salal (*Gaultheria shallon* Pursh.). We used solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy and CuO oxidation to examine whether chemical differences in the humus might be associated with difference in forest productivity after clearcutting. NMR spectra of woody horizons, which were similar for CH and HA sites, were dominated by signals from lignin of decomposed wood. Non-woody humus types were typical of forest litter layers, and were dominated by signals in the O-alkyl region. The differences between CH and HA sites were: (i) higher tannin content in the CH sites, most likely from salal inputs and (ii) higher ratio of carbohydrate to lignin C, indicating less effective decomposition in CH sites. Oxidation with CuO also showed more advanced decomposition in the non-woody horizons of HA than of CH sites. Less effective decomposition possibly due in part to tannin accumulation could contribute to the lower forest productivity on salal-dominated CH sites in this region.

Key words: ^{13}C NMR, CuO oxidation, decomposition, humus, tannin, salal

deMontigny, L. E., Preston, C. M., Hatcher, P. G. et Kögel-Knabner, I. 1993. **Comparaison des horizons d'humus de deux phases écosystémiques dans le nord de l'Ile de Vancouver au moyen de la spectrométrie RMN du ^{13}C RAMPC (CPMAS) et de l'oxydation au CuO.** Can. J. Soil Sci. **73**: 9–25. Une vaste superficie boisée des zones plus humides de la portion nord de l'île de Vancouver est caractérisée par d'épaisses couches d'humus, comportant deux stades écosystémiques distincts: le stade "HA" plus jeune, résultant d'une perturbation, qui est productif après le déboisement, et le stade "CH" de peuplement mûr, où la croissance des plants diminue après cinq à huit ans, à cause de la réinvasion par le salal (*Gaultheria shallon* Pursh.), arbuste de la famille des éricacées. Nous nous sommes servis de la spectrométrie de résonance magnétique nucléaire (RMN) au ^{13}C à l'état solide et de l'oxydation au CuO pour déterminer si les différences chimiques observées dans l'humus étaient associées à la variation de la productivité forestière après déboisement. Les spectres RMN des horizons ligneux, semblables pour les sites CH et HA, étaient dominés par des signaux provenant de la lignine du bois en décomposition. Les types d'humus non ligneux étaient typiques des couches de litière et étaient dominés par des signaux dans la région O-alkylique. Les différences entre les sites CH et HA étaient les suivantes:

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(i) teneur en tanin plus élevée dans les sites CH, liée probablement aux salals et (ii) rapport glucides/carbone de la lignine plus élevé dans les sites CH, reflétant une décomposition moins efficace à cet endroit. L'oxydation au CuO a également révélé une decomposition plus avancée dans les horizons non ligneux des sites HA que dans les autres sites. Une décomposition moins efficace, due peut-être en partie à l'accumulation de tanin, pourrait contribuer à faire baisser la productivité forestière des sites CH dominés par les salals de cette région.

Mots clés: RMN au ^{13}C, oxydation au CuO, décomposition, humus, tanin, salal

Much of the forested land of Northern Vancouver Island is located within the Very Wet Maritime Coastal Western Hemlock biogeoclimatic subzone (CWHbvm) (Pojar et al. 1987). Soils in this region consist of Ferro-Humic Podzols overlain by thick (10–25 cm), compact, lignomors in which greater than 70% of the combined F and H layers consists of wood-derived ligneous material (Green et al. 1993).

The zonal forests form what Lewis (1982) called the *Thuja plicata-Tsuga heterophylla-Abies amabilis-Gaultheria shallon-Rhytidiadelphus loreus* or "salal-moss" ecosystem (S1). This ecosystem association may be divided into two phases: the undisturbed old-growth "CH" phase and the younger "HA" phase. The CH phase consists of somewhat open western red cedar (*Thuja plicata* Donn) and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) with a minor component of amabalis fir (*Abies amabilis* (Dougl.) Forbes) and a dense understory of salal (*Gaultheria shallon* Pursh). The CH phase is the climatic climax community and appears to have remained relatively undisturbed for more than one thousand years, while the HA phase is a seral stage occurring on sites with a history of soil disturbance. Many of the HA stands of northern Vancouver Island originated from a 1908 windthrow event.

When clearcut and planted, sites within the HA phase produce young vigorous stands. In CH stands, however, after clearcutting with or without slashburning, the planted seedlings initially grow well but become chlorotic and stagnant after 5–8 years. This "growth check" is associated with increasing dominance of the ericaceous shrub salal on the CH phase, but not on the HA phase (Messier and Kimmins 1991). Site treatments such as fertilization or site preparation by slash-burning, cultivation, and herbicides have not given consistently satisfactory improvement in productivity (Weetman et al. 1989a,b). The dominance of salal appears to be due to its effectiveness in occupying cutover CH sites and competing for nutrients through its dense network of underground rhizomes and effective mycorrhizal associations (Messier and Kimmins 1991).

Other studies have been undertaken to obtain a greater understanding of ecosystem functioning so that effective site rehabilitation and management programs can be developed. These include investigation of the nutrient-supplying ability of the forest floor in the two phases (Prescott et al. 1993), and the present study of the organic horizons. In this study, the organic horizons occurring in the two ecosystem phases were surveyed and classified in the field. We then used both ^{13}C NMR and CuO oxidation to characterize the organic components in humus types from the two phases and to search for differences which might be associated with differences in forest productivity after clearcutting.

MATERIALS AND METHODS

Sites and Sampling

The study sites were in the submontane Coastal Western Hemlock subzone on Western Forest Products tree Farm Licence 6 on northern Vancouver Island near Port McNeill (50°60′N, 127°35′W). They are characterized as having moderately well to imperfectly drained Duric Humo-Ferric Podzols arising from a sandy-loam glacial till with a blanket and rolling surface expression. Elevations of the sites were similar,

between 85 and 100 m. The HA sites tended to be on ridgetops with zero slope and aspect, while the CH sites were immediately adjacent on lower slopes, with inclines ranging from 11 to 20%. A more detailed account of the region and of similar CH and HA sites may be found in Messier and Kimmins (1991).

A total of 10 sites were located with forest stands typical of the CH and HA phases of this ecosystem association (i.e., 5 CH and 5 HA sites). Within each site, a 30-m transect was run though an area that appeared homogeneous in terms of topography and vegetation. Along this transect, 10 soil pits were dug 3 m apart through the floor and mineral soil to a depth where roots were absent, usually to a root-restricting layer. Forest floor and mineral soil horizons were classified in the field according to the scheme developed by Green et al. (1993).

From each site, a total of one or two samples representative of each humus type were collected in plastic bags. A refrigerated truck was used for field storage and transport; the samples were then stored at 4°C until they were processed. Each sample was sieved at field moisture first through an 80-mm sieve to remove large roots and wood pieces, then through a 40-mm sieve to break up small pieces of wood and remove fine roots. From this field collection, a set of 4–6 representative samples of each horizon type (CH and HA) were selected for further study. These samples, and the litterfall ones were air-dried, ground to pass a 20-mesh sieve (850 μm) and stored in sealed plastic containers at room temperature.

Conifer litter samples were randomly collected within CH and HA plots, and combined to make composite CH and HA samples. Salal litter was sampled in CH plots and one composite sample prepared. Litter samples were air-dried, ground and stored as above.

^{13}C CPMAS NMR Spectroscopy

Dry samples were packed into a bullet-type rotor that was placed in the probe of a Chemagnetics, Inc. M-100 NMR spectrometer operating at 100 MHz for ^{1}H. Solid-state ^{13}C NMR spectra with cross-polarization and magic-angle spinning at 3.5 kHz ("CPMAS" NMR spectra) were obtained as previously described by Hatcher (1987). Dipolar-dephased CPMAS spectra were generated by inserting a delay period of 40–100 μs without ^{1}H 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.

Spectra were divided into chemical shift regions according to chemical types of C as follows: A, alkyl 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. In the context of this paper, the term aromatic C is used to designate, specifically, the non-oxygenated aromatic C occurring at 96–141 ppm, and phenolic, the oxygen-substituted aromatic C at 141–159 ppm. Areas of the chemical shift regions were measured by cutting and weighing and expressed as percentages of total area (relative intensity). The proportions of lignin C and carbohydrate C (C_l, C_c) were then determined using the following procedure (Preston et al. 1990).

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. 1b), which predominates in softwoods. Therefore, the percent 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:

$$C_l = 4.5E + B \quad (1)$$

The 60–96 ppm region (area C) arises from carbons 2 to 6 of cellulose (Fig. 1a) and hemicellulose monomer units, 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:

$$C_c = 1.2\ (C - 1.5E) \quad (2)$$

The ratio of carbohydrate to lignin monomer units (C_m/L_m) is given by:

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

Some problems and uncertainties inherent in analyzing the spectra this way have been described previously (Hemmingson and Newman 1985; Preston et al. 1990). The most notable is that there are problems with peak overlap and lack of completely specific chemical shift regions, for which the use of vertical divisions and correction factors is inadequate. In addition, the analysis of lignin signals is based only on the guaiacyl structural unit, which is the major component of coniferous lignin.

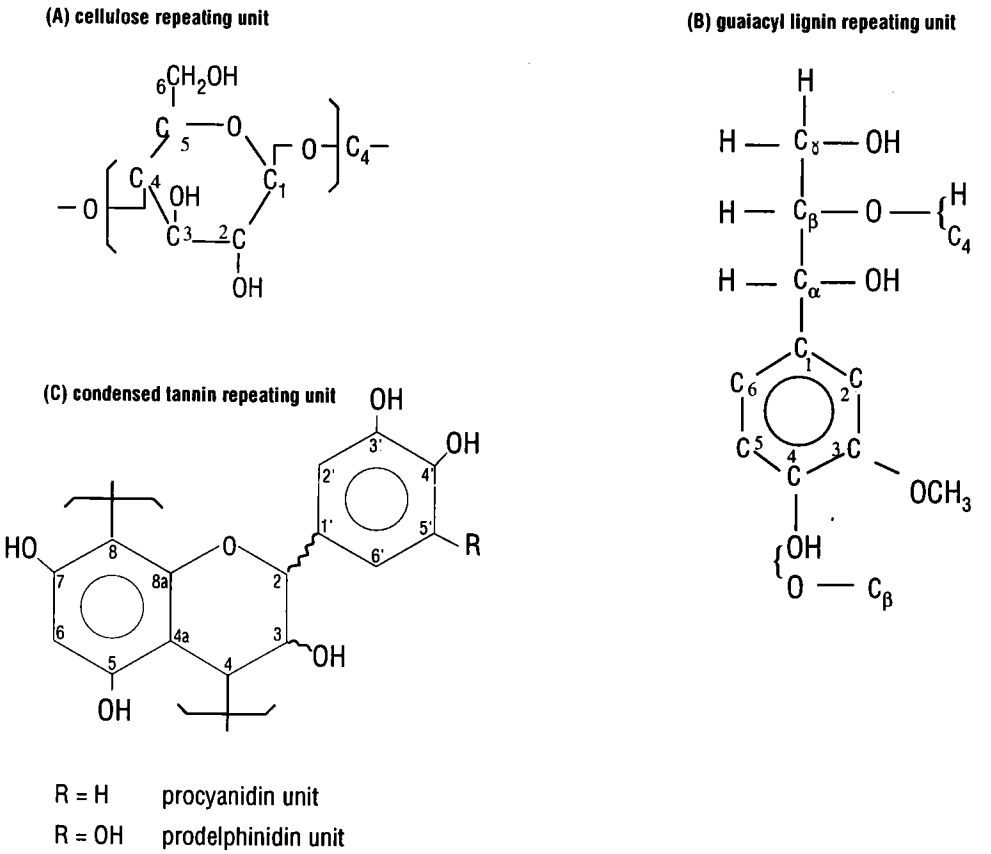

Fig. 1. Structural units of (a) cellulose, (b) lignin and (c) condensed tannins.

This is satisfactory for the woody horizons Fw and Hrw which are almost exclusively the carbohydrate-depleted residue of coniferous wood with little alteration of lignin structures. The results are less meaningful for horizons in which decomposition has proceeded further, resulting in alteration of lignin structures and in non-woody horizons derived from salal and needle litter. For this reason, the analysis was not carried out for one of the horizon types, the highly-decomposed Hhi. Due to time constraints involved in obtaining the NMR spectra it was only possible to run one sample of each horizon type. However, excellent agreement was found between samples of the same horizon type from the two phases.

Cupric Oxide Oxidation

Alkaline CuO oxidation was carried out according to Hedges and Ertel (1982), with extraction of the oxidation products using diposable columns, and separation and quantification by reversed-phase high performance liquid chromatography (RP-HPLC) as described by Kögel and Bochter (1985). Using this procedure, it was found that relative standard deviations for an individual phenol did not exceed 13%. The analysis was carried out on duplicate samples, including the same samples used for CPMAS NMR.

Woody tissues of gymnosperms produce mostly acids, aldehydes and ketones of the vanillyl and p-hydroxyl type, and hardwoods vanillyl, syringyl and p-hydroxyl types. The yields of p-hydroxyl phenols are not used, however, because they can also be derived from non-lignin sources. Non-woody tissues also produce cinnamyl phenols (p-coumaric and ferulic acids) (Hedges and Mann 1979; Kögel 1986). Results are expressed as [V+S+C], the sum of the vanillyl, syringyl and cinnamyl products in mg $(gC)^{-1}$, and also as $(Ac/Al)_v$, the ratio of acid to aldehyde (vanillic acid to vanillin) for the main products from gymnosperm wood and litter.

RESULTS AND DISCUSSION

Classification of Humus Horizons

In the context of this paper, the term "humus" is used to describe any of the organic horizons of the forest floor; characteristic features of each are listed in Table 1. The forest floor was divided into master horizons based on the degree of decomposition (Fox et al. 1987; Green et al. 1993). Litter horizons (L), which were generally very thin (<1 cm) and consisted of the freshly fallen debris of the surrounding vegetation, were removed prior to sample collection of the underlying horizons.

The F and H master horizons were subdivided into two broad categories based on their woody or non-woody nature. The former were given the suffix "w". The woody horizons included an Fw, in which the woody structure held when rubbed between the fingers; an Hrw (residuic), in which the woody structure failed when rubbed between two fingers, but consisting of >20% woody material; and an Hw with <20% woody material. Simple field tests that further distinguished the two humic horizons include the appearance of dark-coloured greasy materials that rubbed out on fingers for Hw but not for Hrw; the reddish colour of Hrw vs. the brownish red colour of Hw, and the more massive and compact structure of Hw.

The non-woody horizons consisted of three types: a matted Fm (mycogenous) horizon with abundant fungal hyphae and plant roots; a well-decomposed Hh (humified) horizon which was >80% amorphous with a massive structure, greasy texture and dark colour; and an Hhi, a very massive, very greasy, black horizon, >95% amorphous, containing intermixed mineral particles (17–35% organic C content) found immediately above the mineral soil. Data from chemical and nutrient analysis (of 4–6 replicates of each horizon type) are presented elsewhere (deMontigny 1992).

NMR Spectroscopy

WOODY HORIZONS. Spectra of woody horizons from CH and HA sites are shown in Figure 2, and the relative proportions of C in the chemical-shift regions in Table 2. The general features of the spectra, and the values in Table 2 indicate that there is little to distinguish samples taken from CH vs. HA sites. There are also only small differences

Table 1. Physical descriptions of organic soil horizons of CH and HA sites

Horizon	Composition	Colour	Structure	Rooting
(a) Non-woody				
Fm	>60% plant <20% amorphous <20% fungi	10R 3–4/4–6 2.5YR 3/4–6 5YR 2.5–3	Compact Matted	Abundant Fine to coarse
Hh	no wood >80% amorphous	10R 2.5–3 2.5YR 1–2 5YR 1–2	Massive Blocky Greasy	Plentiful to abundant
Hhi	>95% amorphous	5YR 2.5–5 7.5YR 0–1	Massive Very greasy blocky to fine granular	Very few
(b) Woody				
Fw	>90% wood	10R 3/4 2.5YR 3/4–6	Woody structure holds	Few
Hrw	<80% wood <20% amorphous	10R 2.5–3 2.5YR 1–2 5YR 1–2	Woody structure fails	Few
Hw	<20% wood >80% amorphous	10R 2.5–3 2.5YR 1.2 5YR 1–2	Crumbly Greasy	Plentiful to abundant

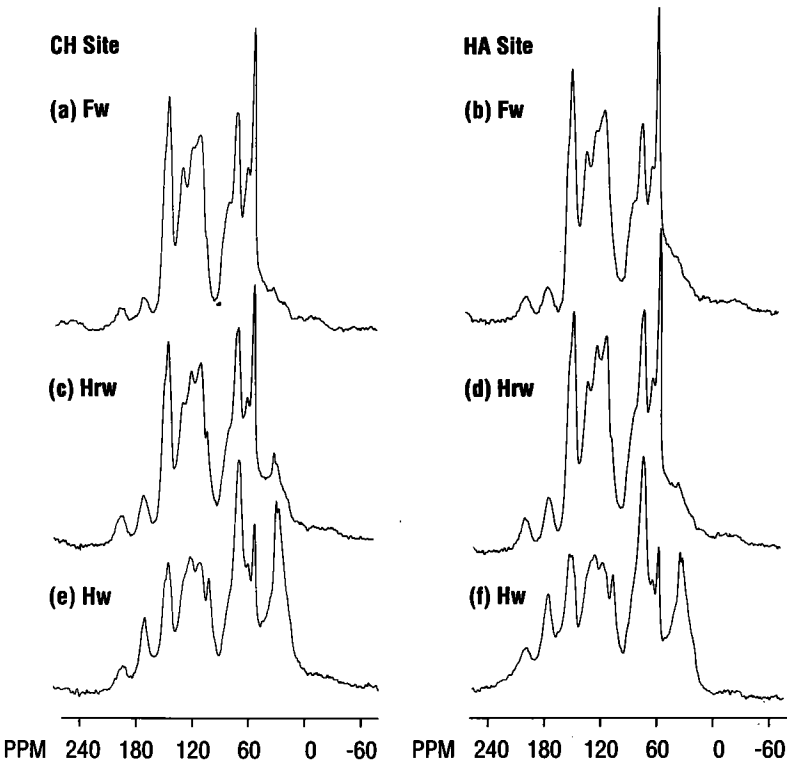

Fig. 2. Carbon-13 CPMAS NMR spectra of woody horizons (Fw, Hrw, Hw) from CH and HA sites.

between Fw and Hrw spectra, but a larger difference between this pair and the Hw spectra.

The spectra of Fw and Hrw horizons (Fig. 2a-d) are similar to those reported previously for well-decomposed gymnosperm wood (Preston et al. 1990). They are dominated by signals typical of the guaiacyl lignin unit (Fig. 1b) which predominates in softwoods; these include methoxyl C at 56 ppm, and aromatic and phenolic C at 110–160 ppm. The phenolic region (141–159 ppm) arises from the sum of guaiacyl C_3 at 148 ppm, free C_4-OH at 146 ppm and C_4 in C_β-O-C_4 ether linkages at 153 ppm (Leary et al. 1986). These components are not resolved but in fact produce a single peak at 148 ppm with a slight shoulder at 153 ppm. This is shown in the expanded spectra of CH and HA Fw horizons in Fig. 3a and b. The aromatic region (96–141 ppm) includes guaiacyl C_1, C_2, C_5 and C_6. O-alkyl C (including carbohydrate) is depleted in these spectra, and the peak at approximately 72 ppm is due largely to the three-C side chain of lignin.

In fresh wood, the O-alkyl region (and in fact, the whole spectrum) is dominated by signals due to carbohydrate, mainly cellulose C (Hemmingson and Newman 1985; Preston et al. 1990). These include the crystalline (65 ppm) and non-crystalline (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 non-crystalline (84 ppm) components of C_4, and the anomeric C_1 at 105 ppm (Fig. 1a). The spectra in Fig. 2 (a-d) show only a poorly resolved shoulder, or no detectable signal for anomeric C. Total carbohydrate C was calculated to be about 4% for the less-decomposed Fw and Hrw, and

Table 2. Relative percentages of C in chemical shift regions of (a) litter, (b) non-woody, and (c) woody humus types by site

		Chemical shift region/PPM Range						
		A 0–50	B 50–60	C 60–96	D 96–141	E 141–159	F 159–185	G 185–210
Humus/site type								
(a) Litter								
	CH	26	5	33	19	9	6	1
	HA	27	4	44	14	6	4	1
(b) Non-woody								
Fm	CH	20	5	32	24	10	6	3
	HA	21	5	29	24	10	7	4
Hh	CH	19	4	28	27	11	8	5
	HA	18	4	27	27	10	9	4
Hhi	CH	38	5	20	18	8	8	4
	HA	25	5	20	24	11	10	5
(c) Woody								
Fw	CH	8	10	27	34	15	3	3
	HA	11	11	23	35	15	3	2
Hrw	CH	14	9	24	33	14	4	3
	HA	10	10	25	34	14	4	3
Hw	CH	23	7	24	27	11	6	3
	HA	17	5	24	28	13	8	5

7.5% for the Hw (Table 3). The ratio of carbohydrate to lignin moieties (C_m/L_m) was in the range of 0.1–0.3. These are similar to values found for highly-decomposed logs of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), western red cedar and western hemlock. By contrast, ratios of 2–3 are found for fresh wood (Hemmingson and Newman 1985; Preston et al. 1990).

Also in contrast to fresh wood, there is a broad region of intensity in the alkyl region (0–50 ppm). This increases from about 10% in the Fw, to 12% in the Hrw, and to 20% in the Hw (Table 2). There is also an increase in resolution; for the Fw spectra, the aliphatic region is a broad shoulder most likely due to selective preservation of waxes and resins in the original wood. The Hrw spectra begin to show a peak at 30 ppm, which is better-defined for the CH site, and for the Hw spectra, this region has a strong, well-defined peak at 30 ppm. The peak at 30 ppm is characteristic of aliphatic $-CH_2-$ units in long chains, such as fatty acids, and is typically seen to increase with increasing decomposition in Folisols and Histosols (Hammond et al. 1985; Preston et al. 1987; 1989). It is thought to be largely a byproduct of production of microbial biomass coupled with incomplete decomposition. The increase in alkyl, O-alkyl, aliphatic and carboxyl C may be the result of microbial or fungal activity (Preston and Ripmeester 1983; Baldock et al. 1990).

There is a greater contrast between the Fw and Hrw spectra vs. the Hw. The Hw spectra (Fig. 2e and f) also have more intensity characteristic of carbohydrate, at 62, 73 and 101 ppm. The carboxyl and carbonyl intensity also increases from Fw to Hrw to Hw; this could be due both to oxidation of lignin and to the production of fatty acids in microbial biomass. Decomposition of lignin, or production of other aromatic structural units is also consistent with a relative decrease in methoxyl, aromatic and phenolic C, and a change in the aromatic region, as the intensity at 130 ppm increases relative to that

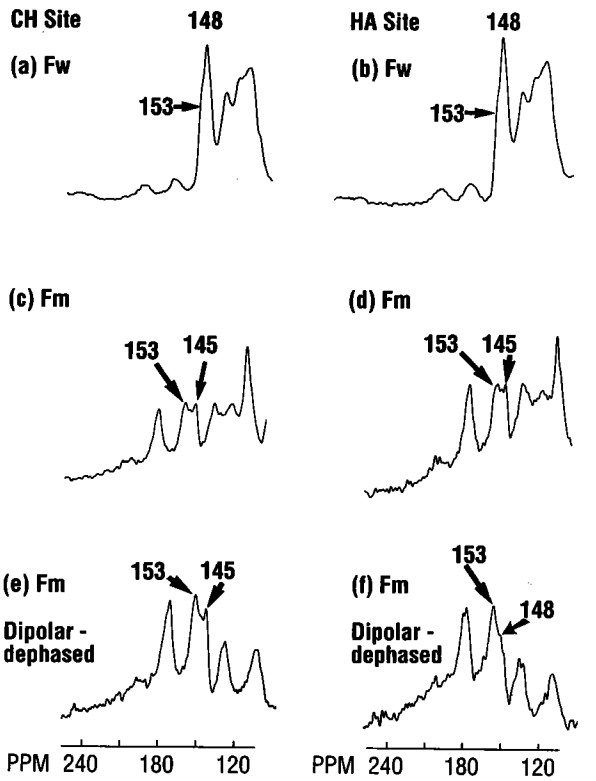

Fig. 3. Expanded ^{13}C CPMAS NMR spectra of the aromatic region for selected horizons and sites: (a) Fw CH and (b) Fw HA; (c) Fm CH and (e) with dipolar dephasing; Fm HA (d) and (f) with dipolar dephasing.

Table 3. Lignin and carbohydrate C (C_l, C_c) as percentage of total C, and ratios of carbohydrate to lignin monomer units (C_m/L_m)

Humus/site type		C_l (% of total C)	C_c (% of total C)	C_m/L_m
(a) Litter				
	CH	43.6	24.5	1.1
	HA	30.9	41.5	2.3
(b) Non-woody				
Fm	CH	48.1	21.1	0.7
	HA	48.7	17.2	0.6
Hh	CH	52.8	13.3	0.4
	HA	50.0	14.4	0.5
(c) Woody				
Fw	CH	78.7	4.7	0.1
	HA	80.2	0	0
Hrw	CH	70.0	4.6	0.1
	HA	74.5	4.2	0.1
Hw	CH	54.5	9.2	0.3
	HA	62.6	5.7	0.2

from 115 to 125 ppm. Total lignin C is calculated to be about 80% for the Fw, 72% for the Hrw, and 58% for the Hw (Table 3).

It is not easy to ascertain the origin of the O-alkyl intensity in the Hw spectra; it may arise largely from the original carbohydrates in woody and non-woody inputs, or there may be greater contribution from microbial activity. However, the usual pattern in organic soils is for plant-derived carbohydrate to decrease while microbial alkyl C increases (Hempfling et al. 1987; Preston et al. 1987, 1989; Kögel-Knabner et al. 1988); this and the good resolution in the spectra suggest that the O-alkyl C most likely derives from original plant inputs.

NON-WOODY HORIZONS. The spectra of the non-woody Fm and Hh horizons (Fig. 4) are

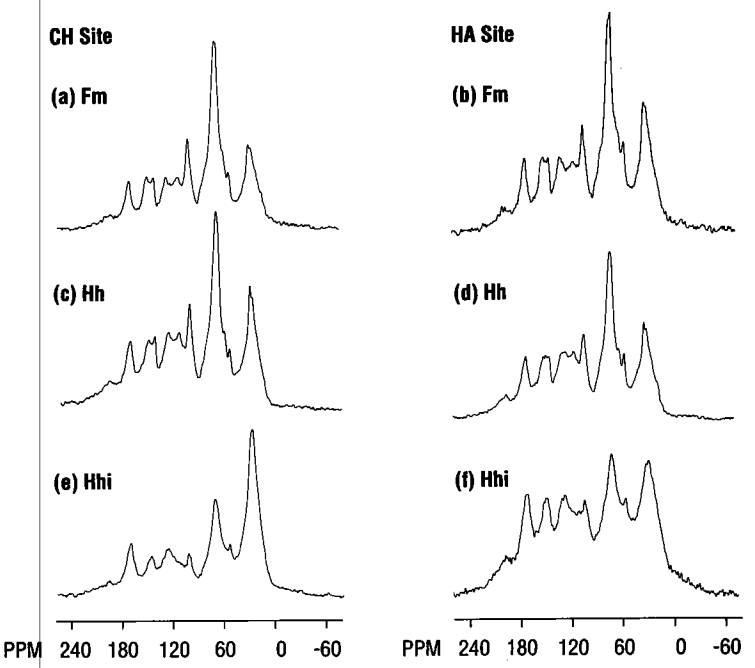

Fig. 4. Carbon-13 CPMAS NMR spectra of non-woody horizons (Fm, Hh and Hhi) from CH and HA sites.

similar to those reported for forest litter layers under conifers (Zech et al. 1987; Kögel et al. 1988). The dominant signal is that from O-alkyl C (Table 1) which accounts for 33–44% of the total C in the litter, and 20–30% in the non-woody horizons (Table 2). Aromatic C accounts for 14–19% of the total C in litter, and somewhat higher proportions (18–27%) in the non-woody horizons. Alkyl C, which would include the cutin, suberin, and highly aliphatic polymers of plant cuticles accounts for about 26% of the total C in the litter, 18–21% in the Fm and Hh, and 25–38% in the Hhi. Compared to the woody samples, the methoxyl C and phenolic C contents are lower, 4–5% and 5–10%, respectively.

From the estimates in Table 3 for these non-woody horizons, total lignin C (C_l) increases with decomposition from Fm to Hh, while total carbohydrate C (C_c) decreases. The ratio C_m/L_m decreases from a high of 2.3 in HA litter to approximately 0.5 for the Hh (values were not calculated for Hhi which was highly decomposed and gave a poorly resolved spectrum). The overall effects of decomposition in the non-woody horizons are a decrease in easily-decomposible O-alkyl C, which would mainly be due to carbohydrates of plant origin, and an increase in alkyl and carboxyl C, most likely derived from microbial biomass.

As was found for the woody horizons, differences between the CH and HA horizons are small. One point of interest is that the HA litter has a considerably higher C_m/L_m ratio than the CH litter. However, the proportions of O-alkyl C and the C_m/L_m ratios are slightly higher in the Fm horizons from CH than from the HA sites. The differences are small, and bear further investigation, but they are consistent with lower litter quality and less effective decomposition in the CH sites.

There is also evidence for tannins in the spectra of the non-woody horizons. Tannins are present in low quantities in most litter materials (Kögel-Knabner et al. 1991) but are difficult to identify in NMR spectra because the peaks occur in the same regions of those

of lignin and carbohydrate (Morgan and Newman 1986; Preston and Sayer 1992). However, a peak due to condensed tannins (C_3' and C_4' of procyanidins and C_3' and $C5'$ of prodelphinidins, Fig. 1c) occurs at 144–145 ppm, in a region that is usually clear in wood and litter spectra. As discussed in the previous section, the phenolic region of the Fw horizon from both CH and HA shows a well-defined peak at 148 ppm, with a slight shoulder at 153 ppm, typical of guaiacyl lignin C_3 and C_4 (expanded spectra in Figs. 3a and b). The Fm and Hh horizons from the CH site, which would have both coniferous and salal litter inputs, show a broad signal presumably resulting from a combination of tannin, guaiacyl and syringyl phenolic inputs. This feature is shown in expanded form for CH and HA Fm horizons in Figs. 3c and d. The feature at 145 ppm is not detectable in the Hhi horizons, probably due to decomposition or complexation of tannins as they are leached through the soil profile.

DIPOLAR-DEPHASED SPECTRA. This technique can be used to examine features that may be masked in the normal CPMAS spectra. During a delay period before signal acquisition, intensity is lost more quickly from carbons that have strong dipolar interactions with protons; i.e., carbons with directly bonded hydrogens. The dipolar interaction is weakened in two cases: for non-protonated C which have a greater separation from hydrogen nuclei, and where there is molecular motion in the solid state. This occurs for methyl C due to methyl group rotation, while long-chain aliphatics can also display sufficient backbone vibrations to weaken the dipolar coupling in the solid state (Opella and Frey 1979).

Dipolar-dephased spectra have proved useful in detecting tannins, as the non-protonated C_4 and C_8 at 108 ppm can be observed in dipolar-dephased spectra in a region that otherwise is masked by aromatic and anomeric CH (Wilson and Hatcher 1988). This provides a useful test for tannins in

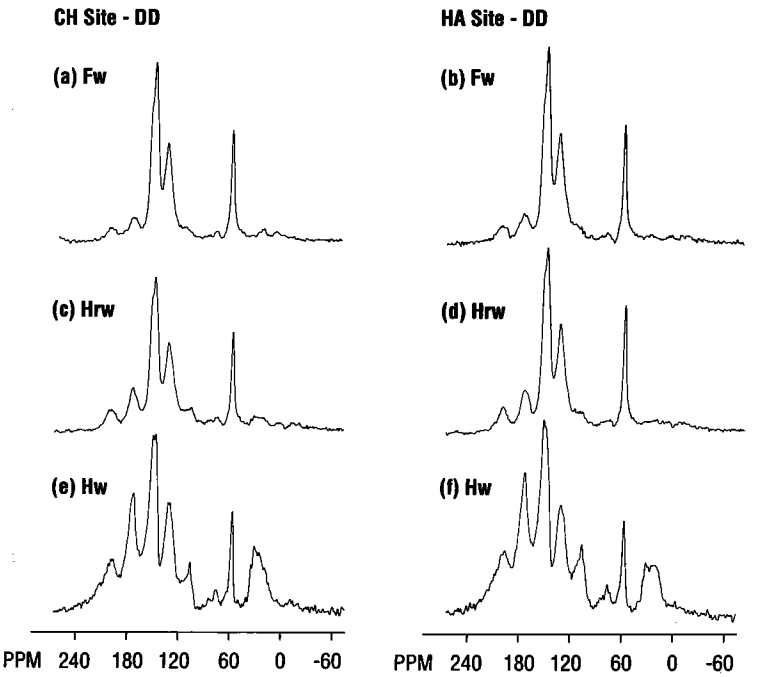

Fig. 5. Dipolar-dephased ^{13}C CPMAS NMR spectra of woody horizons (Fw, Hrw, Hw) from CH and HA sites.

complex matrices, for which the only interference is the ketal C of carbohydrate, which would not likely be a problem for litter and soils.

For the woody horizons, the dipolar-dephased spectra in Fig. 5 (a–d) show typical lignin peaks for methoxyl at 56 ppm, phenolic at 148 ppm with a shoulder at 153 ppm, and non-protonated aromatic C (guaiacyl C_1) at 132 ppm, as well as weaker signals due to carboxyl (172 ppm) and carbonyl (195 ppm) C (Hatcher 1987; Preston et al. 1990). Tannin signals are very weak or absent. For the Hw horizons (Fig. 5e and f), there is some intensity at 108 ppm, but without the other characteristic tannin peak at 144 ppm. There is also considerable residual alkyl intensity for both CH_2 and CH_3, consistent with the presence of long-chain hydrocarbon moieties with considerable molecular motion in the solid state.

For the non-woody horizons, the dipolar-dephased spectra of the Fm and Hh horizons from the CH site (Fig. 6a and c) show the characteristic tannin peaks: a small but clearly-defined peak at 145 ppm partially resolved from the main phenolic peak, as well as a strong peak at 108 ppm. The dipolar-dephased spectra of the Fm and Hh horizons from the HA sites (Fig. 6b and d) lack a clearly-defined peak at 145 ppm, although there is some intensity at 108 ppm. These features are shown in expanded form for the dipolar-dephased spectra of the CH and HA Fm horizons (Fig. 3e and f). Based on the dipolar-dephased spectra, the tannin content appears to be higher for the CH site; however, the differences are small. Quantitative analysis would require analysis of a series of dipolar-dephased spectra with different dipolar dephasing times (e.g., Hatcher 1987), as well as analysis of replicate samples, and some development of protocol for this type of sample.

Dipolar-dephased spectra of the most decomposed Hhi samples (Fig. 6e and f) indicate much lower tannin contents, consistent with the trend shown in the normal spectra (Fig. 4e and f). As was seen for the dipolar-

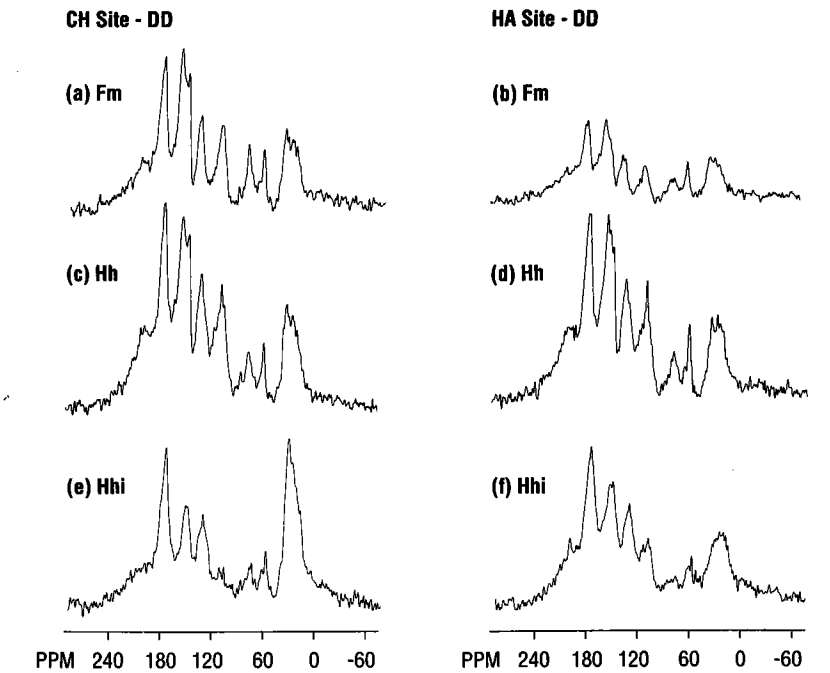

Fig. 6. Dipolar-dephased ^{13}C CPMAS NMR spectra of non-woody (Fm, Hh, Hhi) from CH and HA sites.

dephased Hw spectra, those from the non-woody horizons also show considerable residual intensity from more mobile long-chain aliphatic C. It should also be noted that the presence of residual signal around 75 ppm (O-alkyl CH) for the dipolar-dephased spectra of non-woody horizons indicates insufficient dipolar dephasing time to separate cleanly the two classes of C in these samples. This may be due to higher moisture in these samples than in those from the woody horizons, leading to increased molecular mobility (Hatcher and Wilson 1991).

LITTER INPUTS. To trace the source of the tannins in the organic horizons, some litter inputs and other salal plant parts were examined. The CH and HA litter (Fig. 7b and c) are similar to coniferous litter reported elsewhere (Zech et al. 1987; Kögel et al. 1988), as well as to the Fm horizons except that the resolution is better as there has not been any decomposition. Tannins, if present, are only indicated by the breadth of the phenolic signal with poorly resolved intensity at 145 ppm. The salal litter (Fig. 7a) is different, as it clearly shows a tannin peak at 144 ppm. In addition, the peak at 105 ppm is very large in relation to the O-alkyl signal at 73 ppm, consistent with a sum of anomeric and tannin C.

The spectra of salal flowers, leaves and roots, shown in Fig. 8 (a–c), all have a strong tannin signal at 144 ppm. Interestingly, the tannin content in the leaves is less than in the flowers and roots. Furthermore, the same peak is clearly seen in the litter (Fig. 7a), indicating as expected, that the tannins do not

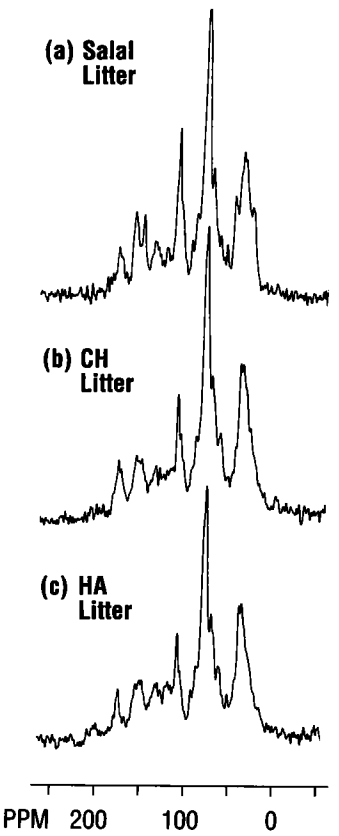

Fig. 7. Carbon-13 CPMAS NMR spectra of litter materials from: (a) CH site salal, (b) CH site coniferous litter and (c) HA site coniferous litter.

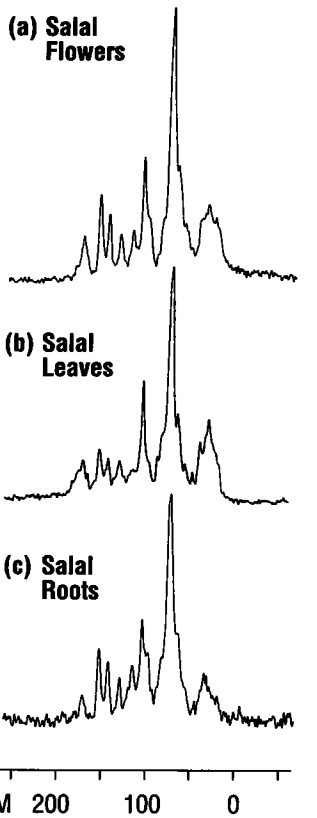

Fig. 8. Carbon-13 CPMAS NMR spectra of: (a) flowers, (b) leaves and (c) roots from salal.

readily decompose. It should also be noted that the spectra in Figs. 7 and 8 all have shoulders at 84 and 100 ppm characteristic of C_4 and C_1 in hemicelluloses.

CuO Oxidation

Results are presented in Table 4 as [V+S+C] in mg (g C)$^{-1}$ and $(Ac/Al)_v$, together with the C content of the samples. The variable [V+S+C] is used as an indicator of lignin content in the different forest floor horizons, although the yields depend on the plant species and types of tissues (Hedges and Mann 1979). The ratio of acid/aldehyde of the vanillyl unit $(Ac/Al)_v$ gives an indication of the degree of lignin biodegradation (Hedges et al. 1988), increasing from about 0.1 in fresh plant leaf litter to values of 1–3 in A horizons.

The data in Table 4 indicate that [V+S+C] does not appear to differ between CH and HA sites for the three non-woody horizons. For the woody horizons, however, [V+S+C] decreases from Fw to Hrw to Hw. This is consistent with the visual assessment used in classifying the woody horizons in the field. There is also a good correlation between [V+S+C] (for both woody and non-woody horizons) and the proportions of lignin C and methoxyl C found by analysis of the NMR spectra (Fig. 9). For the woody horizons, the values of $(Ac/Al)_v$ tend to increase from Fw to Hrw to Hw. Comparing the two site types, the values are similar for CH and HA sites for the Fw and Hrw. For the Hw horizon only, $(Ac/Al)_v$ is higher for HA than CH. This would indicate more effective fungal decomposition in the HA site, possibly due to generally drier conditions (Blanchette et al. 1990). For the non-woody horizons, $(Ac/Al)_v$ increases from Fm to Hh to Hhi horizons for both CH and HA, but the values are higher for the HA than the CH sites from all three horizons, again indicating a more advanced state of lignin decomposition.

In comparing these results with other published work on non-woody forest floor horizons (Kögel 1986; Ziegler et al. 1986), our values of [V+S+C] for non-woody horizons are similar, around 25–30 mg (g C)$^{-1}$. Hedges and Mann (1979) reported a mean value of 30 mg (g C)$^{-1}$ for the yields of CuO oxidation products from non-woody tissues of North American gymnosperms, such as Douglas-fir and red cedar. With increasing depth a slight increase of [V+S+C] is observed. These data therefore suggest only a slight relative accumulation of lignin as compared to the biodegradation of bulk organic matter from non-woody tissues. The values for $(Ac/Al)_v$ for non-woody horizons are also similar, but at the higher

Table 4. Carbon content and yields of phenolics and acid:aldehyde ratios from CuO oxidation of the horizon types (means of duplicate samples)

Humus/site type		C (mg g^{-1})	[V+S+C] mg (g C)$^{-1}$	$(Ac/Al)_v$
(a) Non-woody				
Fm	CH	480	29	0.37
	HA	497	33	0.46
Hh	CH	505	29	0.48
	HA	495	32	0.55
Hhi	CH	376	32	0.88
	HA	350	35	1.14
(b) Woody				
Fw	CH	557	189	0.35
	HA	570	187	0.39
Hrw	CH	553	129	0.69
	HA	552	173	0.64
Hw	CH	537	85	0.69
	HA	531	66	0.93

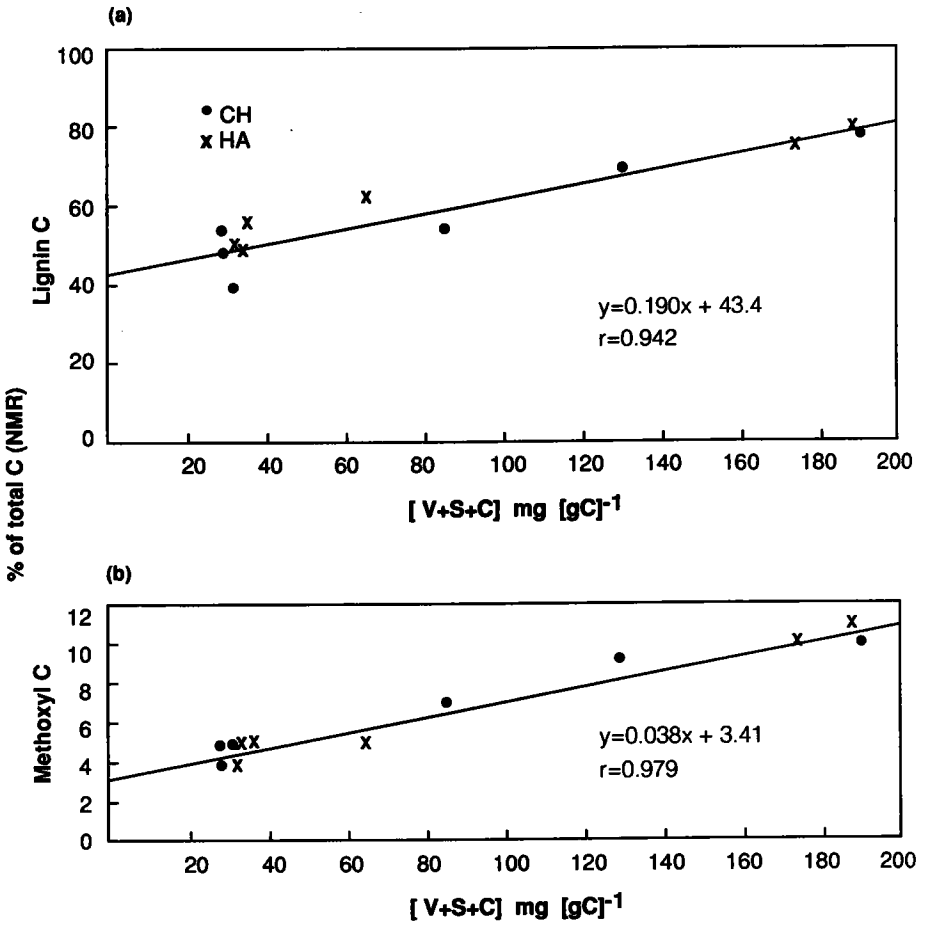

Fig. 9. Correlation of [V+S+C] yields (as mg [g C] $^{-1}$) with NMR determination of (a) lignin C and (b) methoxyl C. All values of [V+S+C] >60 were obtained from woody horizons.

end reported for organic horizons, while the values for the Hi horizons are similar to those reported for mineral horizons, indicative of a high degree of lignin side-chain oxidation in these horizons.

For the woody horizons, [V+S+C] values are higher, but in keeping with the higher lignin component as determined by NMR. Values range from 65 to 189 mg (g C) $^{-1}$, compared to 61 and 85 mg (g C) $^{-1}$ for woody tissues of Douglas-fir and western red cedar, respectively (Hedges and Mann 1979). The elevated lignin phenol yields in the Fw and Hrw horizons as compared with fresh woody tissues point to a selective preservation of lignin compared to polysaccharides during biodegradation of woody tissues in the forest floor. We were not able to locate any reported [V+S+C] data for this type of highly decomposed, ligneous gymnosperm wood residue which is so characteristic of the temperate rainforests of the Pacific Northwest (Preston et al. 1990). For the Fw horizon, $(Ac/Al)_v$ ratios are similar to those reported for organic horizons. They are higher for the Hrw and Hw samples, consistent with the higher carboxyl C content of these two horizons, as determined by NMR.

The CuO oxidation results extend and confirm those from NMR. While they indicate a general similarity in organic components for corrresponding horizons from CH and HA sites, values of $(Ac/Al)_v$ are higher for HA than for CH sites for the Hw and for the non-woody horizons, indicating that lignin decomposition is more advanced in the HA sites. There are good correlations between [V+S+C] and the percentages of lignin and methoxyl C determined by NMR. Results from CuO oxidation are also consistent with the field classification of relative degree of decomposition and wood content.

Site Differences

Neither NMR spectroscopy nor CuO oxidation point to any unusual features of the organic horizons that would explain dramatic differences in seedling performance. The NMR spectra show that the woody horizons are similar to large gymnosperm logs which have undergone extensive decomposition on the forest floor in this environment. Similarly, the non-woody horizons are similar to those widely reported for Histosols and forest organic horizons. The results of the CuO oxidation are also consistent with those reported elsewhere for organic horizons. In this study we found little to distinguish the organic components of similar horizons from the two site types.

Three minor differences were found between CH and HA sites, both largely confined to the non-woody horizons. First, comparison of O-alkyl C content and C_m/L_m ratios indicate that carbohydrates may be more effectively decomposed in the HA sites. Second, $(Ac/Al)_v$ ratios were higher for HA than for CH sites, indicating more extensive decomposition of lignin in the HA sites. Third, tannin contents appeared to be higher in CH than in HA sites. This is supported by the high tannin content in salal, which is much more abundant in the CH phase. It must be emphasized that the differences are small, and to establish statistically significant results would require a much more extensive and costly investigation than was possible in this study. Nonetheless, the trends are all consistent with less effective decomposition in the CH sites.

The higher input and abundance of tannins on the CH site may explain in part the differences in productivity following clearcutting. Tannins have been found to reduce the biodegradability and humification of organic matter by three processes: these are the production of protein-tannin complexes which are much more resistant to microbial decomposition than unaltered proteins; the permeation and coating of non-proteins such as cellulose and hemicellulose by the protein-tannin complexes, giving them considerable resistance to microbial attack; and the inactivation of enzymes important in the process of decomposition (Benoit and Starkey 1968a,b; Zucker 1983). Slower decomposition due to the presence of tannins and generally higher moisture levels could be a factor important to the overall reduction of forest productivity seen on salal-dominated CH sites in this area of northern Vancouver Island.

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